Journal of Chromatography, 125 (1976) 251–263

CHROM. 9584

THE EXCLUSION PROPERTIES OF SOME COMMERCIALLY AVAILABLE SILICA GELS

R. P. W. SCOTT and P. KUCERA
Chemical Research Department, Hoffmann-La Roche Inc., Nutley, N.J. 07110 (U.S.A.)

SUMMARY

The exclusion properties of ten commercially available silica gels have been determined and their relative merits for use as stationary phases in exclusion chromatography discussed. Examples are given of the use of three selected silica gels in the separation of synthetic mixtures of high-molecular-weight standards. The effect of the exclusion properties of silica gel on retention data is considered and a modified retention volume equation that takes into account these exclusion properties is given. The validity of the equation is tested against retention data determined for three different solutes chromatographed on three silica gels of widely different porosities. The use of silica gel as an exclusion medium in conjunction with a column having 250,000 theoretical plates is also demonstrated.

INTRODUCTION

Silica gels are manufactured and supplied for liquid chromatographic purposes with a wide range of surface areas and porosities. In general, the surface area is inversely related to the average pore diameter of a silica gel. Presently, silica gels range from materials with a surface area of 50–100 m^2/g and a mean pore diameter of 300 Å to those with surface areas in excess of 500 m^2/g and a mean pore diameter of 40 Å. The surface area and pore diameter of silica gel imparts specific chromatographic qualities to the material and it has already been shown[1] that the surface area of a silica gel is inversely related to its chromatographic scope. The pore volume and pore diameter, however, have their major effect on the chromatographic properties of the silica gel where substances of large molecular weight are being separated. Owing to the range of pore sizes in any given silica gel, silica gel adsorbents exhibit exclusion properties. These exclusion properties can be used directly to separate substances on a basis of molecular size, but the range of pore diameters also affects the separation characteristics when the silica gel is employed in adsorption chromatography. It is well known that the retention volume of a solute is linearly related to the surface area of the silica gel; however, although a silica gel may have a given surface area, if the pore diameter is such that a molecule is partially excluded, then the solute may be able to come in contact with, perhaps, only a fraction of that surface area. It follows that, under such circumstances, the retention volume will be a fraction of that

expected from the total surface area of the silica gel. This means that solutes of relatively large but significantly different molecular weights will not exhibit the same retention ratios when chromatographed on two silica gels having different pore distributions.

The effective pore diameter will also influence the measurement of the retention volume or capacity ratio (k') value of a solute, as the dead volume for a given solute will not be the sum of the interstitial volume and the total pore volume but the interstitial volume and that proportion of the pore volume that is accessible to the solute concerned.

In this paper the exclusion properties of a number of commercially available silica gels are determined and their suitability for exclusion chromatography examined. Silica gel offers particularly interesting possibilities for exclusion chromatography as, in microparticulate form, very high efficiencies are easily obtainable. Thus, by choosing an appropriate porosity, effective separations on a basis of molecular size are possible.

EXPERIMENTAL

Apparatus

The apparatus used consisted of a Waters Assoc. Model M-6000A high-pressure pump supplied with mobile phase from a glass reservoir. The mobile phase passed from the pump to a sample injection head (Precision Sampling Corp.). The detector employed was a modified LDC UV detector Model 1205 operated at a wave length of 254 nm. The normal cell volume of this detector was between 8 and 10 μl. The path length of the cell, however, was reduced from 1 cm to 3 mm and the diameter of the cell maintained at 1 mm, providing a total volume of 2.4 μl. The inlet tubes to the cell were replaced by a 2-cm length of 0.010 in.-I.D. stainless-steel tubing, having a volume of 1.0 μl and thus the total volume of cell and connecting tube was 3.4 μl. The output from the detector was fed to a 10-mV recorder having a balancing time of 1 sec.

Method

The bulk densities of each silica gel were determined by measuring the volume of a given weight of silica gel when packed, to a bed of minimum volume. These were determined under both wet and dry conditions (the liquid being normal heptane) and the densities are shown in Table I.

Each adsorbent was packed into a 50 cm $\times$ 4.6 mm I.D. stainless-steel column using a slurry method of packing. The packing fluid consisted of a mixture of 25% (v/v) of glycerol in methanol. Packing pressures of 12,000 p.s.i. were used and columns were packed in approximately 3 min.

Subsequent to packing, the columns were reconditioned by passing five dead volumes of ethanol, acetone, ethyl acetate, dichloroethane and *n*-heptane, respectively, through the column. The efficiency of the column was determined using benzene as the solute and *n*-heptane as the mobile phase.

