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# ULTIMATE LIMITS IN HIGH-PRESSURE LIQUID CHROMATOGRAPHY*

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## SUMMARY

Owing to the adiabatic heat of friction, the temperature of an eluent may increase by up to 35° if $\Delta p = 500$ atm. The capacity ratio of a sample decreases with increasing $\Delta p$. Temperature (and consequently viscosity, $D_m$, etc.) gradients exist in axial and radial directions inside the column. Therefore, because of the equipment limitations, $\Delta p_{max}$ is usually less than 500 atm. The value of $u_{min}$ (*i.e.*, the velocity at the minimum of the $h$ *versus* $u$ curve) increases with decreasing practicle size and with decreasing viscosity of the eluent. With $d_p < 5 \mu m$, $h \approx d_p$ while $K \approx d_p^2$. With semi-empirical equations and by experiment, it was demonstrated also that the minimum particle size in HPLC is between 1 and 3 $\mu m$. Up to 240 theoretical plates are generated per second. For routine work, $5 \mu m > d_p > 3 \mu m$ is proposed, except for trace analysis where columns packed with particles of smaller size could be preferable. When $d_p < 5 \mu m$, the air classification of the support and the column packing become sophisticated. The influence of extra-column effects is not negligible.

## INTRODUCTION

In an earlier paper[1], the influence of the particle size (5–35 $\mu m$) of spherical silica on column efficiencies was described. The equivalent particle size, $d_p$, was defined as

$$d_p = \sqrt{10^3 \, K_F} = \sqrt{\frac{10^3 \, F \eta L}{r^2 \pi \, \Delta p}} \tag{1}$$

if the porous support was packed by the balanced density method. In eqn. 1, $d_p$ is given in cm if the flow-rate of the eluent, $F$, is in cm³/sec, the viscosity of the eluent, $\eta$, in Poise, the length of the column, $L$, in cm, the radius of the empty column, $r$, in cm and the pressure drop across the column, $\Delta p$, in dynes/cm² ($\approx 10^{-6}$ atm). The simple relationship $h = A' + C'u$ was found to be an excellent approximation provided that $d_p > 5 \mu m$.

In high-pressure liquid chromatography (HPLC), increasingly smaller particle sizes were required in order to increase the efficiency of the column and the speed of

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analysis. A decrease in $d_p$, however, results in a decrease in the permeability, $K_F$, and the pressure drop over the column increases if linear velocities of the order of mm/sec (or cm/sec) are required. Another problem is that with decreasing $d_p$ the difficulties of packing of the column also increase, because of the larger surface energy of the support. In our experience, the pressure required for the column packing also increases, because this pressure must always be significantly higher than the maximum pressure drop over the column during the analysis. Consequently, it is obvious that for routine work there is a minimum value of $d_p$.

In this paper, some additional facts relating to the ultimate limits of decreasing particle size are discussed. The heat of friction, which is a function of the pressure drop over the column, has some important consequences. Further, the $h$ versus $u$ curve has to be described by the relationship

$$h = A + B/u + Cu \tag{2}$$

if $d_p$ is less than about 5 $\mu$m.

## HEAT OF FRICTION

In an adiabatic system with an incompressible medium, the heat of friction, $Q$, is independent of the stationary and the mobile phases:

$$Q = v\Delta p = mc\Delta T \tag{3}$$

$$\Delta T = \frac{\Delta p}{41.3\,\varrho\,c} = E\Delta p \tag{4}$$

The adiabatic rise in temperature, $\Delta T$ (°C), between the inlet and the outlet of the column increases with increasing pressure drop over the column, $\Delta p$ (atm), and with

