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INFLUENCE OF THE PARTICLE SIZE (5–35 μm) OF SPHERICAL SILICA ON COLUMN EFFICIENCIES IN HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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SUMMARY

The preparation of spherical silica with sieve fractions between 5 and 35 μm is described. Permeabilities (K) and relative peak broadenings (h) have been determined experimentally to be a function of the average particle size (d_p) of the narrow-sieve fractions ($h \approx d_p^{1.3}$). The average particle size can be calculated to a reasonable approximation from the permeability of the column packed by the balanced density method. Simple relationships are proposed for estimating the constants in the equation $h = A + Cu$ as a function of d_p . The maximum sample size is about $2 \cdot 10^{-4}$ g of sample per gram of silica. Some consequences of the pressure limits in high-pressure liquid chromatography are discussed.

INTRODUCTION

It is well known that the speed of analysis in high-pressure (or -performance) liquid chromatography (HPLC) increases with decreasing particle size. The efficiency as a function of the average particle size of the support has been already described¹⁻¹⁴. Different types of stationary phases (silica, glass beads coated with porous silica, diatomaceous earth, uncoated glass beads, etc.) were used in these experiments, and the packing methods (dry, balanced density, viscosity methods, etc.) also differed. The definition of the average particle size of the support is difficult. Hence it is not surprising that sometimes the results of different workers are not in agreement.

Most of the porous supports described in the papers cited above were irregular, *i.e.*, not spherical in shape. In this paper, we describe some chromatographic properties of columns packed with porous spherical silica of narrow-sieve fractions in the range 5–40 μm . The dependence of the height equivalent to a theoretical plate (h) on the linear velocity of the eluent (u) could be described to an excellent approximation by the empirical equation $h = A + Cu$.

If all other parameters are identical, the peak broadening in columns packed with spherical silica is very similar to that obtained with irregular silica. The spher-

ical silica supports, however, have some advantages compared with the "broken" supports:

- (1) Because of the regular shape, the sieve fractions are narrower if a centrifugal air particle classifier is used.
- (2) The reproducibility of the column packing is better¹⁵.
- (3) The specific permeability of columns packed with spherical silica is about 20% higher (*i.e.*, better) than that of columns packed with irregular silica of identical average particle size.

EXPERIMENTAL

Chromatographic apparatus

Self-built equipment was used¹⁵. The maximum flow-rate of the membrane pump was 30 ml/min at 400 atm pressure.

Eluent and samples

The eluent was always *n*-heptane with a viscosity of 0.41 cP at 20°. The inert sample was *n*-octane if the detector was a differential refractometer (Model R401, Waters Ass., Milford, Mass.) and tetrachloroethylene if a UV detector was used. Four aromatic compounds were used as samples: benzene, diphenyl, *o*-terphenyl and nitrobenzene. The sample sizes were between 1 and 10 μg .

Preparation of porous silica beads

The porous silica samples were prepared by hydrolytic polycondensation of polyethoxysiloxane. The procedure has already been described in detail¹⁶⁻¹⁸. To obtain silica beads with different average diameter (d_p), all other surface properties remaining unchanged, only the speed of stirring during the reaction was varied. A 1150-ml volume of polyethoxysiloxane with an average molecular weight of 800 was emulsified in 3.5 l of a solution of ethanol in water (33%, v/v). The speed of stirring was 750, 1000 and 1500 rpm. Ammonia (1.3 mole per 1150 ml of polyethoxysiloxane) was used as the catalyst for the polymerisation reaction.

The surface properties of the different batches with different particle size distribution were nearly identical: specific surface area, $320 \pm 10 \text{ m}^2/\text{g}$; specific pore volume, $1.10 \pm 0.05 \text{ ml/g}$; average pore diameter, 150 Å.

The particle-size distributions of the products are shown in Table I, where it can be seen that the fractions were relatively narrow before the classification procedure. For example, only 10% of the particles were smaller than 8 μm , 50% smaller

TABLE I

PARTICLE-SIZE DISTRIBUTION OF THE SPHERICAL SILICA BEFORE CLASSIFICATION

Speed of stirring (rpm)	$d_{p\ 10}$ (μm)	$d_{p\ 50}$ (μm)	$d_{p\ 90}$ (μm)
1500	8	12	15
1000	10	16	22
750	17	25	38

than $12\ \mu\text{m}$ and 90% smaller than $15\ \mu\text{m}$ if the speed of stirring was 1500 rpm, *i.e.*, the particle-size distribution is symmetrical around the mean value. All measurements described in this paper were made after air particle classification with the silica described above.

By varying the parameters of the basic reaction, the surface properties of the spherical silica can be changed as required. For example, if 1150 ml of polyethoxy-siloxane with a mean molecular weight of 1050 were emulsified in 3.5 l of a solution of ethanol in water (40%, v/v) and 1 mole of ammonia catalyst was added, the surface properties changed to: specific surface area, $370\ \text{m}^2/\text{g}$; specific pore volume, $1.1\ \text{ml/g}$; average pore diameter, $100\ \text{\AA}$.

