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PARTICLE SIZE, PRESSURE AND ANALYSIS TIME IN ROUTINE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

The maximum number of theoretical plates that can be generated in a column can be calculated from the empirical equations $u_{min}\, d_p = 10$ and $h_{min} = 4\, d_p$ (where the minimum linear velocity of the eluent, u_{min}, is given in mm/sec and the average size, d_p, in μm), if the average particle size d_p is based entirely on chromatographic particle measurements. It was demonstrated experimentally that 30-cm silica or reversed-phase columns having over 7000 plates could be packed with $d_p = 10\ \mu$m. Such columns can resolve, with baseline peak separation, mixtures that possess relative retentions greater than 1.12. In routine work, the relative retentions are usually greater and it is advantageous to use only 7.5-cm long columns. Analysis times of 10 min or less can be achieved even with velocities around u_{min}. Consequently, most routine separations require column pressures of less than 50 atm. For research studies and for the optimization of conditions for routine work, columns packed with 5-μm particles are often necessary. The reproducibility of column packing with $d_p < 5\ \mu$m is poor. It was shown that up to 600 plates/cm and over 100 plates/sec (capacity ratio = 1) can be generated on silica columns with $d_p = 3.2\ \mu$m. Such small particle sizes are particularly advantageous for trace analysis because, as our experience indicates, these short and efficient columns can be loaded with samples larger than the amount calculated from the weight of the stationary phase present.

INTRODUCTION

It is well known that the separation time in high-performance liquid chromatography (HPLC) decreases with decreasing particle size. If the pressure drop, Δp, along a column is kept constant (because of equipment limitations), the maximum number of theoretical plates, n_{max}, available at u_{min} (the linear velocity at the minimum of an *h versus u* curve) decreases with decreasing particle size. Furthermore, with decreasing particle size (1) u_{min} increases, (2) the pneumatic resistance for a given column length increases, (3) packing of the column becomes more difficult and (4) the heat of friction due to the high pressure drop cannot always be neglected. These problems were discussed in

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previous publications[1–3], and it was demonstrated that a sieve fraction with a particle size of about 10 μm seems to be an optimal compromise. The main objective of this paper is to demonstrate that most routine separations can be carried out with columns packed with 10-μm particles.

Column packing

During column packing, the sedimentation of the support particles must be minimized, particularly if their diameter is less than 25 μm, and all successful packing procedures make use of this fact. It is well known that the sedimentation velocity, dx/dt, is given by

$$\frac{dx}{dt} = \frac{2r^2 (\varrho_s - \varrho_l)}{9\eta} \quad (1)$$

where r is the radius and ϱ_s the true specific gravity of the support particles, and ϱ_l is the specific gravity and η the viscosity of the dispersing liquid. The differences in the sedimentation velocity due to variations in the particle size can be minimized by: (1) minimizing the difference in both densities, *i.e.*, ϱ_s and ϱ_l, (2) "maximizing" the viscosity η, or (3) using an appropriate combination of both.

The first of these procedures is the balanced density method[1,4–8], which gives good results only when the wetting of the solid by the dispersing liquid is very good (*i.e.*, the wetting angle is approximately zero). If the wetting angle is high, the required specific gravity of the slurry, ϱ_l, can become a function of the pore size distribution (and, sometimes, of the particle size distribution) of the packing material. The wetting angle between a given solid support and a liquid frequently changes from batch to batch, and it is therefore essential to check visually the sedimentation rate of each support sample. Among other factors, the wetting angle is a function of the polarity of the support (*e.g.*, it is different for silica and reversed-phase supports).

The second packing procedure is the viscosity method[8], which is more universally applicable than the first and the choice of dispersing agents is extensive. This aspect can be of special interest if chemically bonded phases are to be packed. However, with increasing viscosity of the dispersing agent, the time required for packing increases. Therefore, as a third alternative, a compromise between the "balanced density" and "viscosity" methods is sometimes the best solution. The columns discussed in this paper were packed by the first two methods[8].

The quality of a column is determined by its efficiency and its lifetime, and our experience indicates that the lifetime of a column is governed primarily by its packing density. This factor is especially important for columns packed with reversed phases. If the packing density is low, the initial efficiency can be up to two times that of a well packed column (to be quantitatively defined later). However, the lifetime of such columns is frequently very short (on the order of 1 h to 5 days). If the packing density is very high, it is almost impossible to destroy the column efficiency by mechanical means. The price for such a long lifetime is a high pneumatic resistance, *i.e.*, the pressure drop along the column is up to twice that of a well packed column. Yet the differences in packing density are small and hard to determine. To produce well packed columns consistently requires both experience and intuition and, furthermore, the problems increase with decreasing particle size. In fact, based on our experience and

that of others, using the packing techniques described above, it can be simply stated that packing columns with 10-μm particles is routine, packing those with about 5-μm particles requires "art", and for packing those with particles smaller than 5 μm nothing short of "black magic" is enough (the rate of success being less than 10%).

