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COMPARATIVE STUDY OF THE PERFORMANCE OF SPHERICAL AND ANGULAR SILICA AND ALUMINA SUPPORTS IN THE 1-10- μm SIZE RANGE

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SUMMARY

Columns packed with four different sets of well characterized packings, spherical silica, angular silica, spherical alumina and angular alumina, varying in mean particle diameter (d_p) between 1 and 10 μm , were compared. On these columns, the chromatographic permeability (K_{t0}), the column resistance parameter (Φ) and the reduced plate height-velocity dependences were determined under identical and comparable conditions. Discrepancies were found for K_{t0} and Φ depending on the type of packing and the particle shape. The courses of the h versus v plot, however, were nearly identical for packings with $d_p > 4 \mu\text{m}$, showing a minimum at $v \approx 5$ and $h = 1.5-2.5$ for solutes of $k' \approx 0.2$ and $h = 2.5-4.5$ for solutes of $k' = 0.5-5.0$. Distinctive deviations in the reduced plots, particularly at $v > 5$, were observed for packings with $d_p < 4 \mu\text{m}$, which may be due to thermal effects caused by a temperature gradient in the column.

INTRODUCTION

In high-performance liquid chromatography (HPLC), it is now commonly accepted that columns regularly packed with 5-10- μm particles provide optimal resolution and speed. On the other hand, it is also well known that a further improvement in column efficiency may be possible by reducing the particle size to 1 μm . In an early paper, Knox and Saleem¹ suggested the use of 2- μm particles as a result of a theoretical study on liquid chromatography (LC). Martin *et al.*² and Halasz *et al.*³ made some extended optimization calculations, taking the minute particle diameter into account. However, there are few published experimental data on the performance of columns packed with 3-4- μm particles^{4,5}, which is not surprising because the prepa-

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ration and operation of columns containing such small particles create various problems, as follows.

(1) If one has batches of suitable microparticles available, the first problem is to size them into narrow cuts and to measure simultaneously their particle size distribution. As sizing by elutriation is limited to particles with $d_p \approx 5 \mu\text{m}$, the only method that can be used is sedimentation, which is a very tedious procedure⁶. Particle size analysis is also extremely difficult: under the microscope the fine particles do not remain in a stable position but move continuously owing to Brownian motion. Applying the photosedimentation technique, the absorption coefficient, which is assumed to be constant at larger d_p values, now becomes strongly dependent on the particle size at $d_p < 5 \mu\text{m}$. Hence the particle size data must be corrected for this dependence.

(2) If narrow-sized microparticles are available, the next step will be to pack the column. In order to produce a homogeneously packed bed, an appropriate slurry should be prepared in order to prevent aggregation of the fine particles. Packing conditions such as flow-rate and pressure should be also optimized.

(3) The most serious problem consists in operating these columns without a decrease in performance owing to extra-column effects. As already pointed out by Knox and Saleem¹ and shown experimentally by Kirkland⁷, the sample should be injected as a sharp pulse in the centre of the packing. Also, the volume of the detector cell and that of connections must be minimized.

(4) A special problem in achieving ultimate performances is the error in the peak width measurement introduced by the relatively large time constant of the detector and recorder. When the response time of the whole system is about 0.3 sec and the peak width of a solute may be a few seconds, this effect then becomes noticeable. No detectors and recorders that have time constants of a few milliseconds are available.

From this discussion, the ultimate limits in HPLC with very small particles are apparent. The purpose of this work was to pack and to characterize columns with microparticulate supports of different origins having particle diameters in the 1–10- μm size range.

EXPERIMENTAL

Supports

The packings used were both home-made and commercial preparations, grouped into four sets, termed spherical silicas, angular silicas, spherical aluminas and angular aluminas (Table I). Each set was of the same origin, as demonstrated by the specific surface area and the pore structure parameters, and differed only in the mean particle diameter.

Packings with $d_p > 5 \mu\text{m}$ were sized into narrow cuts by means of the elutriation technique using an Alpine 100 MZR Zick-Zack Siever (Alpine, Augsburg, G.F.R.). Packings with $d_p < 5 \mu\text{m}$ were classified by means of sedimentation using an appropriate solvent. Sedimentation was also applied to packings of $d_p > 5 \mu\text{m}$ after sieving in order to remove the fines.