TABLE I

CONSTANT $E$ IN EQUATION 4 FOR SOME ELUENTS AT ROOM TEMPERATURE

| Eluent | Specific heat (cal/g) | Density (g/cm³) | E · 100 (°C/atm) | T (°C) |
|---|---|---|---|---|
| Water | 0.999 | 0.9982 | 2.43 | 20 |
| Acetic acid | 0.468 | 1.049 | 4.93 | 20 |
| Methanol | 0.600 | 0.7928 | 5.09 | 20 |
| n-Propanol | 0.586 | 0.8044 | 5.14 | 25 |
| Ethanol | 0.581 | 0.7893 | 5.28 | 25 |
| Acetone | 0.528 | 0.792 | 5.79 | 20 |
| Ethyl acetate | 0.459 | 0.901 | 5.85 | 20 |
| n-Hexane | 0.600 | 0.6603 | 6.11 | 20 |
| Methylene chloride | 0.288 | 1.336 | 6.29 | 20 |
| Chloroform | 0.232 | 1.498 | 6.97 | 20 |
| Isopentane | 0.527 | 0.621 | 7.00 | 20 |
| Tetrachloroethylene | 0.211 | 1.6227 | 7.07 | 20 |
| n-Pentane | 0.542 | 0.6312 | 7.08 | 20 |
| n-Heptane | 0.490 | 0.6838 | 7.23 | 20 |
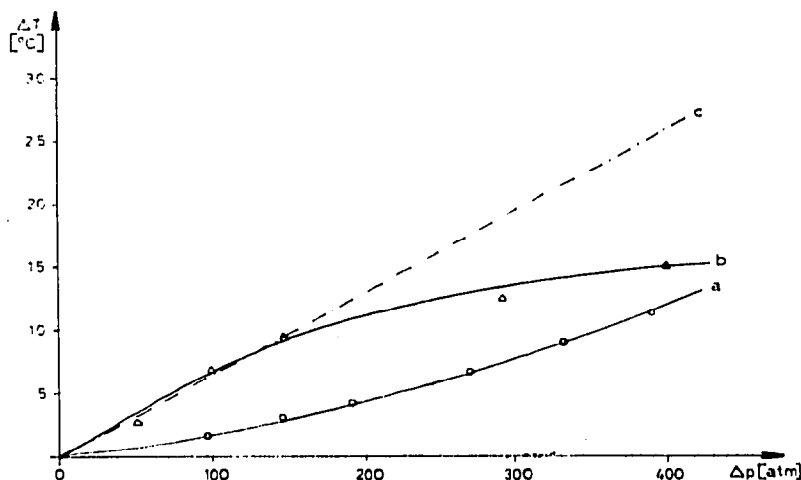
Fig. 1. Temperature gradient in the column. Column material: stainless steel. $L = 30$ cm; I.D. $= 4.2$ mm. The column is screwed directly into the metal block of the injector. a, Experimental values with the column; b, experimental values with needle valve; c, calculated adiabatic values.

decreasing density, $\varrho$ (g/cm³), and specific heat, $c$ (cal/g), of the eluent. The constant $E$ at room temperature is given in Table I for some important eluents. It can be seen in the fourth column that the temperature increase due to the heat of friction is between 5 and 7° if the pressure drop is 100 atm. $E$ is extremely small for water as eluent. In the calculations above, the compressibility of the eluent and the heat expansion of the system were neglected, and also the temperature dependence of $\varrho$ and $c$. The resulting errors are small provided that $\Delta p < 500$ atm.

With thermoelements at the inlet and outlet of the column, $\Delta T$ was determined by repeated experiments. In order to isolate the column, it was wound with asbestos cord. The results are shown in Fig. 1. The dotted line (c) is the calculated value for an adiabatic system, while the full line (a) gives the results of the experiments. Because of the good heat transfer through the metallic connections of the column (i.e., injection system and tubing to the detector), about 50% of the adiabatic $\Delta T$ was measured. This value depends on the type and length of the stainless-steel tube, i.e., the connection between injection system and column, etc. In order to demonstrate these heat effects, the temperature drop across a needle valve was measured as shown in line (b) in Fig. 1. The effective increase in the temperature of the eluent, $\Delta T$, due to the heat of friction decreases (1) with increasing time the eluent spends in the column, (2) with increasing heat conductivity of the tube, (3) with increasing heat conductivity of the junctions to and from the column, (4) with increasing heat capacity of the sampling system, etc. The consequence of the heat of friction inside the column is an axial and a radial temperature gradient in the column.

The axial temperature gradient was demonstrated by coating the stainless-steel column material with a temperature indicator, i.e., with a liquid crystal phase (Licristall, E. Merck, Darmstadt, G.F.R.). This phase was blue at the top, green at the centre and red at the outlet of the tube, for a pressure drop of about 400 atm.