Average particle size, d_p

Column efficiency is a function of the narrowness of the particle size range (width of a sieve fraction) and the average particle size, d_p, of the stationary phase. Unfortunately, d_p is not an unambiguously defined quantity, but depends on the way in which it is determined (sieving, air-classification, sedimentation, centrifugation, measurement with a Coulter counter, microscopy, light scattering, etc.) and the way its average is expressed (number-, volume- or weight-average). Such d_p determinations are carried out on the unpacked stationary phase, and it is questionable whether the values thus obtained characterize realistically the stationary phase inside the column. It is well known that the specific surface area and therefore the surface energy of solids increase essentially linearly with decreasing particle size. To decrease this surface energy, the particles can agglomerate. Thus, the surface energy of 5- to 10-μm particles can become a significant factor during packing, and for particles of size <1 μm agglomeration ("particle bridging") is almost inevitable if the solid is at least partially dry. As an illustration, let us consider two columns packed with a narrow sieve fraction of about 10 μm, but with one of them containing an additional *ca.* 1% by number of 0.1-μm particles. The weight- and volume-averaged d_p values would be virtually identical for both packings, but the efficiency of the column containing the 0.1-μm particles would be lowered considerably.

From the above discussion, it follows that an unambiguous definition of d_p is very important. We have previously proposed[1–3] a definition of d_p that is based entirely on chromatographic measurements:

$$d_p = 41 \sqrt{\frac{F \eta L}{r^2 \pi \Delta p}} \tag{2}$$

where the parameters are given in convenient units, *i.e.*, d_p in μm, flow-rate, F, in ml/min, viscosity η in cP, column length, L, in cm, column radius, r, in mm, and pressure drop, Δp, in atm (if the c.g.s. system is used, the proportionality factor becomes $\sqrt{1000} = 31.6$ instead of 41 in eqn. 2).

Definition of a well packed column

Based on literature data and our experience with 4–40-μm (or perhaps even 3–80-μm) particles, a tolerable definition[2,3] for a "well packed column" seems to be a column whose h *versus* u curve can be described by the following equation, provided that d_p is defined by eqn. 2:

$$h = 3\, d_p + \frac{2\, D}{u} + \frac{d_p^2\, u}{5.3\, D} \tag{3}$$

where the height equivalent to a theoretical plate, h, and d_p are in μm, the diffusion coefficient, D, of the sample in the eluent in 10^{-5} cm^2/sec and the linear velocity of

the eluent, u, in mm/sec. To obtain a valid value for h, all three terms must be summed because the individual terms themselves tend to compensate each other. Eqn. 3 is valid in the velocity range in which virtually all routine separations are carried out, the limitations being imposed by the capabilities of the currently available commercial equipment.

A typical value of D for HPLC in non-aqueous solvents and for samples with molecular weights of roughly 200–600 is $3 \cdot 10^{-5}$ cm²/sec. Substituting this value of D into eqn. 3 yields

$$h = 3\,d_p + \frac{6}{u} + \frac{d_p^2\,u}{16} \tag{4}$$

Eqn. 3 or 4, in conjunction with eqn. 2, defines a well packed column to within $\pm 20\%$ in h at commonly used linear velocities. These velocities, of course, vary with d_p.

Eqns. 3 and 4 can be expressed in dimensionless parameters, in terms of the reduced plate height, h/d_p, and the reduced velocity, $v = ud_p/D$:

$$\frac{h}{d_p} = 3 + \frac{2}{v} + \frac{v}{5.3} \tag{5}$$

Eqn. 4 can be rewritten as

$$\frac{h}{d_p} = 3 + \frac{6}{u\,d_p} + \frac{d_p\,u}{16} \tag{6}$$

Plots of eqns. 5 and 6 are given in Fig. 1. Experience shows that in LC the reduced parameters, to a good approximation, are independent of d_p, as predicted by theory. It should be pointed out again, however, that agreement between theory and practice is valid only for velocities that are typical at present for routine separations. Differentiation of eqn. 6 yields

$$d_p\,u_{min} \approx 10\ \left(\mu\text{m} \cdot \frac{\text{mm}}{\text{sec}}\right) \tag{7}$$

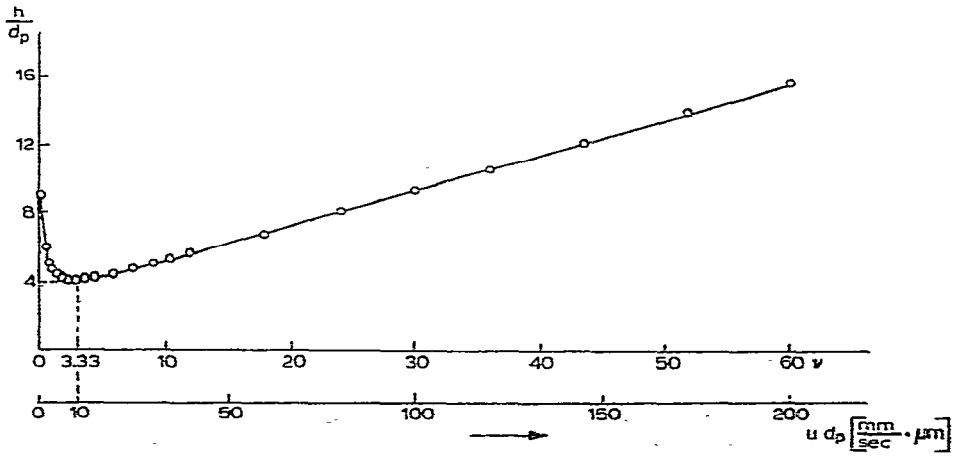

Fig. 1. Plot of reduced plate height, h/d_p, *versus* reduced velocity, v, as calculated from eqns. 5 and 6. For the ud_p-axis, it is assumed that $D = 3 \cdot 10^{-5}$ cm²/sec.