Particle size analysis was performed in two different ways. Spherical packings were characterized by their projected area diameter (d_p^a), which was found by microscopic viewing. The number frequency distribution obtained was converted into a

TABLE I
PROPERTIES OF SUPPORTS

ϵ_p = particle porosity. S_{BET} = specific surface area according to the B.E.T. method using $A_m(N_2) = 0.162 \text{ nm}^2$ per molecule. D = most frequent pore diameter in the relative pore volume distribution curve derived from the nitrogen desorption branch using the method of Pierce.

Support	No.	d_{pw}^{a*} or d_{pw}^{st*} (μm) *	ϵ_p	S_{BET} (m^2/g)	D (nm)
Spherical silica	1	3.8 ± 1.4	0.54	183	10.9
	2	6.1 ± 1.5	0.54	183	10.9
	3	11.0 ± 2.1	0.54	183	10.9
Angular silica	4	2.8 ± 1.3	0.64	475	6.8
	5	5.6 ± 1.8	0.64	475	6.8
	6	10.9 ± 2.5	0.64	475	6.8
	7	2.6 ± 1.5	0.70	278	9.0
	8	5.1 ± 1.8	0.70	278	9.0
	9	9.6 ± 2.0	0.70	278	9.0
Spherical alumina	10	3.8 ± 1.9	0.54	112	10.9
	11	5.5 ± 1.9	0.54	112	10.9
	12	11.0 ± 2.1	0.54	112	10.9
Angular alumina	13	1.8 ± 1.1	0.42	73	2.8
	14	5.1 ± 1.9	0.42	73	2.8
	15	10.4 ± 2.4	0.42	73	2.8

* d_{pw}^{a*} for spherical supports and d_{pw}^{st*} for angular supports.

weight frequency distribution⁶; d_{pw}^{a*} is the 50% value of the cumulative undersize weight distribution. Angular particles were analysed by means of a wide-angle scanning photosedimentometer (WASP). The particle diameter derived corresponds to the Stokes diameter (d_p^{st}); d_{pw}^{st*} is the 50% value of the cumulative undersize distribution, which is called the mean particle diameter. In addition to d_{pw}^{st*} and d_{pw}^{a*} , the standard deviations of the distributions are given.

Packing procedure

Columns were packed by the slurry technique under the following conditions: slurry liquid, tetrachloroethane (reagent grade, E. Merck, Darmstadt, G.F.R.); slurry concentration, 10% (w/w); pump, Orlita MS 80/8 (Orlita, Giessen, G.F.R.); packing pressure, 500 bar. As an immiscible liquid of different density, *n*-heptane was used to pump the plug of slurry from the reservoir into the column.

Equipment

The chromatograph used was a home-built model constructed by Prof. I. Halasz with an Orlita Type S 4 piston pump fitted with a UV photometer of wavelength 254 nm. The optical path of the cell had a volume of about 4 μl . The detector was kindly supplied by Prof. I. Halasz. The columns (100 \times 4.3 mm I.D.) were made of stainless steel, internally polished, to a mirror finish (Schoeller, Hellenenthal, G.F.R.) and terminated by porous stainless-steel frits of pore size $< 2 \mu\text{m}$ and thickness 1 mm (Type PSSH, Pall, Sprendlingen, G.F.R.). The column was thermostated at 298°K. For the connection between the column outlet and the detector, a capillary of I.D.

0.2 mm was used. The dead volume of the connector was about 1.5 μ l. The eluent was *n*-heptane (reagent grade) adjusted to a 33% relative water content.

All measurements were made with the same column, *i.e.*, the column described was emptied after each test, cleaned and re-packed with the next support to be investigated.

Samples were introduced with a syringe (Hamilton HP 305, G. Schmidt, Hamburg, G.F.R.) through a septum. The injection volume was 5 μ l. The samples were tetrachloromethane (to marker) benzene, diphenyl, *m*-terphenyl, *m*-quaterphenyl and *m*-quinquephenyl. The sample concentration was 1 mg/ml.