Particle classification and average particle size

The silicas were sized and graded using a centrifugal air particle classifier (by courtesy of Dr. Krebs, E. Merck, Darmstadt, G.F.R.). The narrow-sieve fractions were recorded microphotographically ($400\times$ enlargement) and the particle-size distribution was determined by the "counting" method. As demonstrated in Fig. 1, the particle-size distribution was an excellent approach to a normal Gaussian type, contrary to the log-normal distribution achieved with irregular silica particles¹³. The average particle size of a sieve fraction is defined as the particle size at 50% probability. In Table II, the size distributions of the air-classified fractions are given. It can be seen from the third column in Table II that the number of particles counted in each fraction decreases with increasing particle size because the photographic enlargement was constant. The arithmetic mean value of the sieve fraction in the second column, as usually given for commercially available products, is greater than the number-averaged \bar{d}_p in the fourth column. The reason is that the arithmetic mean is a weight-averaged value. In the last column in Table II, the standard deviations (σ) are shown

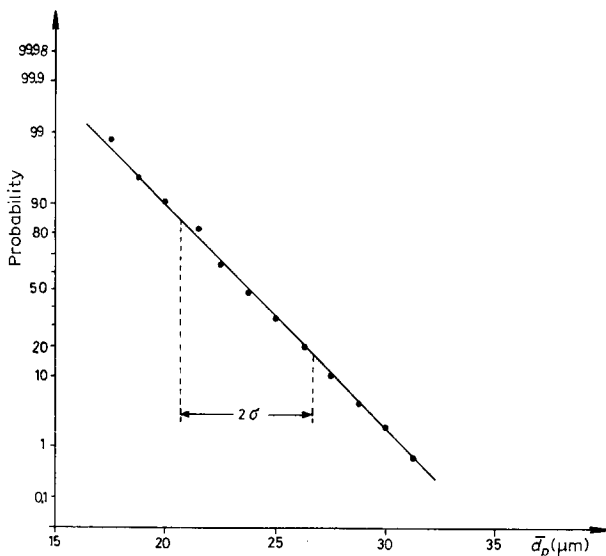


Fig. 1. Probability of the particle size for the sieve fraction 25–32 μm .

TABLE II
SIZE DISTRIBUTION DATA FOR SPHERICAL SILICA

Sieve fraction	Size range (μm)	Particles counted	\bar{d}_p (μm)	σ (μm)	σ (%)
1	5–10	465	5.94	1.48	25
2	10–15	351	11.01	2.40	22
3	15–20	355	16.09	3.26	20
4	20–25	187	23.60	2.80	12
5	25–32	150	24.33	3.00	12
6	32–40	94	33.34	3.46	10

in percentage units of \bar{d}_p . With the 5–10- μm fraction, the lower limits of the air classifier machine are approached, and consequently the standard deviation increases to $\pm 25\%$. With this sieve fraction, however, excellent columns were produced. Similar results were reported with “broad” sieve fractions of irregular silica as the support in HPLC⁸.

Specific permeability

The conventional definition¹⁹ of the (specific) permeability of a packed column is

$$K_F = \frac{F \eta L}{q \Delta p} = \frac{F \eta L}{r^2 \pi \Delta p} \quad (1)$$

where F/q is the “superficial velocity”, *i.e.* the linear velocity of the mobile phase in the empty tube. The calculation of K_F is simple.

In chromatography, however, it is advantageous to define the permeability with the linear velocity of the mobile phase (u), because the retention time is proportional to u (ref. 20), *i.e.*,

$$K = \frac{u \eta L}{\Delta p} = \frac{\eta L^2}{\Delta p t_0} \quad (2)$$

It is sometimes difficult to calculate K because if an inert substance is not known, it is impossible to determine the hold-up time (t_0). By comparing eqns. 1 and 2, it can be shown that

$$K_F = K \varepsilon_T$$

To a reasonable approximation, the total porosity (ε_T) of a regular packed column with a porous support is about 0.82. This value is independent of the particle size of the uncoated support. Both K and K_F values are used in this paper.

The permeability of a column is independent of the temperature provided that the system is isothermal. The heat of friction of the eluent is not negligible²¹ if the pressure drop in the column is greater than 50 atm. With increasing velocity of the eluent (*i.e.*, with increasing pressure drop), the average temperature of the eluent increases and consequently its average viscosity decreases. If the calculations of the permeabilities using eqn. 1 or 2 are made with the “original” viscosity values (*i.e.*,

TABLE III
REPRODUCIBILITY OF K AND h VALUES

Sieve fraction: 5–10 μm . $\bar{d}_p = 11 \mu\text{m}$; $u = 10 \text{ mm/sec}$; $\bar{K} = 1.17 \cdot 10^{-9} \text{ cm}^2 (\pm 8.5\%)$; $\bar{h} = 112.5 \mu\text{m} (\pm 15\%)$.

No.	K (10^{-9} cm^2)	h (μm)
1	1.20	102
2	1.09	129
3	1.14	99
4	1.27	105
5	1.14	110
6	1.17	130

viscosity at the inlet temperature of the column), the calculated results are too high. Otherwise, with increasing flow-rates (*i.e.*, increasing average temperature of the column), decreasing (*i.e.*, worse) permeabilities are determined. The same is also true for the calculation of the total porosity (ϵ_T).