if d_p is given in μm and the linear velocity at the minimum of the curve in Fig. 1, u_{min}, in mm/sec. For example, if $d_p = 10\,\mu$m the minimum of the curve occurs at $u_{min} = 1$ mm/sec. As this a slower velocity than is commonly used, only the ascending branch of the *h versus u* curve used to be presented, which is, of course, linear. Such linearity is particularly true for columns packed with particles with $d_p \geqslant 10\,\mu$m. From the constants in eqn. 4, it is evident that at typical velocities h is, to a first approximation, linear in d_p if $d_p \leqslant 5\,\mu$m and $h \approx d_p^2$ if $d_p > 50\,\mu$m. By substituting u_{min} from eqn. 7 into eqn. 6, we obtain for the h value at the minimum of the *h versus u* curve

$$h_{min} \approx 4\,d_p \tag{8}$$

The specific permeability of a packed column is proportional to d_p^2. Consequently, for $d_p < 10\,\mu$m, h increases approximately linearly with d_p, but the pneumatic resistance of a given column increases with d_p^2. For small particles, therefore, the maximum number of theoretical plates that can be generated with a given maximum pressure decreases with decreasing particle size[2].

The efficiency and analysis time at u_{min}

It will now be demonstrated that most routine separations can be effected with columns packed with 10-μm particles. It should be pointed out again that d_p is not the average particle size claimed by the manufacturer, but rather the value calculated from eqn. 2. A comparison of commercially available "10-μm" silicas revealed a d_p range of 6–12 μm or even greater. The variation in d_p from batch to batch from the same manufacturer is usually smaller, but seldom negligible.

Tables I and II present typical data for routine separations. For the purpose of the following discussion, it will be assumed that the eluent viscosity is 0.4 cP (non-polar eluents), the total porosity, ε_T, of the column is 0.8, and the column diameter

TABLE I

CALCULATED PARAMETERS FOR COLUMNS OPERATED AT MAXIMUM EFFICIENCY

The following typical parameters were used in the calculations: viscosity of the eluent, 0.4 cP; inner diameter of the column, 4.2 mm; total porosity of the column, 0.8.

d_p (μm)	L (cm)	h_{min} (μm)	u_{min} (mm/sec)	F_{min} (ml/min)	n_{max}	Δp (atm)
10	30	40	1	0.68	7500	12
5	30	20	2	1.35	15000	96

TABLE II

CALCULATED PARAMETERS FOR COLUMNS OPERATED AT MAXIMUM EFFICIENCY

d_p (μm)	k'	N_{max}	R	α	t_R (min) ($k' = 2$)
10	2	3333	1.5	1.12	15
10	2	3333	1.0	1.07	15
5	2	6666	1.5	1.08	7.5
5	2	6666	1.0	1.05	7.5

and length are 4.2 mm and 30 cm, respectively. From the data, it can be seen that for $d_p = 10\ \mu m$ at $u_{min} = 1$ mm/sec, about 7500 theoretical plates (corresponding to 3333 effective plates, N, for $k' = 2$) are generated with a pressure drop of only 12 atm for a flow-rate of less than 0.7 ml/min. If the compounds to be separated have capacity ratios, k', of about 2, samples with a relative retention, α, of 1.12 can be separated if a resolution $R = 1.5$ (*i.e.*, baseline separation) is required. The analysis time is 15 min. With $N_{max} = 3333$, even compounds with $\alpha = 1.07$ can be separated if $R = 1$ is sufficient. For smaller relative retentions, the best approach would be to change the properties of the eluent (*i.e.*, change the eluent) and look for higher relative retentions. Only if this approach fails should a column with $d_p = 5\ \mu m$ be tried. With such a column ($L = 30$ cm), a pair of sample components with $\alpha = 1.05$ can be separated, provided $R = 1$ is adequate. Alternatively, a longer 10-μm particle column can be used if a longer analysis time can be tolerated. Most of the separations published recently have involved mixtures in which adjacent peaks had relative retentions of 1.15–1.6 (and greater). As Tables I and II demonstrate, the analysis time, of course, decreases with decreasing particle size. It is questionable, however, whether analysis times shorter than 5–10 minutes are always of great interest in routine work.

APPARATUS AND MATERIALS

Home-built equipment with a UV detector[2] was used. Commercially available eluents were purified by distillation. The stationary phase consisted of porous silica with an average pore size of 100 Å (SI 100, E. Merck, Darmstadt, G.F.R.). The silica was used as received or was chemically bonded with C_{18} bristles (*i.e.*, reversed phase).

RESULTS AND DISCUSSION

In Fig. 2, the column efficiencies obtained with samples having capacity ratios between 0.34 and 1.03 are plotted against the linear velocity. The eluent was methy-