With increasing pressure drop, the average temperature, $\bar{T}$, of the eluent inside

the column increases and consequently its average viscosity, $\bar{\eta}$, decreases. On the other hand, with increasing pressure, $\bar{\eta}$ increases[2]. As a rough approximation, the viscosity increases by 10% if $\Delta p$ increases by 100 atm (except for water and alcohols)[2]. Both of the effects mentioned above could compensate each other. If this is the case, then the flow-rate will be a linear function of $\Delta p$ and the permeability, $K_F$, will be independent of $\Delta p$, assuming that $K_F$ in eqn. 1 is calculated with the viscosity at the inlet temperature of the eluent. In our system, the $K_F$ value determined experimentally was $1.74 \cdot 10^{-10}$ cm$^2$ and was independent of $\Delta p$ up to 400 atm (the variation in $K_F$ was less than 4%). The compensation effect described above, however, only occurs fortuitously. The average temperature of the eluent is a function of the heat conductivity of the column, while the viscosity change due to the pressure is independent of this effect.

With increasing linear velocity, $\bar{T}$ increases and the average interdiffusion coefficient, $D_m$, also increases. The consequence of these effects will be an increase in the rate of mass transfer of the sample with increasing $u$. This could result in a smaller $C$ term in eqn. 2 than expected if the conditions were isothermal. The minimum of the $h$ versus $u$ curve could become broader and the slope of the ascending branch smaller due to the increasing temperature.

Finally, with increasing velocity, the capacity ratio, $k'$, of the samples will decrease because of the increase in $\bar{T}$. This effect becomes important if the heat of sorption of the sample is high. In our experiments with silica as stationary phase and $n$-heptane as eluent, $k'$ decreased by up to 20% if the pressure drop over the column was increased from 10 to 350 atm.

The radial temperature gradient inside the column is a consequence of the poor heat conductivity in a column packed with silica or alumina. The temperature of the mobile phase will be higher at the centre of the column than at the walls. Consequently, the viscosity and therefore the flow-rate of the eluent will be higher at the centre, and the flow profile of the sample will become broader, resulting in increased peak broadening. This effect can be demonstrated by the injection of a coloured sample into a packed glass column where a Poiseuille-type flow profile can be seen. On the other hand, the interdiffusion coefficient of the sample in the centre of the tube increases (owing to the higher temperature), and the result could be a smaller height equivalent to a theoretical plate.

In Fig. 2, the directions of some gradients caused by the pressure drop over the column are presented. Most of these gradients are valid not only in the axial but also in the radial direction. In the theory of chromatography, constant temperature is always assumed, because the diffusion coefficients, viscosities and capacity ratios are a function of temperature. As shown above, in HPLC the temperature distribution inside the column is complicated. Sometimes the influence of some parameters compensate each other. However, it seems to be impossible to develop a valid theory for chromatographic processes if the pressure drop over the column is greater than 50–100 atm.

EXPERIMENTAL

*Chromatographic apparatus*
          Self-built equipment[3] was used. The maximum flow-rate of the membrane
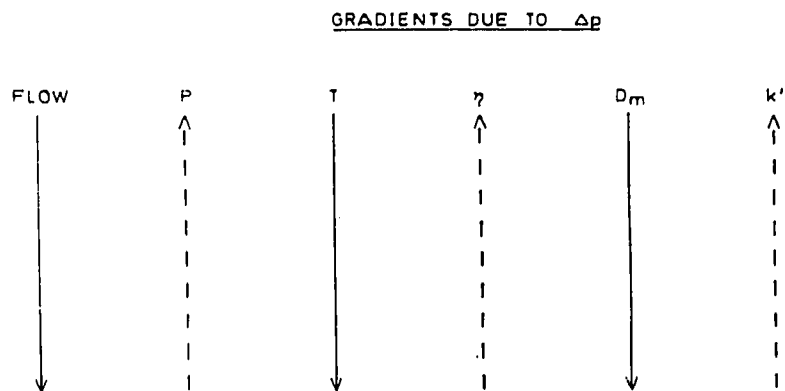
GRADIENTS DUE TO Δp



Fig. 2. Schematic presentation of some gradients due to the pressure drop.

pump was 30 ml/min at 400 atm pressure. The cell volume of the self-built UV detector was 4.5 μl with a 5-mm path length. The noise level was less than $10^{-4}$ absorbance unit at 254 nm.