The mobile phase used in the exclusion measurements was tetrahydrofuran supplied by Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.) and was UV grade. The calibration materials used were benzene, naphthyl benzoate, *d*-α-tocopheryl

acetate, and a series of polystyrene standards covering the range of molecular weights from 2,025 to 655,000 in ten intervals. The polystyrene standards were obtained from Waters Assoc. (Milford, Mass., U.S.A.).

In order to measure accurately the retention volume of each standard, the outlet from the detector was connected directly to a 25-ml Grade A burette. The pump was turned off and time allowed for the pressure to fall. The sample was injected into the column and the burette reading noted. The pump was then started and the burette read again at the peak maximum of the eluted solute. Duplicate measurements were taken and the mean value was considered acceptable if values differed by less than 0.5%.

RESULTS

The physical properties of the ten silica gels examined are shown in Table I. It is seen that they have a wide range of surface areas and porosities and include adsorbents that have different particle diameters. The efficiencies obtained are also shown in Table I and from these the peak capacity of each column was calculated[2]. The efficiency values obtained generally reflect what would be expected from the particle diameters of the respective adsorbents.

The retention volume of each of the standards was measured on each of the silica gel samples and the results obtained plotted as curves relating pore volume to pore diameter are shown in Fig. 1. The pore diameter was taken as the molecular diameter of the polystyrene standards as supplied by the manufacturer. The molecular diameter of benzene was taken from the literature[3] and those of 2-naphthyl benzoate and *d*-*α*-tocopheryl acetate were calculated[3]. The interstitial volume was taken as the retention volume of the standard having a molecular weight of 655,000, and this was subtracted from the retention volume of each other standard for each respective silica gel to give the respective pore volume for that standard.

The pore volume accessible to each standard was then expressed as a percentage of the total pore volume of the respective silica gel that was taken as the difference between the retention volume of benzene and the interstitial volume. Curves relating the percentage pore volume against molecular size are shown in Fig. 2. Any point on the curve in Fig. 2 thus represents percentage of the total pore volume that is accessible to a solute molecule of the respective size. This method of accessing pore volume was originally discussed by Halász at the 1970 International Symposium of Chromatography in Houston. From the curves the molecular exclusion limit was taken as the maximum molecular weight that would penetrate 5% of the pores. The permeation range was taken as the range of molecular weights that could permeate between 5 and 95% of the pore volume. These data are also included in Table I. The first and last 5% of the pore volume were not included as the curves are very flat over these ranges and are thus not usable for practical separations by exclusion chromatography.

DISCUSSION

It is seen from Figs. 1 and 2 that the silica gels examined fall into roughly three groups. The first group comprising of Partisil 10, LiChrosorb 10, Biosil A and HA, having a total pore volume of about 0.6 ml/g and a range of pore diameters

TABLE I

PHYSICAL PROPERTIES OF TEN SILICA GELS

Adsorbent	*Average particle size (μm)*	*Surface area (m^2/g)*	*Shape**	*Mean pore diameter*** *(Å)*	*Pore volume**** *(ml/g)*	*MW exclusion limit§*	*Permeation range MW§§*	*Bulk density (dry) (g/ml)*	*Bulk density (liquid) (g/ml)*	*Mass of silica gel in column (g)*	*N (benzene) §§§*	*Peak capacity*
Partisil 10	10	400+	I	40–50	0.661	$3.40 \cdot 10^4$	140– 34,000	0.465	0.220	3.33	10,000	10
LiChrosorb 10	10	200+	I	60	0.631	$1.90 \cdot 10^4$	200– 19,000	0.465	0.213	3.12	10,100	9
Silarex II	10	300	I	130	0.894	$3.00 \cdot 10^4$	230– 30,000	0.382	0.201	2.83	19,600	15
Spherosil 10	10	200+	S	—	1.040	$1.35 \cdot 10^5$	520–135,000	0.387	0.295	2.65	5,064	8
Biosil A	20–44	200+	I	<100	0.550	$1.10 \cdot 10^4$	190– 11,000	0.497	0.359	3.07	3,734	5
Biosil HA	<44	200+	I	<100	0.520	$1.10 \cdot 10^4$	220– 10,000	0.519	0.372	3.10	8,002	7
Sil-LC	44	—	I	—	0.566	$8.40 \cdot 10^3$	190– 8,400	0.564	0.448	3.27	1,840	4
Porasil C	37–75	50–100	S	300	0.966	$1.80 \cdot 10^5$	1,000–180,000	0.404	0.377	2.65	1,799	5
Porasil A	37–75	350–500	S	<100	1.107	$2.30 \cdot 10^4$	180– 23,000	0.424	0.395	2.44	1,406	4
CPG-10	37–75	177	I	75	0.560	$1.96 \cdot 10^4$	240– 19,600	0.486	0.411	2.91	2,326	3

* I = irregular, S = spherical.