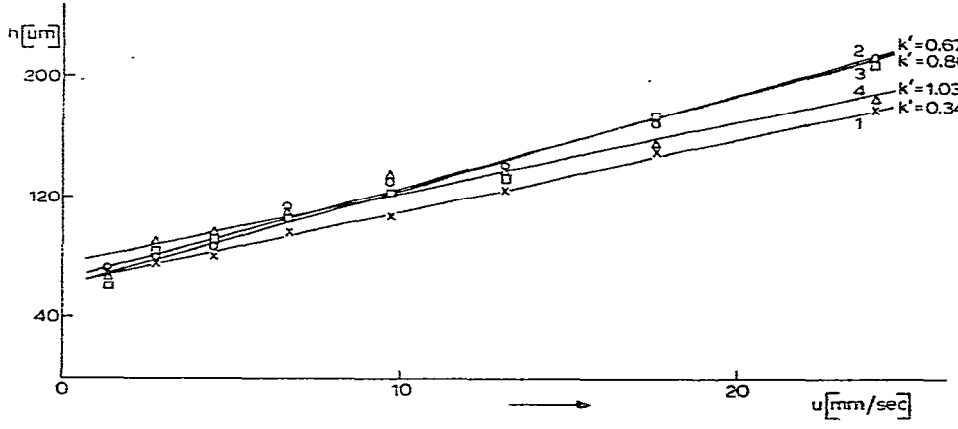

Fig. 2. Plots of *h versus u* on silica SI 100 ($d_p = 13.5\ \mu m$). Column length, 30 cm; I.D., 4.2 mm. Eluent, methylene chloride–propanol-2 (95:5, v/v). Samples: 1, thiogenal ($k' = 0.34$); 2, secobarbital ($k' = 0.67$); 3, vinylbarbital ($k' = 0.80$); 4, barbital ($k' = 1.03$). $K_F = 1.8 \cdot 10^{-9}$ cm². Temperature, ambient.

lene chloride–propanol-2 (95:5, v/v), and the average particle size (eqn. 2) was 13.5 μm. All measurements were carried out at ambient temperature. The linear velocity was varied from 1 to 25 mm/sec, which is typical for routine applications with $d_p = 13.5\ \mu$m. The value of u_{min} is 0.74 mm/sec (eqn. 7).

As expected, the curves display acceptable linearity and are more or less independent of the k' values. It is an empirical fact, for which at present there is no theoretical basis, that for "excellent" columns the h *versus* u curves are independent of k' for a wide range of capacity ratios. But even for experts in column packing the probability of producing an "excellent" column is less than 10%. The results shown in Fig. 2 were obtained with an above-average, but not "excellent", column. The data points in Fig. 2 are replotted in Fig. 3 in terms of the (dimensionless) reduced plate height, h/d_p. The solid line represents the values calculated by means of eqn. 5, where $u = Dv/d_p = 3v/13.5$, assuming that $D = 3 \cdot 10^{-5}$ cm²/sec and $d_p = 13.5\ \mu$m. It is evident that acceptable agreement exists between the calculated and measured values in the commonly used velocity range. The pressure required to achieve a particular velocity is also shown in Fig. 3.

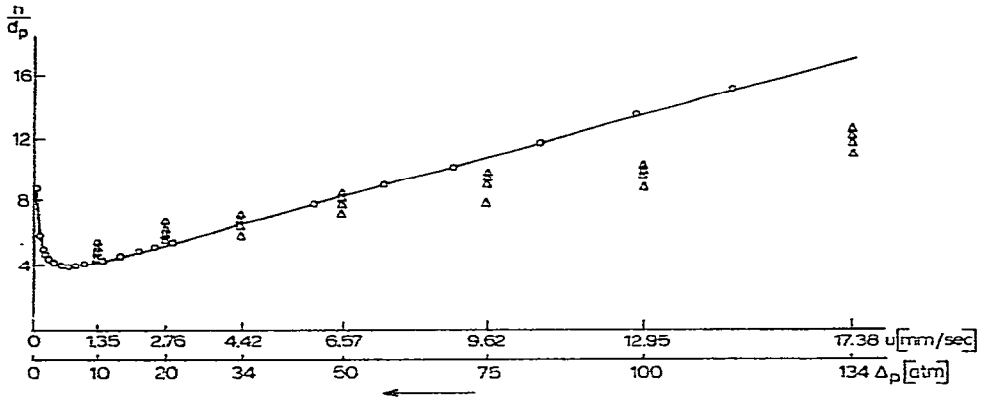

Fig. 3. Plots of h/d_p *versus* u. Parameters as in Fig. 2. The solid line was calculated by means of eqn. 5, assuming that $D = 3 \cdot 10^{-5}$ cm²/sec and $d_p = 13.5\ \mu$m.

A separation of barbiturates on the same column is illustrated in Fig. 4. The linear velocity was 1.1 mm/sec, *i.e.*, 50% higher than u_{min}, and the pressure drop was only 7.5 atm. For the pair of compounds with the smallest relative retention (α = 1.19), secobarbital and vinylbarbital, the resolution $R_{2,3}$ was 1.62, *i.e.*, better than baseline resolution. In general, if the resolution exceeds 1.5 only time is wasted. To optimize the separation time, the resolution should be reduced, *i.e.*, the column efficiency is actually excessive. To reduce the column efficiency, either the column length can be decreased or the linear velocity increased. Based on experience, the minimum column length should be greater than 7 cm if $d_p \leqslant 10\ \mu$m. Otherwise, the inlet and outlet turbulences inside the column reduce its efficiency, as calculated from an h *versus* u curve determined with a longer column.

Unfortunately, packed columns 7–10 cm in length are almost unavailable from commercial sources. Therefore, a more practical means of optimizing the analysis