## Eluents and samples

The eluents were n-heptane and n-pentane with viscosities of 0.41 and 0.23 cP, respectively, at 20°. All measurements were made at room temperature. Before use, the eluents were dried over molecular sieves. Their water contents were not controlled except in the measurements where the influence of the pressure drop over the column (i.e., the heat of friction) on the capacity ratios was determined. The inert sample was n-octane if the detector was a differential refractometer (Model R 401, Waters Ass., Milford, Mass., U.S.A.) and 1,1,2,2-tetrachloroethylene ($C_2Cl_4$) if a UV detector was used. Four aromatic compounds were selected as samples: benzene ($C_6H_6$), diphenyl (Diph.), o-terphenyl (Terph.) and nitrobenzene (NB). The sample sizes were between 1 and 10 μg.

## Stationary phase

'The stationary phase was always porous spherical silica with a specific surface area of 350 $m^2/g$ and with an average pore size of 150 Å as described previously[1]. If the particle size of the support, $d_p$, is less than 5 μm, it is extremely important to remove fine particles ($d_p <$ 1 μm). The air-classified sieve fraction of silica was suspended in a water–acetone (1:1) mixture in order to strip the fine powder. The equivalent particle size, $d_p$, of the fraction was 4.2 ± 0.2 μm as calculated by eqn. 1. The average particle size was 4–5 μm as determined by optical methods.

## Column packing

All columns were packed in drilled stainless-steel tubes with 4 mm I.D. and the balanced density packing method was used as described previously[3]. The asymmetry ($A_s^2$) of the peaks[3] was always less than 2, and mostly less than 1.5. Three columns with different lengths were used: 29 cm (column I), 22 cm (II) and 7.5 cm (III). The specific permeabilities were independent of the column length, $K_F =$ (1.75 ± 0.15)·$10^{-10}$ $cm^2$.

TABLE II

CONSTANTS IN THE EQUATION $h = A + B/u + Cu$

Eluent: $n$-heptane.

| $L$ (cm) | Sample | $k'$ | $A$ ($\mu m$) | $B$ ($10^{-5}$ cm²/sec) | $C$ (msec) | $\sqrt{\dfrac{B}{C}}$ (mm/sec) |
|---|---|---|---|---|---|---|
| 29 | C₂Cl₄ | 0 | 20.3 (±7%) | 2.89 (±14%) | 7.19 (±5%) | 0.63 |
| | C₆H₆ | 0.46-0.50 | 19.7 (±8%) | 3.85 (±13%) | 5.57 (±7%) | 0.83 |
| | Diph. | 1.47-2.02 | 21.3 (±3%) | 2.48 (±9%) | 2.29 (±8%) | 1.04 |
| | Terph. | — | — | — | — | — |
| | NB | 12.5-14.9 | 33.0 (±6%) | 1.76 (±55%) | 4.93 (±10%) | 0.60 |
| 22 | C₂Cl₄ | 0 | 14.0 (±16%) | 10.9 (±10%) | 0.48 (±91%) | 4.77 |
| | C₆H₆ | 0.44-0.48 | 17.4 (±21%) | 9.3 (±19%) | 0.12 (±575%) | — |
| | Diph. | 1.1-1.3 | 14.9 (±17%) | 9.5 (±13%) | 0.61 (±84%) | 3.95 |
| | Terph. | 2.6-3.3 | 15.1 (±10%) | 8.1 (±9%) | 1.60 (±18%) | 2.25 |
| | NB | — | — | — | — | — |
| 7.5 | C₂Cl₄ | 0 | 16.5 (±5%) | 6.59 (±7%) | 1.25 (±9%) | 2.30 |
| | C₆H₆ | 0.4-0.5 | 14.1 (±4%) | 6.68 (±4%) | 1.16 (±6%) | 2.40 |
| | Diph. | 1.3-1.7 | 12.6 (±5%) | 4.68 (±7%) | 1.36 (±5%) | 1.86 |
| | Terph. | 3.3-4.6 | 11.5 (±8%) | 3.57 (±13%) | 2.25 (±5%) | 1.26 |
| | NB | 11-14 | 12.1 (±5%) | 7.17 (±5%) | 1.08 (±7%) | 2.58 |