Calculations

Column performance data (K_{r0} , Φ , h , ν and k') were calculated using the basic equations^{8,9}. The diffusion coefficients (D_m) of the solutes were estimated by the Wilke-Chang equation to be as follows: benzene 3.75, diphenyl 2.53, *m*-terphenyl 2.00, *m*-quaterphenyl 1.70 and *m*-quinquephenyl $1.50 \cdot 10^5$ cm²/sec at 295° K.

RESULTS AND DISCUSSION

Particle size characterization

As this was a comparative study dealing mainly with the effect of the mean particle diameter on the performance of columns, some initial comments about the definition and estimation of d_p are necessary. The most direct and precise information about d_p is obtained by microscopy, particularly when the particles are totally spherical. As the evaluation of d_p is based on a statistical procedure, a sufficient number of particles, say between 500 and 700, should be counted. The result can be plotted as a relative or cumulative undersize number distribution, as is shown for a spherical silica in Fig. 1. The number distribution can then be converted into a weight distribution using the relationship

$$w_i \approx n_i d_{pi}^3 \quad (1)$$

where w_i is the weight of the particle, n_i the number of particles counted and d_{pi} the particle diameter¹⁰. It is well known that the weight-averaged quantity is larger than the number-averaged quantity. This is exemplified in Fig. 1 on comparing the d_p^{a*} values at 50% of the cumulative undersize distribution of a spherical silica; the d_p^{a*} value differs from that of d_{pn}^{a*} by a factor of 1.68. This difference may be larger or smaller depending on the type of packing.

Because the estimation of d_p^a of angular particles by microscopy is erratic, the particle size analysis of the angular particles is based on the Stokes equation using the WASP. d_p is then expressed as the Stokes diameter (d_p^{st}), which corresponds to the free-falling diameter of a sphere in a laminar flow region. In other words, the diameter of an angular particle is expressed in terms of an equivalent diameter of a sphere.

As two different techniques are employed in the evaluation of the mean particle diameter, depending on the particle shape, these diameters cannot be considered to be equivalent and extreme caution is therefore required in comparing the performance data of angular and spherical particles. For this reason, the packings

cumulative undersize distribution

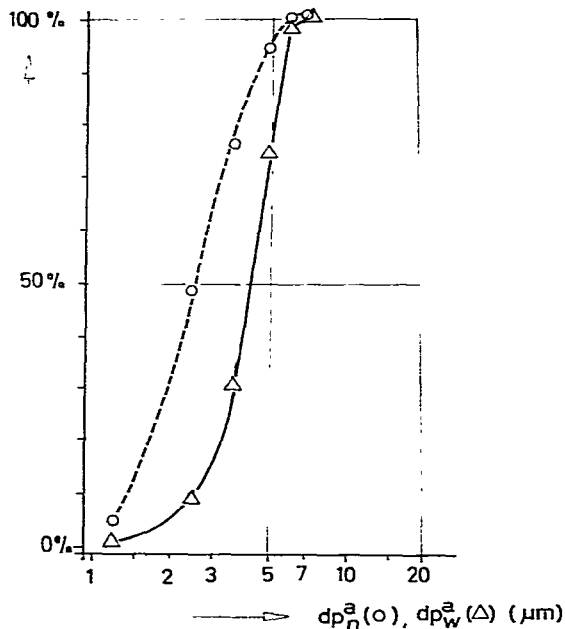


Fig. 1. Cumulative undersize frequency distribution of a spherical silica packing estimated from microscopic viewing. Number of particles counted: 720. \circ , Cumulative number frequency distribution; Δ , cumulative weight frequency distribution assuming $w_i \approx n_i d_{pi}^3$.

used in this study were grouped into the four sets according to the type of packing and the particle shape. Although the main interest was in particles with $d_p < 5 \mu\text{m}$, those with $d_p > 5 \mu\text{m}$ were also investigated because the three packings of a given set with varying d_p were obtained from the same large batch. The fact that the packings of a given set exhibit the same pore structure is particularly important with respect to the chromatographic permeability (K_{t0}).