Reproducibility of column packing

All columns were packed in the same stainless-steel tube (30 cm \times 4 mm). The column material was drilled (or, after some use, re-polished). The balanced density packing method^{9,10,22} was used, as described in detail previously¹⁵. In an accepted column the asymmetry (As^2) of the peaks¹⁵ was always less than 2, and mostly better than 1.5. In Table III, the reproducibilities of the K and h values (at $u = 1 \text{ cm/sec}$) are shown. The columns were packed repeatedly in the same tube with the same material with a nominal sieve fraction of 10–15 μm (fraction 2). It can be seen that the standard deviation for the permeability ($\pm 8.5\%$) is much less than that for h ($\pm 15\%$). These results are in good agreement with earlier results¹⁵.

Permeability as a function of the particle size

The permeabilities (K_F) of columns packed with the six sieve fractions as specified in Table II are given in Table IV. The permeabilities are average values and the numbers of independent tests made are given in the last column. As demonstrated in column 4 in Table IV, the reproducibility of the total porosity (ϵ_T) is poor. One reason

TABLE IV
SPECIFIC PERMEABILITIES OF THE SIEVE FRACTIONS

Sieve fraction	\bar{d}_p (μm)	K_F (10^{-10} cm^2)	ϵ_T	No. of independent tests
1	5.94	3.66	0.83	4
2	11.01	10.3	0.88	6
3	16.09	22.3	0.92	3
4	23.60	57.5	0.90	3
5	24.33	60.0	0.89	3
6	33.34	84.9	0.91	3

could have been the inaccurate analysis determination of the empty column volume. By using a regression method, the value of the constant β from the equation

$$K_F \approx (\bar{d}_p)^\beta \quad (4)$$

was calculated to be 1.93 ± 0.11 . Using the weight-averaged mean, *i.e.*, the arithmetic mean of the sieve fraction, $\beta = 2.1 \pm 0.21$. The value of β is, of course, a function of the packing method. To a rough approximation, it can be assumed that K_F (or K) is proportional to d_p^2 . Our results are not in disagreement with those of other workers^{6,13,23,24}.

It can be calculated from the data in Table IV that the values

$$K_F = \frac{\bar{d}_p^2}{1080} \quad (5)$$

and

$$K = \frac{\bar{d}_p^2}{910} \quad (6)$$

are reasonable approximations (with a standard deviation of about 12%) for estimating the permeability if \bar{d}_p (number-averaged mean) is known. The reverse situation, however, is much more important: the permeability can easily be measured and the average particle size has to be determined. The main problem is to establish the kind of average of the particle size that is wanted. Without discussing this problem¹³ and the problem of number- or weight-averaged mean particle size, it is proposed to define an average particle size, d_p , for columns packed with spherical silica using the balanced density packing method by the equation

$$d_p^2 = \frac{K_F}{1000} = \frac{F \eta L}{10^3 r^2 \pi \Delta p} \quad (7)$$

This definition is of great help if sieve fractions under $5 \mu\text{m}$ are used and electron microscopic measurement should be necessary for determining \bar{d}_p . The permeabilities of columns packed by the conventional "dry" method are worse by a factor of about 2 than those packed by the balanced density method⁸.

Another method for the calculation of d_p could be the extrapolation of calibration graphs from eqn. 5 or 6 in the direction of smaller particle sizes. It is questionable which of the methods for the determination of d_p is the most accurate. The simplest experimental method, however, is that based on eqn. 7.

COLUMN EFFICIENCY

The h versus u plots were determined for different samples with different capacity ratios in columns packed with the sieve fractions 1–6. The maximum pressure drop over the column was 400 atm. The linear velocities of the *n*-heptane eluent varied for the columns packed with larger particles between 1 and 40 mm/sec. For the 5–10- μm sieve fraction, the maximum velocity obtained was 15 mm/sec because of pressure limitations. All measurements were made at room temperature and the water contents

TABLE V

A TERMS OF EQN. 8 AS A FUNCTION OF \bar{d}_p

Spherical silica; inert sample.

Sieve fraction	\bar{d}_p (μm)	A (μm)	A/d_p
1	5.94	28.2	6.06
2	11.01	47.7	4.58
3	16.10	144.0	11.35
4	23.60	116.0	5.80
5	24.30	139.0	5.84
6	33.30	152.0	4.70

of the *n*-heptane eluent and of the silica were not controlled and, as a consequence, the capacity ratio of the samples varied, as shown in Tables VI and VII.

In the velocity range used ($u = 1\text{--}45$ mm/sec), the simple relationship

$$h = A + Cu \quad (8)$$

is an excellent approximation if spherical silica is used in the particle-size range 5–35 μm and the balanced density packing method is used.

In Table V, the calculated A terms in eqn. 8 of an inert peak are given as a function of \bar{d}_p . As shown in the last column in Table V, the A term is, to a first approximation, 5–6 times greater than the number-averaged mean particle size [$A \approx (5\text{--}6)\bar{d}_p$] with the exception of sieve fraction 3. This factor is more or less independent of the capacity ratio of the sample. Well packed columns can be characterized by the fact that $A \approx 5 d_p$, where d_p is the weight-averaged mean particle size. From the data of Table V, it can be calculated that the slope of the $\log A$ versus $\log \bar{d}_p$ graph is not unity (as assumed in the first approximation), but it is 1.15 ± 0.17 . This value is much higher (better for the routine of chromatography) than the value of 0.6 reported earlier for columns dry-packed with irregular-shaped silica¹. The reason for this discrepancy is, of course, the different packing methods used.