*Column efficiency*

For every column, the $h$ values were determined for a given sample using at least 12 velocities, and every measurement was repeated at least three times. The constants in eqn. 2 were calculated and are given tabulated in Tables II and III for

TABLE III

CONSTANTS IN THE EQUATION $h = A + B/u + Cu$

Eluent: $n$-pentane.

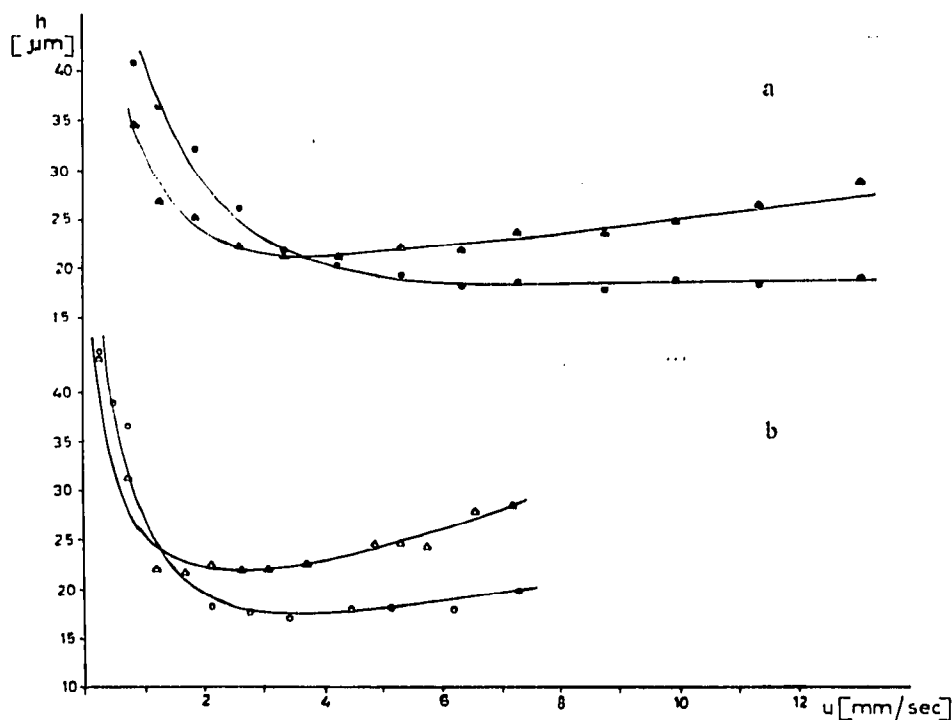| $L$ (cm) | Sample | $k'$ | $A$ ($\mu m$) | $B$ ($10^{-5}$ cm²/sec) | $C$ (msec) | $\sqrt{\dfrac{B}{C}}$ (mm/sec) |
|---|---|---|---|---|---|---|
| 29 | C₂Cl₄ | 0 | 17.3 (±9%) | 6.30 (±14%) | 3.83 (±6%) | 1.28 |
| | C₆H₆ | 0.4-0.44 | 13.2 (±11%) | 8.87 (±9%) | 3.67 (±5%) | 1.55 |
| | Diph. | 1.42-1.61 | 13.4 (±7%) | 7.01 (±7%) | 2.94 (±4%) | 1.54 |
| | Terph. | 3.5-4.0 | 17.7 (±8%) | 4.41 (±18%) | 2.14 (±9%) | 1.44 |
| | NB | 10.7-12.1 | 8.7 (±38%) | 14.0 (±20%) | 4.93 (±8%) | 1.69 |
| 22.5 | C₂Cl₄ | 0 | 14.7 (±12%) | 21.4 (±11%) | 0.50 (±35%) | 6.54 |
| | C₆H₆ | 0.5-0.6 | 15.8 (±13%) | 24.3 (±11%) | 0.029 | — |
| | Diph. | 1.5-1.8 | 13.0 (±9%) | 22.7 (±7%) | 0.44 (±27%) | 7.18 |
| | Terph. | 3.6-4.7 | 12.2 (±6%) | 18.6 (±5%) | 1.15 (±6%) | 4.02 |
| | NB | — | — | — | — | — |
| 7.5 | C₂Cl₄ | 0 | 26.8 (±8%) | 4.25 (±37%) | 0.29 (±58%) | 3.83 |
| | C₆H₆ | 0.5-0.6 | 13.2 (±4%) | 11.0 (±4%) | 0.69 (±7%) | 3.99 |
| | Diph. | 1.9-2.05 | 12.0 (±6%) | 7.17 (±8%) | 0.74 (±7%) | 3.11 |
| | Terph. | 5.3-5.8 | 12.2 (±6%) | 5.44 (±11%) | 0.99 (±5%) | 2.34 |
| | NB | 13.8-14.9 | 11.7 (±5%) | 10.7 (±5%) | 0.46 (±10%) | 4.82 |