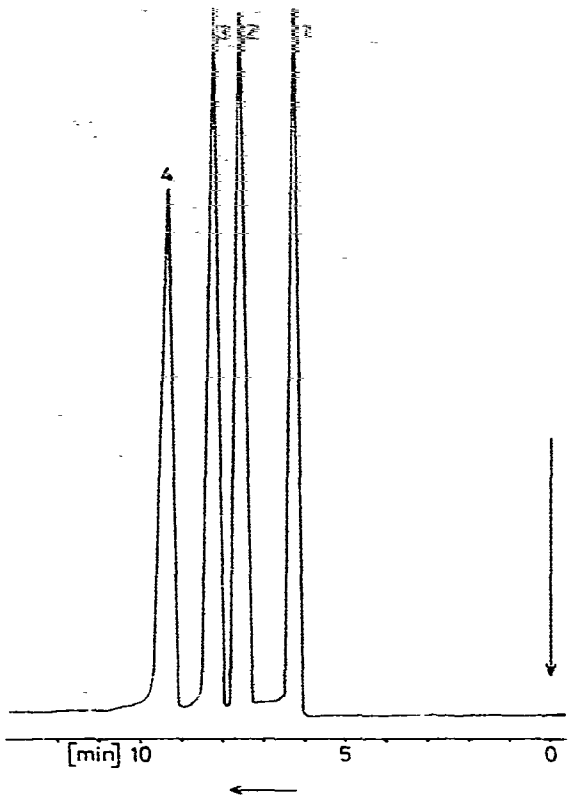

Fig. 4. Separation of barbiturates on silica. Parameters as in Fig. 2, except: $u = 1.1$ mm/sec, $\Delta p = 7.5$ atm and $R_{2,3} = 1.62$.

time, t_R, is to keep the column length the same but to decrease the efficiency by using a faster flow-rate. Such optimization can be achieved only at the expense of increased pressure (Figs. 5–7). In Fig. 5, $R_{2,3} = 1.47$ (essentially a baseline separation) is attained with $\Delta p = 10$ atm in 8 min. In Fig. 6, the analysis time is only 45 sec, but the pressure has risen to 100 atm. Of course, t_R can be reduced even further (25 sec), at the expense of a higher pressure (185 atm) and lower resolution (0.71), as shown in Fig. 7.

All of the results discussed above are well known, and perhaps trivial, but these facts are seldom applied in routine work. Thus:

(1) The maximum number of plates, n_{max}, is generated at u_{min}. Both can be estimated from eqns. 7 and 8.

(2) For conventional columns ($L = 30$ cm or 1 ft.) and typical relative retentions ($\alpha > 1.2$), peak separation better than baseline resolution is generally attained if the linear velocity is u_{min}. The analysis time can be optimized by reducing the column efficiency by using faster flow-rates. This is usually done intuitively, and the result is a high pressure drop over the column. It is for this reason that commercial equipment sometimes includes excessively high pressure pumps and attendant fittings, injection system, etc., which increase its price considerably.

(3) It would be better to reduce the conventional column length because sepa-

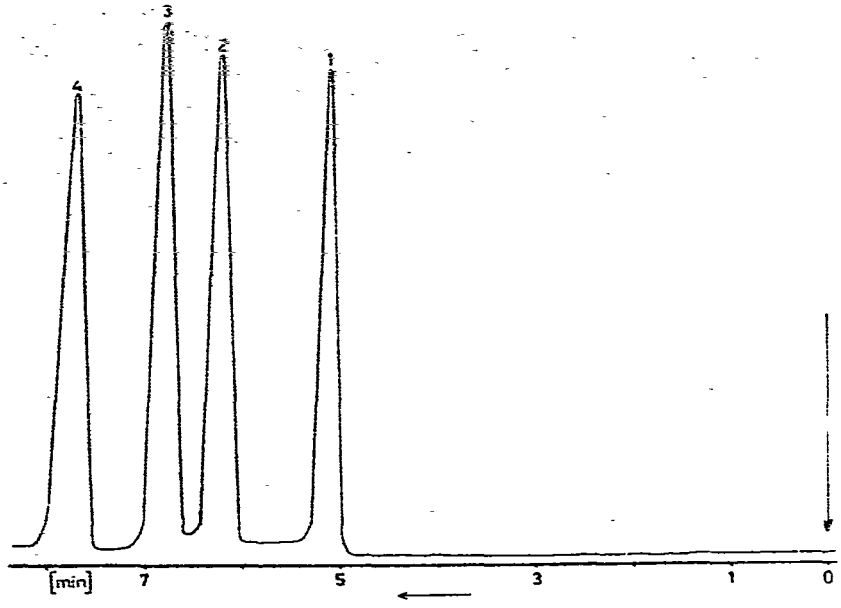

Fig. 5. Separation of barbiturates on silica. Parameters as in Fig. 2, except $u = 1.33$ mm/sec, $\Delta p =$ 10 atm and $R_{2,3} = 1.47$.

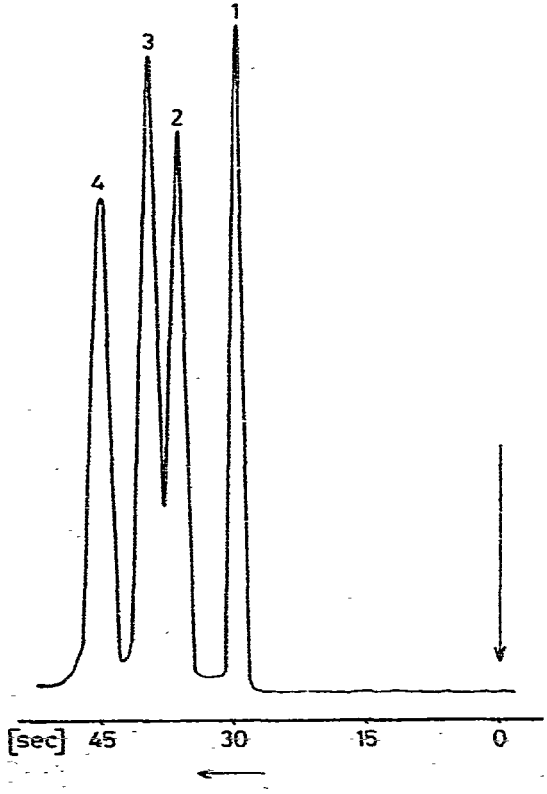

Fig. 6. Separation of barbiturates on silica. Parameters as in Fig. 2, except $u = 13.6$ mm/sec, $\Delta p =$ 100 atm and $R_{2,3} = 0.89$.