In Tables VI and VII, the constants A and C are given for different samples as a function of the mean particle size of the support. The figures in parentheses are the standard deviations in percentage units. With every column, the h values were

TABLE VI

A TERMS FOR DIFFERENT SAMPLES IN MICROMETRES

Spherical silica. Eluent: *n*-heptane.

No.	\bar{d}_p (μm)	Tetra-chloroethylene ($k' = 0$)		Benzene		Diphenyl		<i>o</i> -Terphenyl		Nitrobenzene	
		A	k'	A	k'	A	k'	A	k'	A	k'
1	5.94	28.2 (17%)	0.29	25.6 (10%)	0.32	36.7 (15%)	0.66	28.1 (40%)	1.26	67.7 (11%)	4.50
2	11.0	47.7 (5%)	0.32	43.8 (5%)	0.32	50.4 (5%)	0.77	50.5 (3%)	1.53	69.3 (6%)	5.15
3	16.1	144.0 (16%)	0.32	158.0 (25%)	0.32	182 (24%)	0.76	240 (14%)	1.51	—	—
4	23.6	116 (21%)	0.30	129 (15%)	0.30	131 (10%)	0.81	123 (6%)	1.58	184 (41%)	4.34
5	24.3	139 (17%)	0.40	126 (20%)	0.40	148 (12%)	1.10	100 (34%)	2.30	142 (6%)	8.20
6	33.3	152 (28%)	0.33	161 (30%)	0.33	261 (7%)	0.89	308 (15%)	1.77	280 (23%)	8.10

TABLE VII

C TERMS OF EQN. 8 FOR DIFFERENT SAMPLES IN MILLISECONDS

Spherical silica. Eluent: *n*-heptane.

No.	\bar{d}_p (μm)	Tetra chloroethylene ($k' = 0$)		Benzene		Diphenyl		<i>o</i> -Terphenyl		Nitrobenzene	
		<i>C</i>	k'	<i>C</i>	k'	<i>C</i>	k'	<i>C</i>	k'	<i>C</i>	k'
1	5.94	1.8 (56%)	0.29	2.2 (25%)	0.29	2.1 (57%)	0.66	9.8 (25%)	1.26	6.9 (23%)	4.50
2	11.00	6.3 (5%)	0.32	5.8 (4%)	0.32	7.0 (4%)	0.77	12.7 (1%)	1.53	5.7 (8%)	5.15
3	16.10	9.0 (13%)	0.32	9.7 (22%)	0.32	13.4 (17%)	0.76	18.3 (10%)	1.51	—	—
4	23.60	13.4 (10%)	0.30	13.6 (8%)	0.30	21.0 (3%)	0.81	37.0 (1%)	1.58	25.0 (16%)	4.34
5	24.30	15.0 (7%)	0.40	14.0 (8%)	0.40	21.0 (4%)	1.10	43.0 (4%)	2.30	22.0 (2%)	8.20
6	33.30	24.0 (9%)	0.33	23.0 (10%)	0.33	32.0 (3%)	0.89	56.0 (4%)	1.77	41.0 (7%)	8.10

determined for a given sample using at least 8–10 velocities and every measurement was repeated at least three times. Unexpectedly high *A* terms were determined in the column packed with sieve fraction 3 ($\bar{d}_p = 16.1$). Unfortunately, this result was reproducible when new columns were repeatedly repacked. No explanation was found for this result. Large deviations in the k' values are common if the water content of the *n*-heptane eluent is not carefully controlled.

Dependence of the *C* term on particle size

In Fig. 2, the *C* terms are plotted as a function of $\log(\bar{d}_p)$ for an inert and for a retarded sample. The calculated exponents of the relationship

$$C \approx (\bar{d}_p)^\beta \quad (9)$$

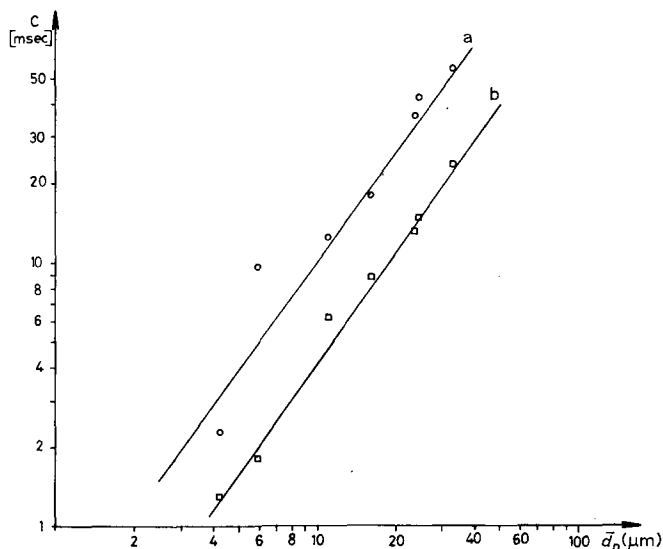


Fig. 2. Mass transfer (*C*) terms for spherical silica. Eluent: *n*-heptane. (a) *o*-Terphenyl ($k' = 1.3\text{--}3.2$); (b) tetrachloroethylene ($k' = 0$).