Fig. 3. Plots of $h$ *versus* $u$ on spherical silica ($d_p$ = 4.2 $\mu$m). Column length: 22 cm. Eluents: (a) $n$-pentane; (b) $n$-heptane. Samples: ●, ○, benzene; ▲, △, $o$-terphenyl. $K_F$ = 1.74·10$^{-10}$ cm². 

$n$-heptane and $n$-pentane as mobile phases. The standard deviations are given as a percentage of the average tabulated values in parentheses. In Fig. 3, $h$ *versus* $u$ plots are shown for different eluents and different samples in column II ($L$ = 22 cm). All of the curves have a minimum and can be described by eqn. 2. At a constant velocity, the peak broadening of a given sample is less with $n$-pentane than with $n$-heptane as eluent. The $h$ *versus* $u$ curves for tetrachloroethylene and diphenyl are between the curves of the two other samples shown in Fig. 3. In Fig. 4, results similar to those in Fig. 3 are shown. The column in this instance is only 7.5 cm in length, however. The constants in eqn. 2, as calculated from the experimental results, are given in Tables II and III. For better understanding, it should be pointed out that in eqn. 2 the following units are used: $h$, $\mu$m; $A$, $\mu$m; $B$, 10$^{-5}$ cm²/sec; $C$, msec; and $u$, mm/sec.

The reproducibility of column packing described by the $h$ *versus* $u$ curves becomes poorer with decreasing particle size because of the increasing surface energy of the support. The reproducibility of the constants in eqn. 2 is especially poor if the column length is varied, as demonstrated in Tables II and III. The constancy of the $A$ term is acceptable. Increasing $B$ terms, however, are mostly compensated for by decreasing $C$ terms, and consequently the peak broadening in the neighbourhood of the minimum is roughly independent of the column length. Similar effects were found in terms of the reproducibility of column packing with constant column length.
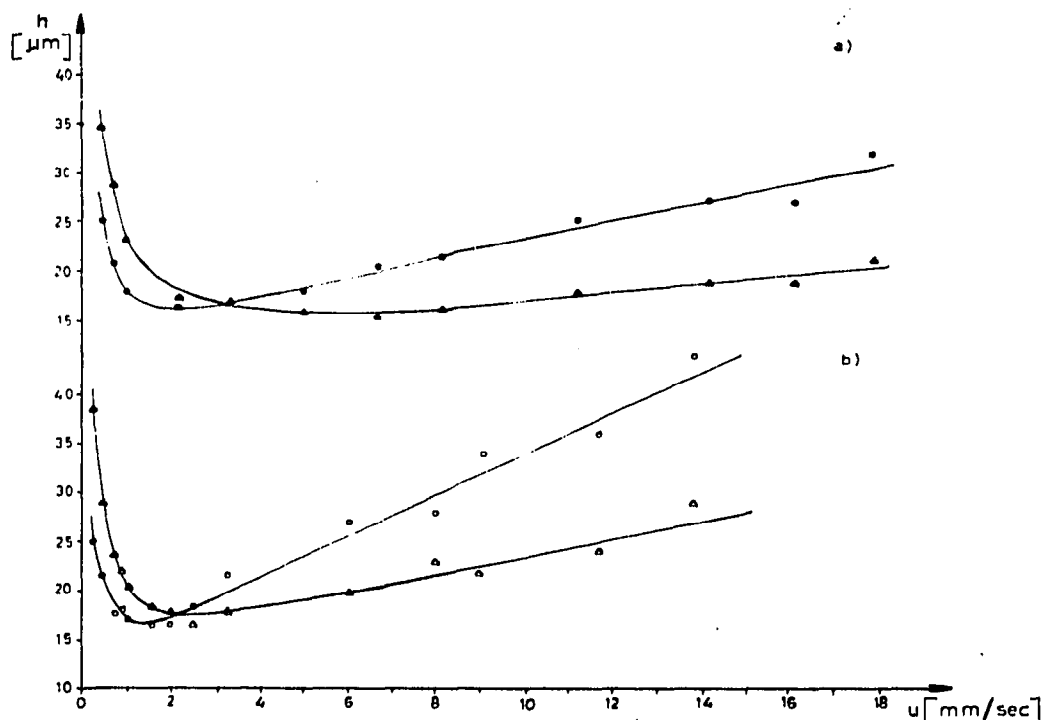
Fig. 4. Plots of $h$ versus $u$ on spherical silica ($d_p$ = 4.2 cm). Column length: 7.5 cm. Eluents: (a) $n$-pentane; (b) $n$-heptane. Samples: ●, ○, $o$-terphenyl; ▲, △, nitrobenzene. $K_F$ = $1.74 \cdot 10^{-10}$ cm$^2$.