** Given by the manufacturer.

*** Exptl. determined as total pore volume/g of silica gel.

§ Determined from exptl. data as the MW corresponding to 5% of the total pore volume.

§§ Determined from exptl. data as the MW range corresponding to 5–95% of the total pore volume.

§§§ Calculated from exptl. data assuming the same peak width of benzene for all solutes in the permeation range.

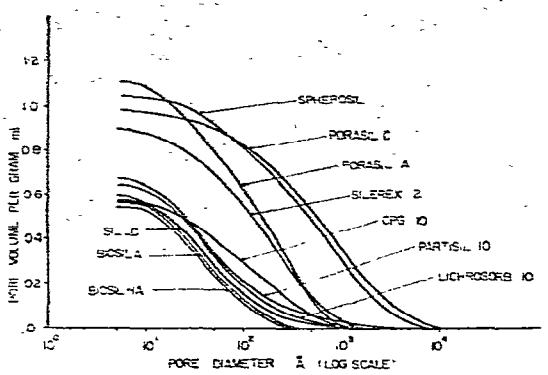


Fig. 1. Curves relating pore volume to pore diameter for different silica gels.

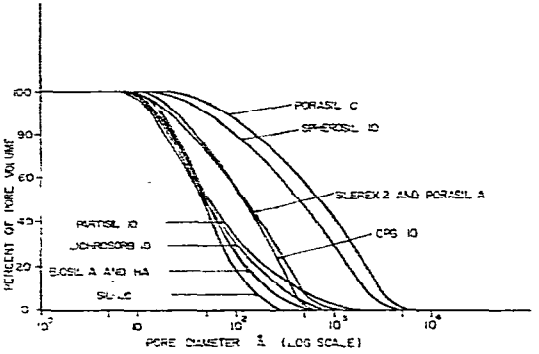


Fig. 2. Curves showing pore size distribution as percentage of total pore volume for different silica gels.

from about 12 to 400 Å; the second group comprising Controlled Pore Glass (CPG) 10, Silarex 2, 10 and Porasil C, having a total pore volume ranging from 0.55 ml/g to 1.5 ml/g and a range of pore diameters from about 20 to 1000 Å; the third group comprising of Porasil C and Spherosil 10 having a total pore volume of about 1 ml/g and a pore diameter range of about 50 to 5000 Å. It is interesting to note that the intermediate range comprising of CPG 10, Silarex 2, 10 and Porasil C, although having similar ranges of pore diameters, are, in fact, quite different forms of silica, the Controlled Pore Glass, having a relatively small pore volume of 0.55 ml/g and, at the other extreme, Porasil, having a pore volume of about 1.6 ml/g. The characteristics of these three groups can be summarized by their exclusion limits and permeation ranges in Table I.

The adsorbent providing the highest efficiency in each group was taken to demonstrate their different exclusion characteristics. The three chosen were Spherosil 10, Silarex 2, 10 and Partisil 10, each having particle diameters of 10 μm. Examples of the separation of the standards having mean molecular diameters of 1100, 240, 49.5, 27.1 and 7.4 Å on the three chosen adsorbents are shown in Fig. 3. It is seen that the large-pore Spherosil 10 is suitable for separating substances having molecular diameters lying between 240 and 11,000 Å; solutes having diameters of less than