K_{t0} is derived from t_0 , the elution time of an unretained but totally permeating solute that penetrates not only the interstitial volume of the column but also the internal volume consisting of the pore space. In this respect, it is important that the pore structure parameters of the packings of each set are identical.

It can be seen from Table I that the standard deviation (σ) of the cumulative frequency distribution is about 20–30% for particles with d_p in the range 5–10 μm . For smaller particles, σ increases considerably, sometimes to about 50%, as shown for packing No. 13. The reason for this increase is that the sedimentation procedure applied did not permit a sharper cut of the fines, otherwise a significant loss in mass would have to be taken into account.

Chromatographic permeability (K_{t0}) and column resistance factor (Φ)

In Table II the parameters K_{t0} and Φ are listed for all of the columns investigated. These parameters are related by the equation

$$\Phi = \frac{d_p^2}{K_{t0}} = \frac{d_p^2 \eta L^2}{\Delta p t_0} \quad (2)$$

TABLE II

CHROMATOGRAPHIC PERMEABILITY (K_{t_0}), PERMEABILITY ACCORDING TO THE KOZENY-CARMAN EQUATION (K_{KC}) ($\epsilon_0 = 0.4$) AND FLOW RESISTANCE PARAMETER (Φ) FOR ALL COLUMNS INVESTIGATED

Conditions as given under *Equipment*. Sample: tetrachloromethane ($k' \approx 0$).

Type of packing	No.	d_{pw}^{as} or d_{pw}^{st} (μm)	$K_{t_0} \times 10^{10}$ (cm^2)	$K_{KC} \times 10^{10}$ (cm^2)	Φ
Spherical silica	1	3.8 ± 1.4	2.48	1.44	$560 \pm 10\%$
	2	6.1 ± 1.5	5.44	3.72	674
	3	11.0 ± 2.1	15.39	12.10	956
Angular silica	4	2.8 ± 1.3	0.55	0.78	1266
	5	5.6 ± 1.8	1.86	3.14	1673
	6	10.9 ± 2.5	8.08	11.88	1676
	7	2.6 ± 1.5	0.36	0.68	1873
	8	5.1 ± 1.8	2.26	2.60	1176
Spherical alumina	9	9.6 ± 2.0	6.80	9.22	1343
	10	3.8 ± 1.9	2.72	1.44	574
	11	5.5 ± 1.9	8.67	3.03	355
Angular alumina	12	11.0 ± 2.1	23.97	12.10	493
	13	1.8 ± 1.1	1.09	0.32	287
	14	5.1 ± 1.9	4.67	2.60	555
	15	10.4 ± 2.4	17.93	10.82	623

where η is the viscosity of the eluent, L the column length, Δp the pressure drop and t_0 the retention time of a totally permeating solute defined as unretained ($k' \approx 0$)^{8,9}. While K_{t_0} is a parameter determined by experimental data, the dimensionless factor Φ involves an additional quantity d_p , which is derived by separate particle size analysis as mentioned above. K_{t_0} for a given column can be compared, to a first approximation, to the quantity K_{KC} , the permeability according to the Kozeny-Carman equation:

$$K_{KC} = \frac{d_p^2}{180} \cdot \frac{\epsilon_0^3}{(1-\epsilon_0)^2} \quad (3)$$

where ϵ_0 is the interstitial porosity of the column. Assuming $\epsilon_0 = 0.4$ for a well packed bed, eqn. 3 gives

$$K_{KC} = \frac{d_p^2}{1000} \quad (4)$$

Inspection of the values of K_{t_0} and K_{KC} in Table II indicates that (i) for spherical silicas $K_{t_0} > K_{KC}$ while for all angular silicas $K_{t_0} < K_{KC}$ and (ii) for both spherical and angular alumina the K_{t_0} values are larger than the K_{KC} values by a factor of 2-3.