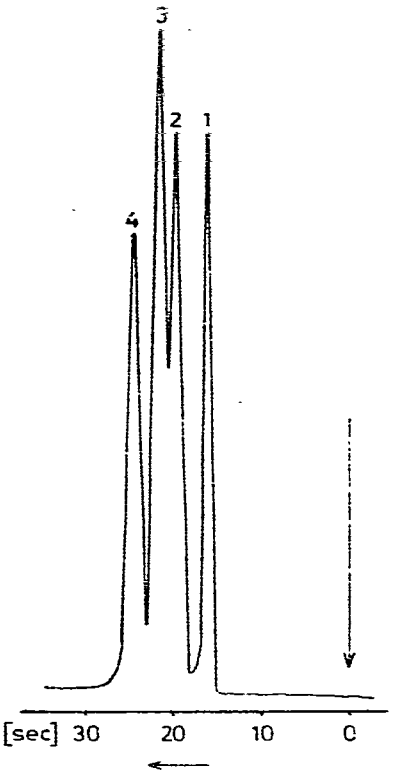

Fig. 7. Separation of barbiturates on silica. Parameters as in Fig. 2, except $u = 24.6$ mm/sec, $\Delta p = 185$ atm and $R_{2,3} = 0.71$.

rations with $\alpha = 1.2$ could still be accomplished on a 10-cm column packed with particles with $d_p = 10\ \mu$m in a reasonable period of time (<5 min) and the required pressure would be less than 50 atm.

(4) A short column has a further advantage over a "long" column. Identical resolution would be achieved with both columns if they possessed equal plate numbers, but h would be smaller for the short column. Moreover, since a decrease in peak height is a concomitant effect of peak broadening, which varies directly with column length, for equal h values higher peaks would be generated with the shorter column. The consequence of the overall result, which is a product of both of these effects, is that the minimum detectable sample concentration decreases with decreasing column length if the columns are loaded with identical sample sizes. This result is always an important consideration in quantitative and qualitative trace analyses.

(5) A third means of reducing the required pressure for optimal resolution is to increase the particle size. A 1-ft. column can be dry-packed with 30-μm particles as in gas chromatography[9]. Although this column packing technique is simple, the increased detection sensitivity discussed above would be lost.

(6) If the relative retention, α, and the required resolution, R, are fixed, the number of theoretical plates, n, needed for such a separation increases with decreasing capacity ratios, k', because to a good approximation

$$R = \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{1 + k'} \sqrt{n} = \frac{\alpha - 1}{\alpha} \sqrt{N} \quad (9)$$

Hence, the resolution is proportional to the square root of the effective plate number, N. Figs. 4–7 demonstrate, however, that even with relatively small capacity ratios ($k' = 0.3$–1.0), the analysis can be completed within 15 min.

(7) The diffusion coefficient, D, of a given sample component decreases with increasing eluent viscosity, η. In a given eluent, D varies approximately inversely with $\sqrt{M}$, the molecular weight of the solute. In the analytically important velocity range where $u > u_{min}$, h increases with decreasing D, as can be seen from eqn. 3. For high values of M or η (*e.g.*, water), or especially of both, more efficient columns (for example, those packed with 5-μm particles) may be required. Such efficient columns are necessary, of course, for research work and for the development of optimal conditions for routine analyses.

To demonstrate the effect of increasing eluent viscosity on h *versus* u curves, some organic acids were eluted with a water–methanol–acetic acid mixture on a C_{18} chemically bonded stationary phase, with $d_p = 10.9\ \mu$m (compare Fig. 8 and Figs. 4–7). Experience shows the efficiencies of silica and reversed-phase columns to be essentially the same under otherwise identical conditions. The data in Fig. 8 are in reasonable accord with the values calculated from eqn. 3.

A separation of five acids at $u_{min} = 1$ mm/sec is displayed in Fig. 9. A pressure drop of only 25 atm was sufficient and the analysis took less than 20 min, although the resolution was unnecessarily high ($R = 1.71$). A somewhat less than baseline separation ($R = 1.38$) required less than 3 min (Fig. 10), although the inlet pressure had increased to 220 atm.