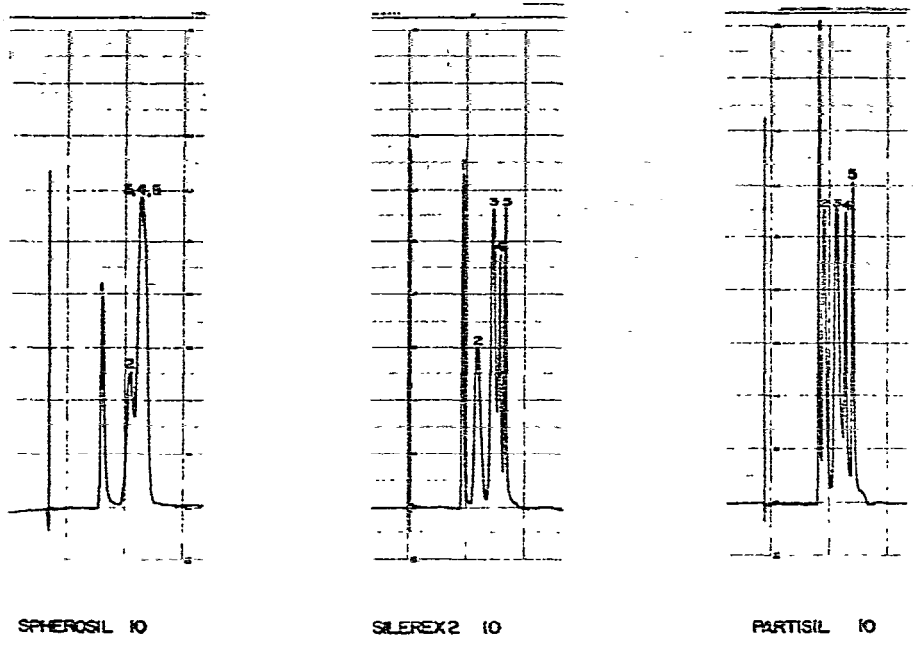


Fig. 3. Exclusion chromatograms from different silica gels. Column length, 50 cm; mobile phase, tetrahydrofuran. Molecular size of standards (Å), (1) 11,000; (2) 240; (3) 49.5; (4) 27.1; (5) 7.4.

240 Å being crowded together and unresolved. The adsorbent having an intermediate range of pore diameters, Silarex 2, 10 would be appropriate for separating substances having molecular diameters between 27 and 240 Å. Little pore volume is available for solutes having molecular diameters greater than 240 Å or less than 27 Å. At the other extreme, Partisil is suitable for separating substances having relatively small diameters covering the range of 5 to about 100 Å.

It would be noted that the peaks for the polystyrene standards (1, 2 and 3) in Fig. 3 are very broad. This is due to the standards not being single compounds but a group of compounds of close, but not identical, molecular weight. The peaks for these standards are thus composed of a number of different solute bands combined together to provide one composite peak.

It is of interest to determine the resolving power of these three columns. Assuming that baseline separation will be obtained when the peak maximum of the two solutes is four standard deviations apart, then the distance between the peak maxima (D) in plate volumes of mobile phase will be given by

$$D = 4\,n^{\frac{1}{2}}\,(v_i + v_j)$$

where n is the efficiency in theoretical plates, v_i is the interstitial volume of a plate and v_j is the pore volume of a plate accessible to the solute. Now the retention volume of a solute ($V_{o(j)}$) is given by

$$V_{o(j)} = n\,(v_i + v_j) = V_i + V_j$$

TABLE II

MASS RESOLUTION ($\Delta M''$) FOR THREE DIFFERENT ADSORBENTS

Adsorbent	V_t (ml)	V_j (ml)	*MW at* V_j	$\left\|\frac{\Delta M}{\Delta V_j}\right\|$ (ml^{-1})	n	ΔM
Partisil 10	3.25	1.10	1,600	3,000	10,000	522
Silarex 2, 10	3.19	1.27	5,200	8,000	19,600	1,019
Spherosil 10	3.27	1.38	18,000	27,500	5,064	7,188

where V_t and V_j are the interstitial column volume and the pore volume accessible to the solute, respectively.

If $\Delta M/\Delta V_j$ is the rate of change of solute molecular weight with retention volume, then the minimum difference in molecular weight ($\Delta M''$) between two substances that are completely resolved is given by

$$\Delta M'' = \frac{4\,(V_t + V_j)}{n^{\frac{1}{2}}} \cdot \frac{\Delta M_{(V_j)}}{\Delta V_j} \tag{1}$$

In Table II, values for $\Delta M''$ (mass resolution) for each of the three selected columns are given together with the data necessary for calculating them from eqn. (1). V_t was experimentally determined and V_j was taken at the midpoint of the curve relating molecular weight to pore volume for each silica gel; $\Delta M/\Delta V_j$ was taken as the slope of these curves at V_j. In Fig. 4, the curves relating mass resolution ($\Delta M''$) to column efficiency calculated from eqn. 1 for each selected silica gel, illustrate the importance of having extremely high efficiencies if good molecular-weight discrimination is to be achieved. Even with columns having efficiencies of 30,000 theoretical