A more realistic picture is obtained by considering the Φ values. Φ usually varies between 500 and 1000 for well packed columns^{8,9}, and the values for most of the columns studied fall in this range except for some of the angular silica packings which show slightly larger Φ values, is in accordance with their low chromatographic permeabilities.

h versus v dependences

There are various ways of describing the dependence of the plate height on the flow velocity^{10,11}. For the presentation of our data we preferred to use reduced parameters, namely the reduced plate height (h):

$$h = \frac{H}{d_p} \quad (5)$$

and the reduced linear velocity (v):

$$v = \frac{u d_p}{D_m} \quad (6)$$

where d_p is the mean particle size of the packing and D_m the diffusion coefficient of the solute. According to the concept of reduced parameters, which was introduced by Giddings¹² and widely advocated by Knox and co-workers¹³⁻¹⁵, h is proposed to be dependent on the reduced velocity by adding the contributions of three dispersion mechanisms to give

$$h = B/v + A v^{1/3} + C v \quad (7)$$

A is a contribution from the flow in the intraparticle space, B represents the influence of diffusion and C is the contribution due to the rate of mass transfer of the solute between the mobile and stationary phases^{8,9}. A logarithmic plot of the h versus v dependences shows that the B term dominates at low and the C term at high reduced velocities. In the intermediate region, the function passes through a minimum, where the A term dominates. The values of A , B and C for a particular column can be derived from the experimental h versus v plot by curve fitting. Both the position of the minimum and the values of the three constants are a measure of the efficiency of the column. The minimum should be in the range $1 < v < 10$ and for equally well packed columns the constants will be $B = 2$, $A = 1$ and $C = 0.05$ (refs. 8-10).

Figs. 2-5 show the logarithmic h versus v plots for the four different sets of packings. Each figure gives the data obtained on the three packings of the given set for a retained and an unretained solute. It should be noted that each point presented is the average of two measurements on the same column and under the same conditions after emptying and re-packing the column.

Comparison of the family of curves in Figs. 2-5 indicates that the minima fall in the expected range of $1 < v < 10$. For an unretained solute, h varies between 1.5 and 2.5 in the region of the minimum, which reflects a high performance. Retained solutes with $k' = 0.5-5.0$ gave h values up to about 5 in the minimum region. There is no distinct difference in the shapes of the curves depending on the type of packing, the particle diameter or the particle shape; as will be seen later, this statement is valid only for particles with $d_p > 4 \mu\text{m}$. Inserting $B = 2$ for well packed columns, it can be seen that the real course of all curves at low reduced velocities is flatter than that of the function $h = B/v$ for $B = 2$. On the other hand, at high velocities the constants A and C cannot be derived from the experimental curves with high accuracy because v was measured only to within about 50.

Particular attention is drawn to the columns packed with particles with $d_p < 4$

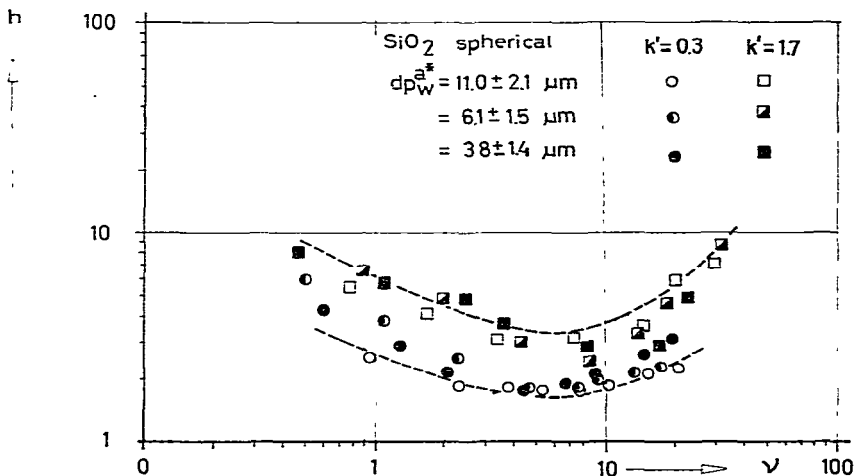


Fig. 2. Reduced plate height–reduced velocity plots for a retained and nearly unretained solute on a series of spherical silica packings (Nos. 1–3, Table I).