Probably new packing methods are required if the particle size of the support is much less than $5\,\mu$m. The ageing-procedure of these small-particle columns will be faster because of the high surface energy of the particles. The results with the columns described in this paper were reproducible when the "dry" column was used again after a few months.

The shape of the $h$ versus $u$ curves can be described by eqn. 2, as demonstrated by the values in Tables II and III. The standard deviations of the calculated constants $A$, $B$ and $C$ average about $\pm 10\%$. The accuracy of the calculation decreases rapidly if the $C$ terms become less than $10^{-3}$ sec because (a) small differences between large numbers are taken and (b) the equipment limits are reached. In our experience, $h$ values as low as $10$–$15\,\mu$m can be determined with the apparatus described above. If the relative peak broadening becomes smaller, the influence of the extra-column effects (i.e., injection, broadening inside the connecting pipes, volume of the detector) becomes comparable to (or greater than) the broadening inside the column itself.

The minima of the $h$ versus $u$ curves were predicted or have been described earlier[4-7]. Huber[5] described a sharp minimum in the $h$ versus $u$ curve at a linear velocity of about 0.1 mm/sec if the particle size of the support was about 30 $\mu$m. These velocities are of limited importance from the point of view of routine analytical work. However, if the particle sizes are less than $5\,\mu$m, the minimum in the curve appears at velocities that are significant in routine work. It is difficult to increase the velocity here because of the limitation in pressure and because of the increasing heat of friction

caused by the increased pressure drop over the column. It should be borne in mind that in routine work one always has to work at least at the minimum or better on the ascending branch of the $h$ versus $u$ curve. This minimum as a function of $d_p$ is discussed later.

As demonstrated in Figs. 3 and 4, the shape and the position of the $h/u$ curves in a column are, to a first approximation, independent of the capacity ratio of the sample if the column is "well packed". Similar results were achieved with columns packed with greater[1] or smaller particle sizes than those considered in this paper. This is unexpected from the point of view of the theory of chromatography.

The capacity ratio of a sample is an extremely sensitive function of the water content of the $n$-heptane or $n$-pentane eluent if the column is packed with silica. Because the water content was not controlled, the $k'$ values for a given sample and eluent varied from column to column (from time to time), as shown in Tables II and III. In a given column, on a given day, the capacity ratio of a sample decreased monotonically with increasing linear velocity, i.e., with increasing pressure drop and consequently with increasing heat of friction. The extreme values of $k'$ are given in Tables II and III.