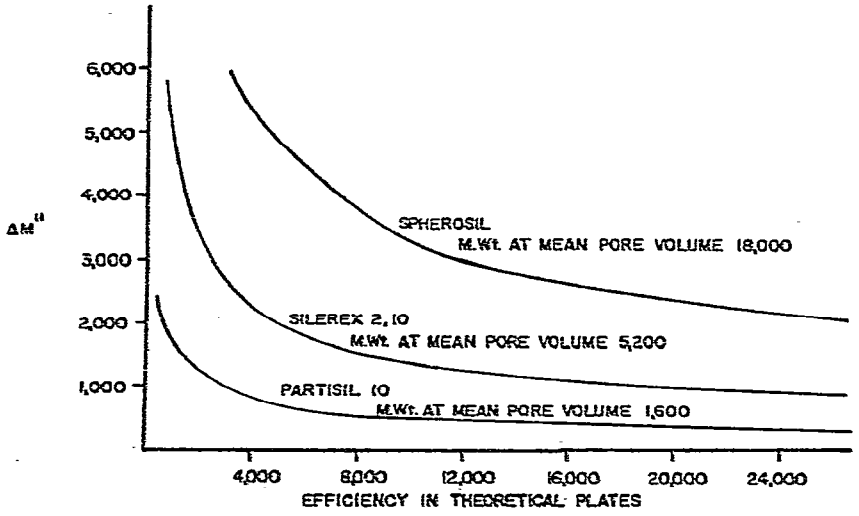
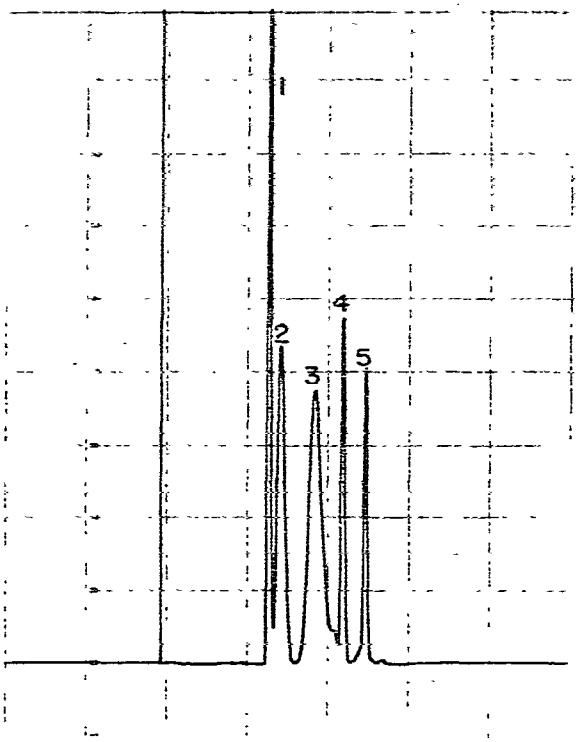


Fig. 4. Curves relating resolution ($\Delta M''$) to column efficiency for the three standard columns.



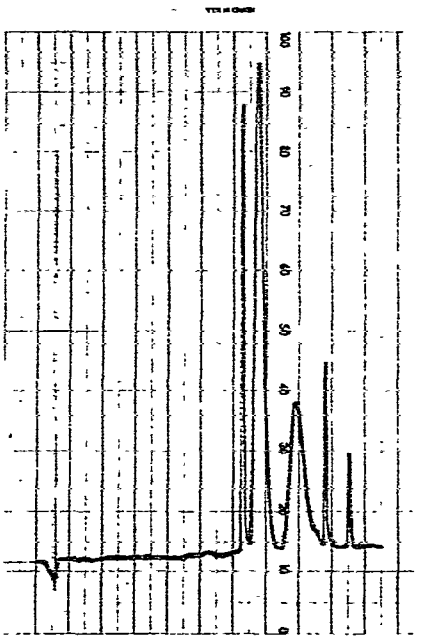


Fig. 5. Exclusion chromatogram from high-efficiency silica gel column. Column, 2 m × 1 mm I.D.; stationary phase, Partisil 20. Molecular size of standards (Å), (1) 11,000; (2) 240; (3) 49.5; (4) 27.1; (5) 7.4.