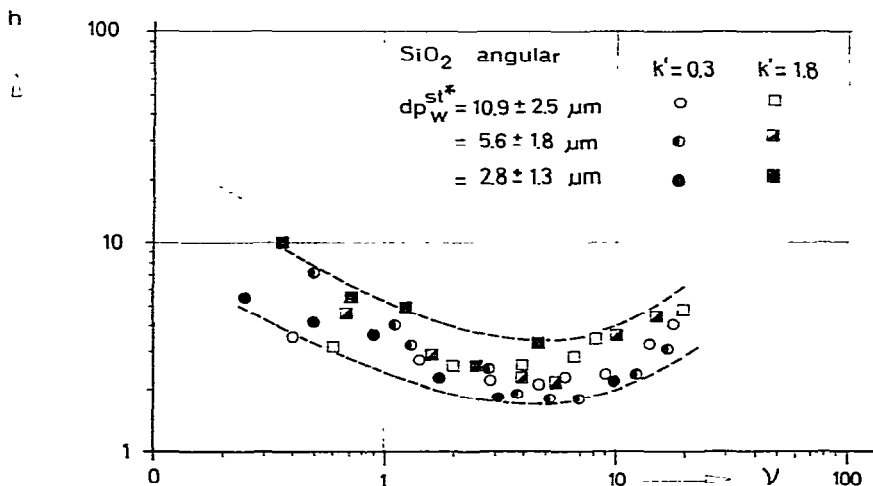


Fig. 3. Reduced plate height–reduced velocity plots for a retained and nearly unretained solute on a series of angular silica packings (Nos. 4–6, Table I).

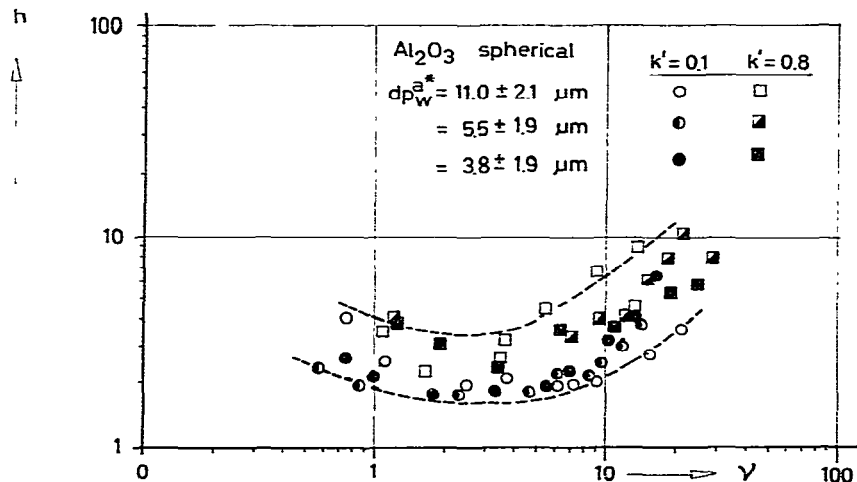


Fig. 4. Reduced plate height–reduced velocity plots for a retained and nearly unretained solute on a series of spherical alumina packings (Nos. 10–12, Table I).

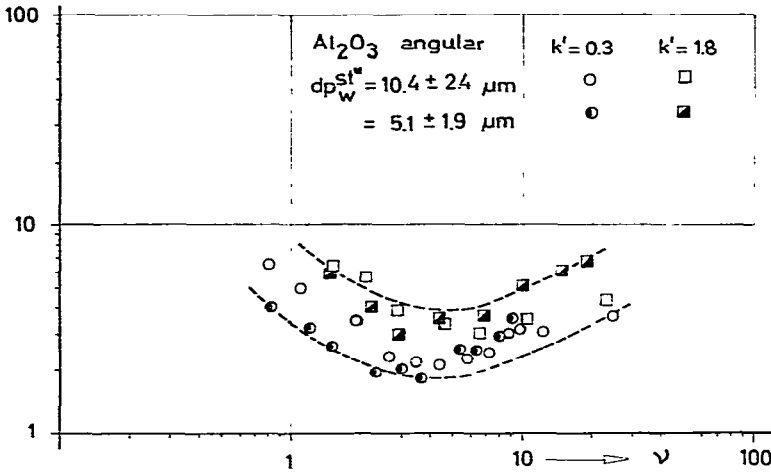


Fig. 5. Reduced plate height-reduced velocity plots for a retained and nearly unretained solute on a series of angular alumina packings (Nos. 14-15, Table I).