TABLE VIII

EXPONENTS OF THE EQUATION $C \approx \bar{d}_p^\beta$ Eluent: *n*-heptane.

Sample	β
Tetrachloroethylene	1.41 ($\pm 5\%$)
Benzene	1.38 ($\pm 5\%$)
Diphenyl	1.56 ($\pm 4\%$)
<i>o</i> -Terphenyl	1.37 ($\pm 14\%$)
Nitrobenzene	1.48 ($\pm 21\%$)
	Av. 1.44 ($\pm 10\%$)

are shown for different samples in Table VIII. To a rough approximation, the sample-averaged value of β is 1.5 (i.e., $C \approx \sqrt{\bar{d}_p^3}$).

Dependence of C on capacity ratio

In eqn. 8, it is assumed that the mass transfer in the mobile phase (C_m) is much slower than that "in" the solid stationary phase (C_s), i.e., $C = C_m \gg C_s$. It can be expected that C will be inversely proportional to the diffusion coefficient of the sample in the mobile phase (D_m). This D_m value can be calculated by using the empirical relationship of Wilke and Chang²⁵:

$$D_m = 7.4 \cdot 10^{-8} \cdot \frac{T M^{0.5}}{\eta V^{0.6}} \quad (10)$$

where T is the temperature ($^\circ\text{K}$), M and η are the molecular weight and viscosity, respectively, of the eluent and V is the mole volume of the sample. It cannot be expected that the dependence on capacity ratio of the empirical constant, C , in eqn. 8 will be identical with the function described by Golay³⁰ for the mass transfer term in the mobile phase in open tubes. To a first approximation, it can be assumed that $C \approx E$, where E is a function of k' and is, of course, not dimensionless. It can be assumed that

$$C = E \cdot \frac{\bar{d}_p^{1.44}}{D_m} \quad (11)$$

The constants C in eqn. 8 were calculated from experimental data with *n*-heptane as the eluent for the samples tetrachloroethylene, benzene, diphenyl, *o*-terphenyl and nitrobenzene in columns packed with different sieve fractions of spherical silica. The diffusion coefficients were calculated from eqn. 10 in order to determine E from eqn. 11. The results have been described in detail elsewhere²⁶. The constant $E = 3.4 \cdot 10^{-3}$ [$\text{cm}^{0.56}$] was independent of the capacity ratios, if a deviation of $\pm 20\%$ was allowed, especially if the k' values were less than 2. To judge the quality of a column packing, the C terms should be calculable with

$$C = 3.4 \cdot 10^{-3} \cdot \frac{\bar{d}_p^{1.44}}{D_m} \quad (11a)$$

where C has the dimension of seconds, if \bar{d}_p is given in centimetres and D_m in cm^2/sec units. If the \bar{d}_p values of the narrow sieve fractions are in the range $2\text{--}20\ \mu\text{m}$ and an error of $\pm 50\%$ is allowed, the following simplified approximation is possible:

$$C = \frac{d_p^{3/2}}{2 D_m} \quad (12)$$

In this equation, C can be calculated in milliseconds if d_p (the weight-averaged mean particle size as usually given for commercially available products) is given in micrometres and D_m in $10^5\ \text{cm}^2/\text{sec}$ units. This unusual unit for D_m is proposed because the diffusion coefficients of the samples (with molecular weights less than 1000) in liquids are of the order of $10^{-5}\ \text{cm}^2/\text{sec}$.

The C values calculated by using eqn. 12 were compared with those determined experimentally. The deviation of the calculated from the measured C values are shown in Table IX. The error in D_m as calculated by the Wilke and Chang equation²⁵ is, of course, included in the data in Table IX, as well as the error in the determination of d_p and that in the calculation of C from experimental data. Furthermore, it is assumed that the columns are packed by the balanced density method.

In our experience, the h versus u curves achieved with irregular and spherical silica are similar if the sieve fractions are similar. Eqn. 12 could be a rough approximation also for columns packed with irregular-shaped silica.

TABLE IX

DEVIATION OF THE CALCULATED AND EXPERIMENTAL C VALUES (%)*

Eluent: *n*-heptane. D_m values as calculated with the Wilke and Chang equation²⁵ are in ($10^5\ \text{cm}^2/\text{sec}$) units: tetrachloroethylene (3.16); benzene (3.75); diphenyl (2.53); *o*-terphenyl (2.00); nitrobenzene (3.24).

d_p (μm)	Tetrachloroethylene	Benzene	Diphenyl	<i>o</i> -Terphenyl	Nitrobenzene
5.94	+27	-12	+36	-63	-68
11.0	-8	-16	+3	-28	-1
16.1	+14	-11	-5	-12	-
23.6	+35	+12	+8	-23	-29
24.3	+26	+14	+13	-30	-16
33.3	+27	+11	+19	-14	-28

* $[(C_{\text{exp.}}/C_{\text{calc.}}) \times 100] - 100$; $C_{\text{exp.}}$ values were calculated with eqn. 12.