Fig. 6. Chromatogram of a series of molecular weight standards from a column of 250,000 theoretical plates. Column, 10 m × 1 mm I.D.; mobile phase, tetrahydrofuran; flow-rate, *ca.* 30 μl/min; adsorbent, Partisil 20; solutes, molecular weight standards.

plates, molecular-weight discrimination of 350, 960 and 3,000 at mean molecular weights of 1,500, 5,000 and 20,000, respectively, is the minimum that can be obtained on the different silica gels. In Fig. 5 a chromatogram of the standard mixture obtained from a column of 40,000 theoretical plates illustrates the type of separations obtained using Partisil 20 as the exclusion medium.

In Fig. 6, an exclusion chromatogram is shown obtained from a column having a quarter of a million theoretical plates. The column was 10 m long and 1 mm I.D. slurry packed with Partisil 20. The chromatogram is for the same set of standards as that used in Fig. 4. The chromatogram shown in Fig. 7 is for a mixture of benzene, ethylbenzene, butylbenzene, hexylbenzene, octylbenzene and decylbenzene. The charge on the column was 0.2 μl of a solution containing approximately 3% of each solute; the total mass of each solute being about 6 μg.

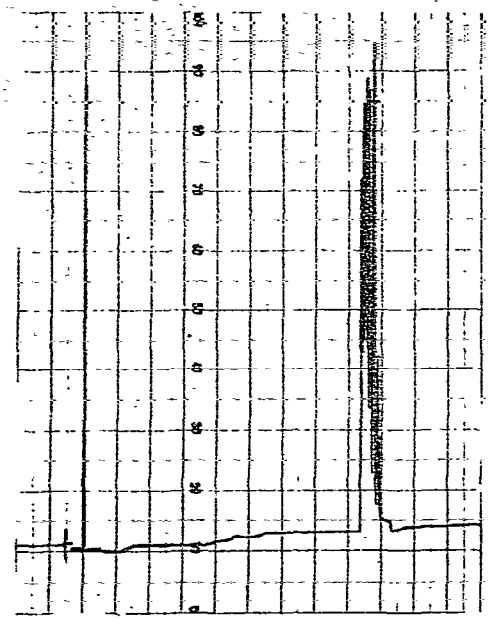


Fig. 7. Chromatogram of a series of alkylbenzenes from a column of 250,000 theoretical plates. Column, 10 m × 1 mm I.D.; mobile phase, tetrahydrofuran; flow-rate, *ca.* 30 μl/min; adsorbent, Partisil 20; solutes: benzene, ethylbenzene, butylbenzene, hexylbenzene, octylbenzene, decylbenzene.

The advantages of high efficiencies in exclusion chromatography are clearly seen; solutes having molecular weight differences equivalent to only two carbon numbers can be well resolved and it is obvious that a difference of one carbon number could be easily detected. The molecular weight of decylbenzene is 218 and thus one methylene group would represent 6.4% of the molecular weight.

In Fig. 8 the same column was used to separate a mixture of methyl phenyl siloxane polymers with a trimethylsilyl terminal group. From the expanded chromatogram on the right of the figure it is seen that each polymer can be identified as a specific peak. The last polymer has a molecular weight of 1800 and as each monomer has a molecular weight of 137 a discrimination in molecular weight of 7.6% was achieved.

Partisil 20 has a surface area of about 400 m^2/g. If a silica gel having a surface area of 800 or more m^2/g was employed and a similar column of high efficiency, then owing to reduced pore size, a discrimination of 1 carbon atom should be obtainable, up to a molecular weight of about 1,000. At molecular weights up to 500, it is likely that the column would be able to discriminate between different sized atoms. Such a column could provide approximate molecular weight data. The system, if compared with the mass spectrometer, would only provide molecular weights and no fragmentation patterns to help in structural elucidation. On the other hand, as the system is also a separation technique, pure samples would not be required. However it should be noted that exclusion chromatography, in fact, separates on a basis of molecular size and thus the shape of the molecule would have to be taken into account to determine