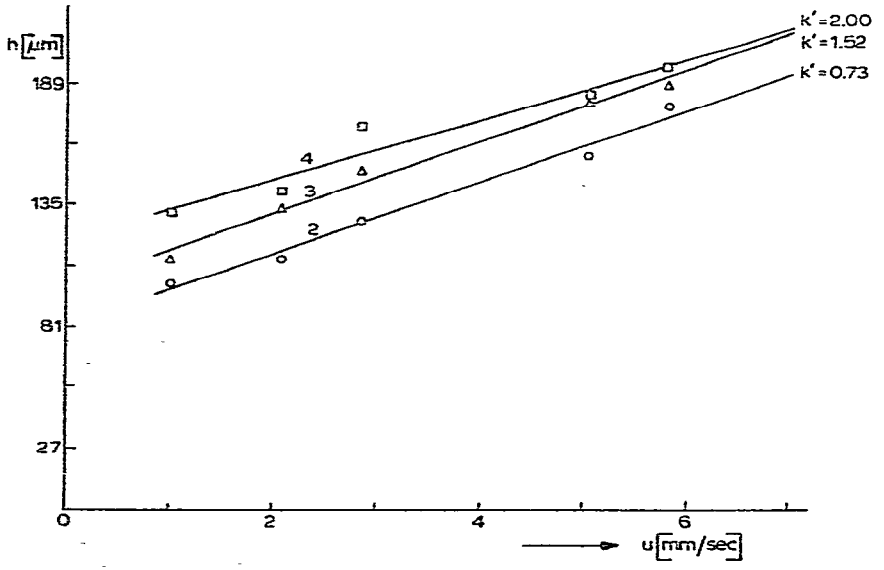

Fig. 8. Plots of h *versus* u on a reversed phase ($d_p = 10.9\ \mu$m). Stationary phase, C_{18} chemically bonded on silica (SI 100). Column length, 30 cm; I.D., 4.2 mm. Eluent, water–methanol–acetic acid (55:30:15, v/v/v). Samples: 2, caffeic acid ($k' = 0.73$); 3, *p*-coumaric acid ($k' = 1.52$); 4, *m*-coumaric acid ($k' = 2.0$). $K_P = 1.2 \cdot 10^{-9}$ cm^2. Temperature, ambient.

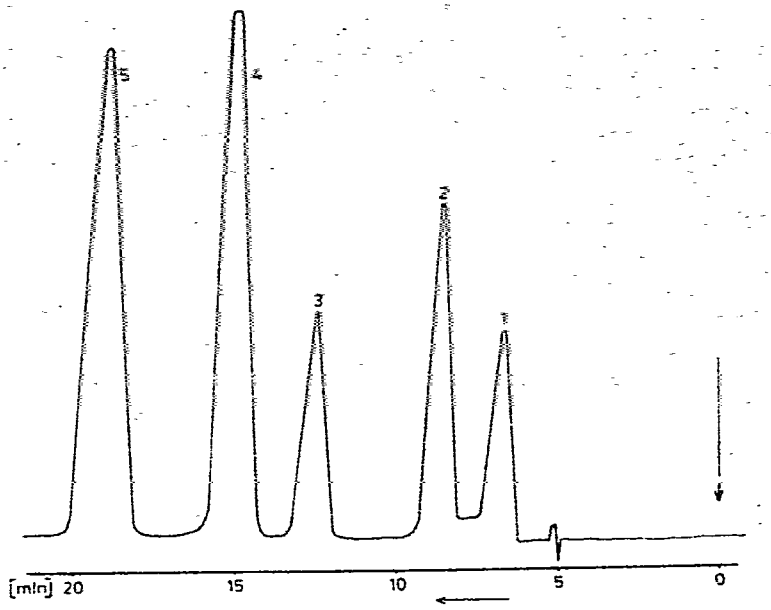

Fig. 9. Separation of organic acids on a reversed phase. Parameters as in Fig. 8, except for the following. Samples: 1, chlorogenic acid ($k' = 0.38$); 2, caffeic acid ($k' = 0.73$); 3, *p*-coumaric acid ($k' = 1.52$); 4, *m*-coumaric acid ($k' = 2.0$); 5, *o*-coumaric acid ($k' = 2.82$). $u = 1$ mm/sec; $\Delta p = 25$ atm; $R_{3,4} = 1.71$.

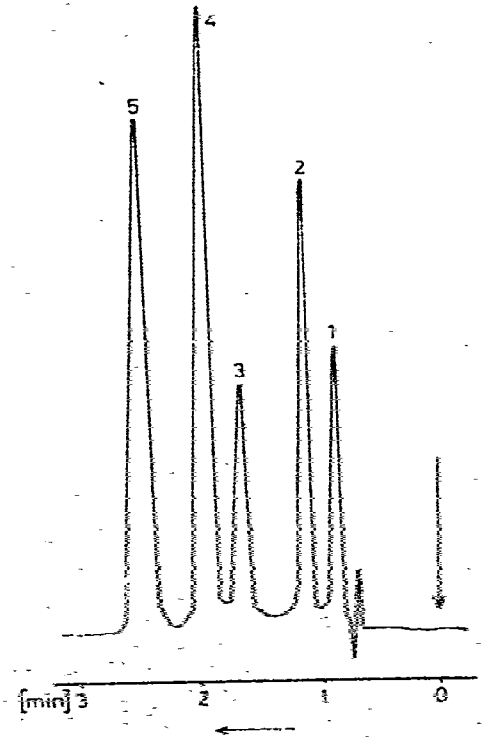

Fig. 10. Separation of organic acids on a reversed phase. Parameters as in Fig. 9, except $u = 7.5$ mm/sec, $\Delta p = 220$ atm and $R_{3,4} = 1.38$.