Dependence of h on particle size

By statistical evaluation of the experimental data as discussed above, the dependence

$$h \approx d_p^{1.3} \quad (13)$$

was found when the velocity of the eluent was constant. In Fig. 3, h versus \bar{d}_p semi-logarithmic plots are shown for the samples benzene and *o*-terphenyl. In Table X, the exponents of eqn. 13 are shown for different samples. The average value is $1.3 \pm 11\%$.

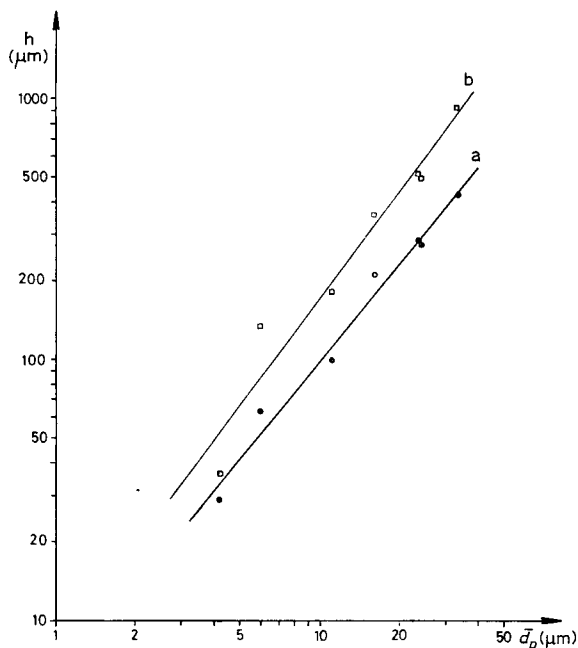


Fig. 3. Semilogarithmic h versus \bar{d}_p plots for spherical silica. Eluent: *n*-heptane, $u = 1$ cm/sec. (a) Benzene; (b) *o*-terphenyl.

Dependence of h on capacity ratio

As shown above, to a rough approximation the constant A in eqn. 8 is independent of D_m , while the constant C is inversely proportional to D_m . Consequently, with increasing linear velocity (*i.e.*, $Cu > A$), h will become increasingly inversely proportional to the diffusion coefficient. In Table XI, the height equivalents to a theoretical plate are given for different samples at a linear velocity of 1 cm/sec as a function of \bar{d}_p . Dividing the h values in Table XI by the calculated diffusion coefficients as given in Table IX, the standard deviation of the average h value for a given particle size is 53%. In this calculation, the h/D_m ratios are greater by a factor of 1.9 than the average values for terphenyl, which is a consequence of the error in D_m if

TABLE X
EXONENTS OF THE EQUATION $h \approx \bar{d}_p^\beta$
Eluent: *n*-heptane.

Sample	β^*
Tetrachloroethylene	1.16 (8%)
Benzene	1.24 (7%)
Diphenyl	1.38 (8%)
<i>o</i> -Terphenyl	1.36 (12%)
Nitrobenzene	1.34 (18%)
Av. 1.3 ($\pm 11\%$)	

* Standard deviations are given in parentheses.

TABLE XI

h VALUES (μm) AT $u = 1$ cm/sec WITH *n*-HEPTANE AS ELUENT

d_p (μm)	Tetrachloroethylene	Benzene	Diphenyl	<i>o</i> -Terphenyl	Nitrobenzene
5.94	68.4	63.0	73.0	133.0	136.5
11.0	112.5	99.0	122.0	179.0	143.0
16.1	226.0	210.0	263.0	356.0	248.0
23.6	303.0	286.0	368.0	513.0	463.0
24.3	261.0	275.0	336.0	494.0	354.0
33.3	416.0	424.0	611.0	919.0	709.0

calculated with the Wilke and Chang equation²⁵. Excluding the *h* values of *o*-terphenyl, the standard deviation of the average decreases to 31%. To summarize, at the usual linear velocities (*i.e.*, 1 cm/sec or higher) used in routine work, it is an acceptable approximation that *h* is inversely proportional to D_m if \bar{d}_p is between 6 and 34 μm .

Pressure required per unit plate

As shown above, $K \approx (\bar{d}_p)^2$ and $h \approx (\bar{d}_p)^{1.3}$. From eqn. 2 with $L = hn$, it follows that

$$\frac{\Delta p}{n} = u \eta (\bar{d}_p)^{-0.7} \approx \frac{u \eta}{(\bar{d}_p)^{2/3}} \quad (14)$$

The pressure drop required per unit plate ($\Delta p/n$) at constant linear velocity increases with decreasing particle size.

Speed of analysis

The number of theoretical (or effective) plates generated per unit time:

$$\frac{n}{t} = \frac{u}{h(1+k')}$$

or

$$\frac{N}{t} = \frac{u}{h} \cdot \frac{(k')^2}{(1+k')^3} = \frac{u}{H(1+k')} \quad (15)$$

always increases with decreasing particle size of the support because, with u constant, the relationship $h \approx d_p^{1.3}$ is valid. In routine work, a given number of plates is required owing to the given relative retentions, and very rapid analysis is required. Consequently, provided that the required inlet pressure is available, the smallest possible particle size of support is optimal. However, if the limitation of pressure is serious, it might be necessary to increase the particle size in order to decrease the required pressure per unit plate. It is then disadvantageous if the column has to be elongated in order to achieve the number of plates required. Consequently, the time of analysis increases if the linear velocity is kept constant. A further disadvantage of elongation of the column is that the peak heights become smaller and the minimum detectable sample size decreases.