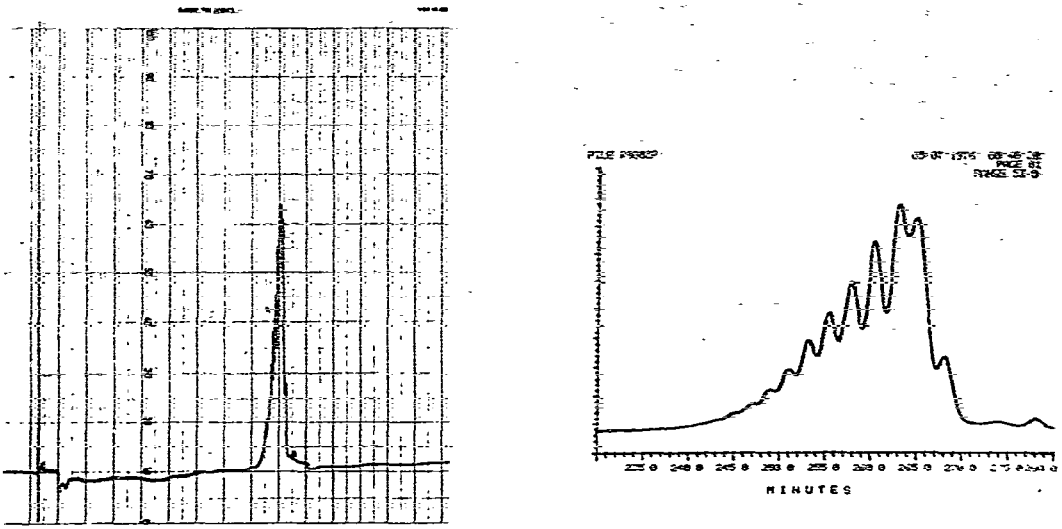


Fig. 8. Chromatogram of poly(phenyl siloxane) with trimethylsilyl terminal.

its molecular weight from its exclusion properties. Alternatively the column could be calibrated in terms of molecular weight for substances of a similar shape or geometry, and in this case the molecular weight could be obtained directly from the calibration curve. The method of injection and detector volume contributions to band variance are extremely critical when using microbore columns. The standard deviation of a peak eluted from a column is given by

$$\sigma = V_r/n^{\frac{1}{2}}$$

For the above high-efficiency column V_0 was 7.1 ml and n was 253,000

$$\text{thus, } \sigma = \frac{7.7}{(253{,}000)^{\frac{1}{2}}} = 15.3\ \mu\text{l}$$

It follows that even a detector having the reduced dimension described will still significantly affect the actual efficiencies obtainable from such columns.

The pore volume and pore size of a silica gel can also significantly affect the retention characteristics of a solute under elution conditions. The retention volume of a solute (V_r) is normally described by the following equation:

$$V_r = V_0 + KA$$

where V_0 is the dead volume and includes the interstitial volume (V_i) and the total pore volume V_p, thus,

$$V_0 = V_i + V_p$$

and

$$V_{r(J)} = V_i + V_p + KA$$

Now, if the solute has a significant molecular weight or size, then it will only be able to enter pores of a size greater than itself. Thus, if the pore volume accessible to the solute molecule is V_j, then

$$V_{r(j)} = V_i + V_j + KA_j$$

where A_j is the surface area associated with the pore volume V_j it follows that

$$k' = KA_j/(V_i + V_j)$$

Further, the separation ratio α between two solutes A and B will be

$$\alpha = \frac{K_B A_{j(B)}}{K_A A_{j(A)}}$$

It is seen that if either k' or α is determined on two silica gels having a different distribution of pore sizes, different values of k' and α will be obtained for the two solutes as A_j and V_j for the respective solutes will differ for each silica gel. In fact, because silica gel always has a distribution of pore diameters, it acts as a programmed stationary phase. Irrespective of the polarity of the solute or solvent, silica gel will elute solutes having larger molecular weight (or strictly larger molecular size) more rapidly than solutes of a smaller molecular weight having the same polarity.

The effect of pore size distribution on retention ratios can be tested experimentally. The three columns packed with Partisil 10, Silarex 2, 10 and Spherosil 10 were employed to separate the solutes benzyl acetate, *d*-α-tocopheryl acetate and naphthyl benzoate by an elution procedure using 3.5% (w/v) ethyl acetate in heptane as the mobile phase. The interstitial volume, pore volume and retention volume for each solute on each column are given in Table III. The values of α for each pair of solutes calculated by different procedures is shown in Table IV.