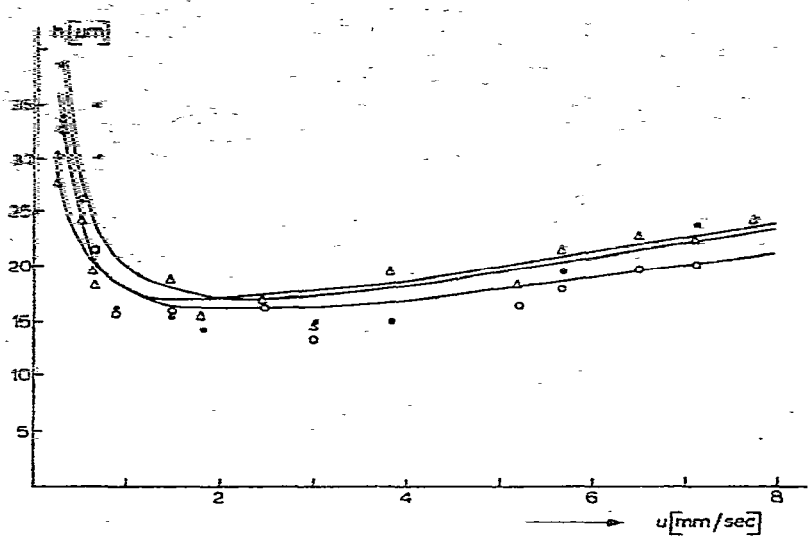

Fig. 11. Plots of h *versus* u on spherical silica ($d_p = 3.2\,\mu$m). Column length, 7.5 cm; I.D., 4.2 mm. Eluent, *n*-heptane. Samples: △, *o*-terphenyl; ○, diphenyl; ●, inert. $K_F = 1 \cdot 10^{-10}$ cm². Temperature, ambient.

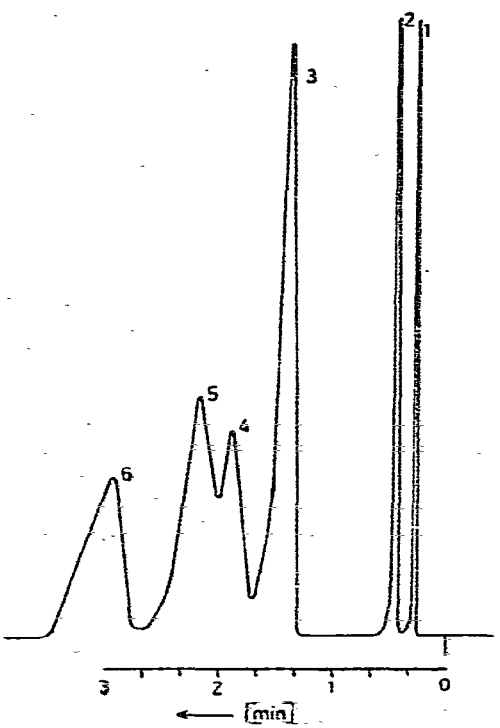

Fig. 12. Separation of barbiturates on spherical silica ($d_p = 3.2\,\mu$m). Column length, 7.5 cm; I.D., 4.2 mm. Eluent, methylene chloride–ethyl acetate (95:5, v/v). Samples: 1, inert; 2, thiobarbital; 3, secobarbital; 4, amobarbital; 5, vinylbarbital; 6, barbital. $K_F = 1 \cdot 10^{-10}$ cm². Temperature, ambient. $u = 4.2$ mm/sec; $\Delta p = 105$ atm.

Efficiency of columns packed with 3.2-μm silica

The low rate of success (less than 10%) in packing a "good" column with 3.2-μm silica was pointed out earlier. The data for the best of about 15 columns packed are presented in Fig. 11. Because of its low specific permeability, the column was only 7.5 cm long. As the viscosity of the eluent (*n*-heptane) and the molecular weights of the sample components were low, more than 600 theoretical plates/cm were generated at u_{min}. As predicted by eqn. 7, the small particle size causes u_{min} to shift to higher values ($u_{min} = 3.3$ mm/sec). The high column efficiency in this instance (Fig. 12) would have permitted more barbiturates to be separated than on a column packed with 13.5-μm silica (Figs. 4–7). The analysis was carried out at $u = 4.2$ mm/sec with a pressure drop of only 105 atm in about 3 min. The lifetime of such highly efficient columns is as long as that of more conventional columns ($d_p = 10\,\mu$m). Because of the sophisticated packing techniques required, however, columns with $d_p < 5\,\mu$m are not recommended for routine applications.

The loadability (*i.e.*, the maximum sample size that causes no loss in efficiency) of silica columns is about 10^{-4} g per gram of support. In our experience, highly efficient columns such as those described in Figs. 11 and 12 have a higher loadability, and hence are very suitable for use in trace analysis.

LIST OF SYMBOLS

d_p (μm)	average particle size as defined in eqn. 2
h (μm)	height equivalent to a theoretical plate
h_{min} (μm)	h at the minimum of the h *versus* u curve
k'	capacity ratio $\left(= \frac{t_R - t_0}{t_0}\right)$
n	number of theoretical plates
n_{max}	n at u_{min}
Δp (atm)	pressure drop over the column
r (mm)	radius of the column
t_R (sec)	time of analysis
t_0	hold-up time of the inert peak
u (mm/sec)	linear velocity of the eluent
u_{min} (mm/sec)	u at the minimum of the h *versus* u curve
$\bar{w}$ (sec)	average peak width
D (10^5 cm^2/sec)	interdiffusion coefficient in the mobile phase
F (cm^3/min)	flow-rate
K_F (cm^2)	specific permeability $\left(= \frac{F \eta L}{r^2 \pi \Delta p}\right)$
L (cm)	column length
M	molecular weight of the sample
N	number of effective plates
N_{max}	N at u_{min}
R	resolution $\left(= \frac{\Delta t_R}{\bar{w}}\right)$

α	relative retention $\left(= \frac{k'_2}{k'_1}\right)$
ϱ_l (g/cm^3)	specific gravity of the dispersing liquid
ϱ_s (g/cm^3)	true specific gravity of the support
ν	reduced velocity $(= ud_p/D)$
η (cP)	viscosity of the eluent
ε_T	total porosity

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