With a given maximum inlet pressure and with a given column geometry, the linear velocity increases if an eluent with a lower viscosity is used. Consequently, the speed of analysis increases with decreasing viscosity of the mobile phase. However, if eluents of similar chemical constitution are used (because of the solubility of the mixture to be separated), the boiling point of the eluent generally decreases with decreasing viscosity and the probability of bubble formation in the detector or in the pump increases. Furthermore, especially if columns packed with very small particles are used, the increase in the diffusion coefficients of the sample molecules in the eluent with decreasing viscosity is not negligible. With increasing D_m , the second term in the Van Deemter equation (*i.e.*, B/u) cannot be neglected, as was done in eqn. 8. This problem will be discussed in a following paper.

It should be pointed out again that all of the results presented in this paper are valid only if $5 \mu\text{m} < d_p < 35 \mu\text{m}$ and the columns are packed by the balanced density method.

The speed of analysis in columns packed with material of different particle size and for different samples can be calculated from the h values shown in Table XI if the linear velocity is 1 cm/sec. Combining eqns. 8 and 15, it is evident that the optimum speed of analysis is achieved if $A \ll Cu$. The A terms in micrometres are high compared with the C terms in milliseconds, as was shown in Tables VI and VII. Consequently, with decreasing d_p (and decreasing permeability), it becomes more and more difficult to achieve the velocity in a given column length required for $Cu \gg A$.

Loadability or maximum sample size

With increasing sample size, the h values increase and the capacity ratios (k') decrease if all other parameters are kept constant. In Fig. 4, the height equivalent to a theoretical plate for different samples is plotted as a function of the sample size (s). The column (30 cm \times 4 mm) was packed with spherical silica ($d_p = 16.1 \mu\text{m}$). The

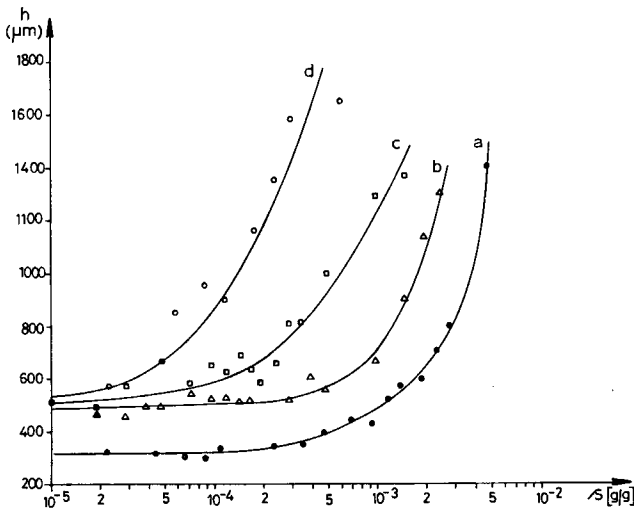


Fig. 4. Peak broadening as a function of the sample size. (a) Benzene; (b) diphenyl; (c) *o*-terphenyl; (d) nitrobenzene. Spherical silica ($d_p = 16.1 \mu\text{m}$); column length, 30 cm; I.D., 4 mm; eluent, *n*-heptane; $u = 1$ cm/sec.

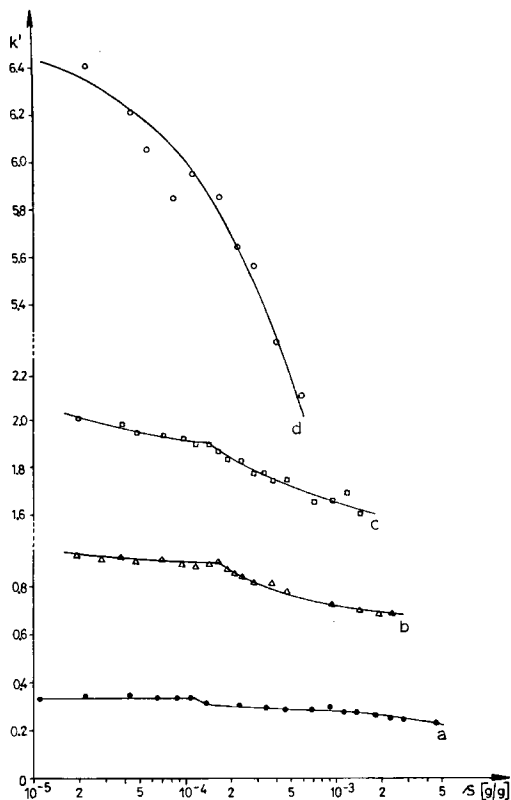


Fig. 5. Capacity ratios as a function of the sample size. Parameters as in Fig. 4.

sample size is given in the dimensionless units of grams of sample per gram of stationary phase. In Fig. 5, the capacity ratios of the four samples are shown as a function of the sample size. The maximum sample size (or loadability of a stationary phase) can be determined with different definitions, for example:

(a) the sample size where the h value is doubled compared with the h determined with the minimum detectable sample size;

(b) the sample size where k' decreases by 10% compared with that determined with the minimum detectable sample size.