In procedure A, α values were calculated in the normal way as the ratio of the corrected retention volume of the respective solutes obtained by subtracting the sum of the interstitial volume and total pore volume from the retention volume of each solute. It is seen that the α values obtained for each solute pair differs by as much as 15% between the different adsorbents. In procedure B, the same method was used, but the dead volume was taken as the sum of the interstitial volume and the pore volume for each respective solute. Procedure B results in little improvement in the consistency of the α values for each silica gel, variations of up to 13% still being obtained. In procedure C, an attempt was made to compensate for the different surface areas associated with the accessible pore volume of each solute for each respective silica gel. The assumption was made that the pores were spherical and thus the surface area associated with them was proportional to the radius of the pore and thus the square of the cube root of the respective pore volume.

$$\text{thus, } \alpha_{AB} = \frac{(V_{r(A)} - V_i - V_{j(A)})}{(V_{r(B)} - V_i - V_{j(B)})} \cdot \left(\frac{V_{j(B)}}{V_{j(A)}}\right)^{\frac{2}{3}}$$

This equation can only be a very crude approximation to a realistic correction procedure as the model used is based on a number of unverified assumptions. However,

TABLE III

RETENTION DATA FOR SOLUTES CHROMATOGRAPHED ON SILICA GELS OF DIFFERENT POROSITIES

Solute	*Partisil 10*				*Silarex 2, 10*				*Spherosil 10*			
	V_o	V_i	V_j	V_r	V_o	V_i	V_j	V_r	V_o	V_i	V_j	V_r
2-Naphthyl benzoate	5.45	3.25	1.99	11.94	5.72	3.19	2.45	10.30	6.02	3.27	2.73	8.45
d-α-Tocopheryl acetate	5.45	3.25	1.59	14.12	5.72	3.19	2.24	12.53	6.02	3.27	2.60	9.73
Benzyl acetate	5.45	3.25	2.08	19.55	5.72	3.19	2.47	15.59	6.02	3.27	2.73	11.19

TABLE IV

RETENTION DATA FOR THREE DIFFERENT ADSORBENTS

Procedure A, α calculated using benzene as dead volume for each solute; procedure B, α_j calculated using respective dead volume of each solute; procedure C, α_j' is corrected α_j value assuming spherical pore model.

Solute pair	*Relative retention*		*Adsorbent*			*% Aver. diff.* α
			Partisil 10	*Silarex 2, 10*	*Spherosil 10*	
Benzyl acetate–*d*-α-Tocopheryl acetate	A	α	1.63	1.45	1.40	15.40
	B	α_j	1.53	1.40	1.34	13.41
	C	α_j'	1.28	1.31	1.30	2.31
d-α-Tocopheryl acetate–2-naphthyl benzoate	A	α	1.34	1.49	1.53	13.07
	B	α_j	1.39	1.51	1.58	12.86
	C	α_j'	1.61	1.60	1.63	1.86
Benzyl acetate–2-naphthyl benzoate	A	α	2.17	2.16	2.13	1.86
	B	α_j	2.12	2.12	2.14	0.94
	C	α_j'	2.06	2.11	2.14	3.80

using this procedure, the retention ratios for all three solutes differ by less than 4% between all three silica gels.

It should be also noted that the porosity of the support will affect the linear velocity. The values for the linear velocity of the mobile phase used in the various HETP equations usually pertain to the interstitial volume between the particles where the mobile phase is flowing and not to the static liquid within the pores. Thus linear velocity should be determined from the retention time of a completely excluded peak. Velocities calculated from the retention of a non-adsorbed solute that can penetrate the pores may give velocities less than half the value obtained for a completely excluded peak.

CONCLUSIONS

Silica gels are available in a wide range of pore diameters and pore volumes and for exclusion chromatography the correct material must be chosen to suit the molecular-weight range or molecular-size range of the solutes to be separated. Silica gel can be a very effective stationary phase for exclusion chromatography as in the microparticulate form high efficiencies are readily obtainable. Employing microbore columns having efficiencies of a quarter of a million theoretical plates permit separation on a basis of molecular weight or size with a discrimination of 6% of the mean molecular weight of the species. A carefully selected series of silica gels that would each provide optimum separation for solutes of a specific molecular-weight range would be a valuable aid to the practicing chromatographer. Such silica gels, however, should be supplied with the pertinent exclusion characteristics and performance data to permit a simple selection of the appropriate material for a particular application. Employing very-high-efficiency columns with the material of appropriate pore size can provide an approximate method for determining molecular weights.

Owing to the variation in pore diameter between different silica gels, retention data obtained by the normal elution procedure cannot be compared directly between one adsorbent and another, particularly for solutes having molecular weights in excess of 150. Using the pore volume available to a particular solute, approximate corrections can be applied to improve the correlation of retention data between one adsorbent and another.

REFERENCES

1 R. P. W. Scott and P. Kucera, *J. Chromatogr. Sci.*, 12 (1974) 437.
2 R. P. W. Scott, *J. Chromatogr. Sci.*, 9 (1971) 449.
3 L. Pauling, *The Nature of the Chemical Bond*, Cornell University Press, 1960.