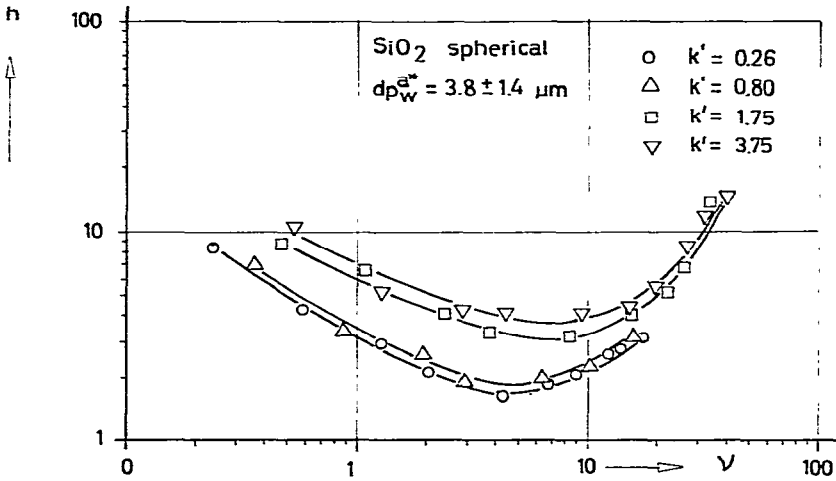


Fig. 6. Reduced plate height-reduced velocity plots for retained and unretained solutes on a spherical silica packing (No. 1, Table I).

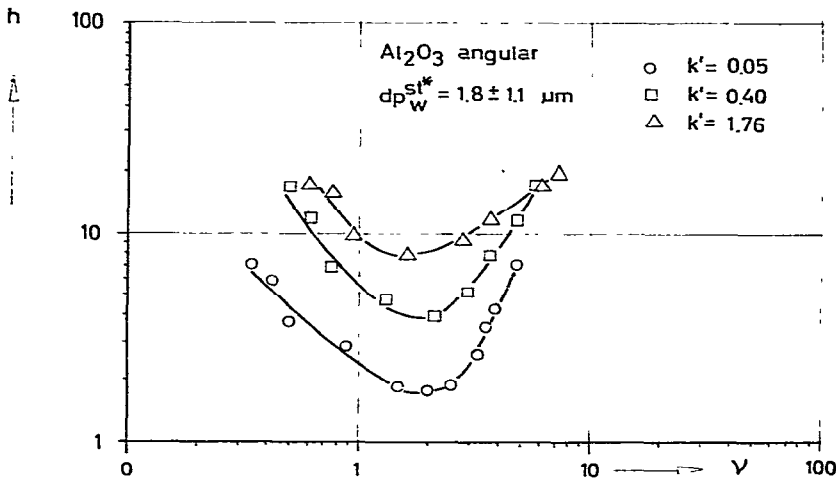


Fig. 7. Reduced plate height-reduced velocity plots for retained and unretained solutes on an angular alumina packing (No. 13, Table I).

μm , and the results with two packings can be considered. Fig. 6 shows the h versus ν dependence for a spherical silica with $d_{pw}^a = 3.8 \pm 1.1 \mu\text{m}$ for a series of solutes. Again the minimum is around $\nu \approx 5$, giving excellent h values of 1.6–5 for the range of capacity factors. In contrast to the h versus ν plots for packings with $d_p > 4 \mu\text{m}$, the slope of the curve in Fig. 6 at higher reduced velocities ($\nu > 10$) has a tendency to become independent of the capacity factor. Simultaneously, the plate height in this region increases with ν much more than is observed for other packings. The course of the slope can be approximated by $h = C\nu$ and $C = 1.0$, which corresponds to poor mass transfer.