In Table XII, the loadability is shown for both definitions. The limits are similar in both columns, but definition (b) seems to be more severe than (a). The maximum sample size for porous silica is about $2 \cdot 10^{-4}$ g of sample per gram of silica. It was shown experimentally that this value is independent of the sieve fraction of the support provided that the silica was prepared by a given method. Experience in our and in other laboratories²⁷ has shown that the loadability of commercially available porous silica is always about or greater than 10^{-4} g/g provided that their specific surface area is greater than $50 \text{ m}^2/\text{g}$ and the average pore size is great enough not to exclude the sample molecules.

The units of loadability (g/g) could be misunderstood. The weight of the stationary phase (and consequently the sample size) can be increased by increasing either

TABLE XII
LOADABILITY OF THE POROUS SPHERICAL SILICA
Parameters as in Fig. 4.

Sample	$k'_{max.}$ *	Loadability (10^{-4} g/g)	
		Method (a)	Method (b)
Benzene	0.3	17	2.0
Diphenyl	0.9	17	3.0
<i>o</i> -Terphenyl	2.0	6.0	2.5
Nitrobenzene	6.3	1.5	1.8

* Capacity ratio with the minimum detectable sample size.

the inner diameter or the length of the column. If the column is too short, for example, shorter than 4 cm packed with $d_p > 5 \mu\text{m}$, inlet and outlet turbulences of the eluent may result in unusually large peak broadening. If the column length is increased excessively, the inlet of the column will be overloaded; when the inner diameter of the column is increased, the problems of sampling increase and it becomes more and more difficult to distribute the sample uniformly over the whole cross-section of the column at the inlet.

Because of the reasons mentioned above, the loadability as given in this paper is limited to the usual and "symmetrical" column geometry. The inner diameter of the column should be between 2 and 8 mm and the length between 5 and 100 cm. The fact that the loadability is proportional to the weight of the stationary phase as determined experimentally is not in agreement with the theory of the "infinite diameter column"^{28,29}.

CONCLUSION

The preparation and experimental chromatographic properties of spherical porous silica in the particle size range 5–35 μm have been investigated. Permeabilities and peak broadenings in columns packed with spherical and irregular-shaped silica are similar if the average particle sizes are similar. To check the quality of a column (packed by the balanced density method) in HPLC, the following procedure is proposed. The average particle size (d_p) can be calculated as a function of: (1) the column geometry (L, r); (2) the viscosity of the eluent (η); and (3) the chromatographic parameters ($F, \Delta p$) as proposed in eqn. 7. The constants of the simple relationship $h = A + Cu$ can be estimated by using $A \approx 5 d_p$ and $C \approx (d_p)^{3/2}/2 D_m$ (eqn. 12). The error involved in these calculations may be as great as $\pm 50\%$, partially because of the inaccuracy of the diffusion coefficient calculated by the empirical relationship of Wilke and Chang²⁵. It seems to be meaningful to define the average particle size in terms of the permeability of the column because in order to obtain a large number of plates or a high speed of analysis a great pressure drop over the column must be tolerated.

With decreasing d_p , the permeability decreases more rapidly ($K \approx d_p^2$) than the relative peak broadening ($h \approx d_p^{1.3}$). Consequently, the pressure drop per unit plate increases with decreasing particle size if the linear velocity of the eluent is kept constant. With decreasing particle size ($d_p < 5 \mu\text{m}$), the B/u term of the Van Deemter

equation is not negligible. The inlet pressure required for a given linear velocity increases and so the heat of friction also increases. The speed of analysis, however, always increases with decreasing particle size, provided that the particle size is greater than $5 \mu\text{m}$. Because of the reasons mentioned above and because of the higher precision of the equipment required with decreasing particle size (*i.e.*, smaller dead volume of the detector, shorter full-scale time of the recorder, problems of the column packing, etc.) it seems that the optimum particle size of the support in HPLC should not be much less than $5 \mu\text{m}$.

To give a more complete picture, some results are plotted in Figs. 2 and 3 achieved with a column packed with $\bar{d}_p = 4.2 \mu\text{m}$. These and some further experimental results and their consequences will be discussed in a following paper, where the minimum particle size of the support optimal in routine HPLC will be estimated.

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SYMBOLS

- A = constant in eqn. 8;
- C = constant in eqn. 8, mass transfer;
- D_m = interdiffusion coefficient of the sample in the eluent;
- \bar{d}_p = average particle size as defined in eqn. 7;
- \bar{d}_p = number-averaged mean particle size;
- E = constant in eqn. 11;
- h = height equivalent to a theoretical plate;
- K = permeability defined with the linear velocity in eqn. 2;
- K_F = permeability defined with the superficial velocity in eqn. 1;
- k' = $(t_R - t_0)/t_0$ = capacity ratio;
- L = column length;
- M = molecular weight of the eluent;
- N = number of effective plates;
- n = number of theoretical plates;
- Δp = pressure drop across the column;
- r = radius of the column;
- T = temperature ($^{\circ}\text{K}$);
- t_0 = retention time of an inert peak;
- t_R = retention time of a retarded peak;
- $u = \frac{L}{t_0}$ = linear velocity of the eluent;
- V = molecular volume of the sample;
- β = exponent in eqns. 4 and 12;
- η = viscosity of the eluent;
- ε_T = total porosity;
- σ = standard deviation.

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