The specificity in the course of the h versus ν function for packings with $d_p < 4 \mu\text{m}$ is apparent in Fig. 7 for the packing with the smallest particle size. The minimum obtained is around $\nu \approx 2$, showing an excellent plate height for the unretained solute, whereas for retained solutes at $\nu \approx 2$ h increases considerably with k' . This column, which has a length of 10 cm and I.D. 4.3 mm, gives 31,000 theoretical plates for the unretained solute and only 7000 theoretical plates for the solute with $k' = 1.76$ at the minimum. A separation of a mixture of compounds is shown in Fig. 8.

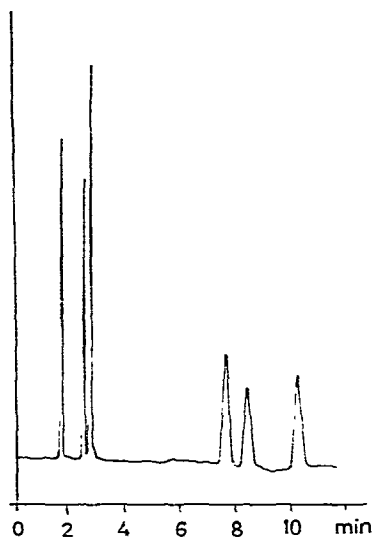


Fig. 8. Separation of a synthetic mixture on a column packed with angular alumina with $d_{pw}^a = 1.8 \pm 1.1 \mu\text{m}$. Conditions as given under *Equipment*, with a pressure drop of 200 bar. Order of elution: 1, benzene; 2, naphthalene; 3, diphenyl; 4, phenanthrene; 5, pyrene; 6, *m*-terphenyl.

The peculiar behaviour in the h versus ν plot at high reduced velocities again could be established experimentally. The slope of the curve in Fig. 7 is steeper than that in Fig. 6, indicating a poorer mass transfer on increasing the flow-rate. Additionally, there is a deviation in the course of the h versus ν function at low reduced velocities compared with those in Figs. 2–6: while the slope of h versus ν in Fig. 7 at $\nu < 1.0$ for the unretained solute can be well approximated by $h = B/\nu$ for $B = 2$, the other two retained solutes gave considerable higher B values that increased with k' .

The large dependence of h on k' of the solute at low velocities is contrary to that predicted theoretically according to the equation

$$B = 2 \gamma' (1 + k') \quad (8)$$

where γ' is the tortuosity factor of the packing¹⁶. For the dependence of the C term on k' , it is assumed that

$$C = q' \cdot \frac{k'}{(1 + k)^2} \cdot \frac{D_m}{D_s} \quad (9)$$

where q' is a constant, D_m the diffusion coefficient of the solute in the mobile phase and D_s the diffusion coefficient of the solute in the stationary phase¹⁶. Neither eqn. 7 nor 8 describes the experimental dependences.

If one discusses the effects observed with the two columns packed with small-diameter particles in terms of column dispersion, one has to ensure that extra-column contributions are excluded. A simple calculation shows that under the given conditions the contributions of the sample volume and the cell volume of the detector to the total standard deviation of the peak (σ_v) are small. A critical point in the study is the influence of the poor response time of the measuring device on the peak width, because the absolute H values are very small (a few micrometres). As the peculiar slope of the curves in Fig. 7 is correlated to the capacity factor, we also studied the constancy of k' as a function of flow-rate under the given conditions. Again we could not find a variation in k' with the flow-rate. The deviation at high reduced velocities may be due to thermal effects. Under the given conditions, the column cannot be considered to be an adiabatic system and there is a heat loss that creates an axial and radial temperature gradient. However, there are no equations available that describe the overall influence of this gradient on the various dispersion mechanisms.

On the basis of these results a plausible explanation of the exceptional behaviour in the column performance of packings with $d_p < 4 \mu\text{m}$ cannot yet be given and further comprehensive studies are required.

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