The $B$ term in eqn. 2 describes the longitudinal diffusion inside the column; theoretically, $B = 2\gamma D_m$. The factor $\gamma = 0.6$ was proposed by Snyder[8]. The dispersion of the $B$ terms for a compound in a given eluent is great, as demonstrated in Tables II and III. Particularly high $B$ values were calculated for column II (compensated for by relatively small $C$ terms). If the average $B$ terms, including some other results not described in this paper, are compared with the diffusion coefficients as calculated by the Wilke–Chang equation[9], it seems to be a simple and acceptable approximation to assume that $\gamma = 1$. In the following discussion, the assumption $B = 2 D_m$ is made. It should be pointed out, however, that extremely careful experiments are required if $D_m$ is to be determined by means of liquid chromatographic methods.

The mass transfer term $C$ in eqn. 2 is about 1–2 msec for $d_p = 4.2\,\mu$m. In liquid chromatography, the mass transfer in the mobile phase is usually much slower than that "in" the stationary phase (i.e., silica). Consequently, the ratios of two $C$ terms for a given sample when using the same two eluents in the same column should be constant. This ratio should be proportional to the viscosity ratios of the eluents. As can be seen in Tables II and III, as expected, the $C$ terms with $n$-pentane as eluent are always less than those with $n$-heptane as eluent. Unfortunately, the ratios of the $C$ terms are not constant and are not equal to the viscosity ratios of the two eluents. It is possible that the precision of our equipment (or our own experimental skill) did not permit the required precision when calculating these mass transfer terms.

*Efficiency and speed of analysis*

The maximum number of plates is generated at the minimum of the $h$ versus $u$ curve ($h_{min}$, $u_{min}$, $n_{max}$). The speed of analysis is characterized by the number of plates, $n/t$, or the number of effective plates, $N/t$, generated per unit time:

$$\frac{n}{t} = \frac{u}{h(1 + k')} \tag{5}$$

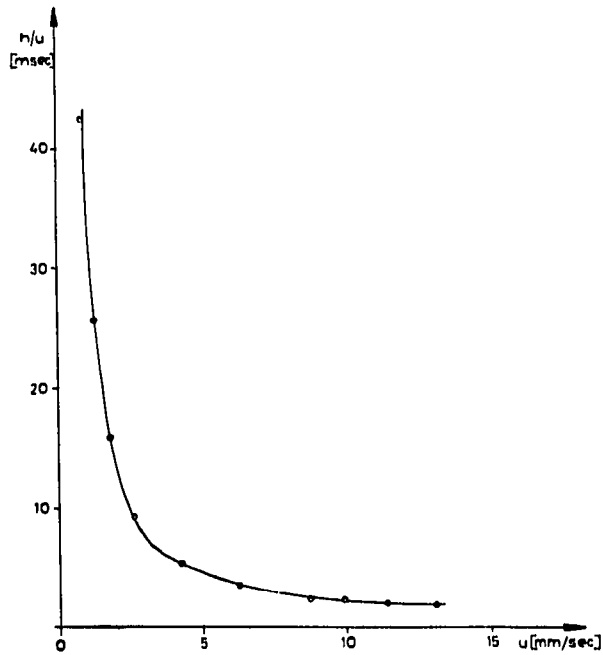$$\frac{N}{t} = \frac{u}{h}\,\frac{(k')^2}{(1 + k')^3} \tag{6}$$

Fig. 5. $h/u$ versus $u$ curve in column II ($L = 22$ cm). Eluent: $n$-pentane. Samples: tetrachloroethylene; benzene; diphenyl; $o$-terphenyl; nitrobenzene.

In Fig. 5, the $h/u$ values are plotted against $u$ for column II ($L = 22$ cm) with $n$-pentane as eluent. This curve is approximately independent of the capacity ratio of the sample, as demonstrated earlier in Fig. 3. The optimum speed of analysis, $u_{opt}$, is achieved at $2 u_{min}$ ($u = 1$ cm/sec). The required pressure drop is 260 atm with $n$-pentane as eluent. The $h_{opt}$ values are not very different from $h_{min}$ because of the small $C$ term ($h_{min} \approx 20 \mu$m).

The resolution is proportional to the effective number of plates. In Fig. 6, the
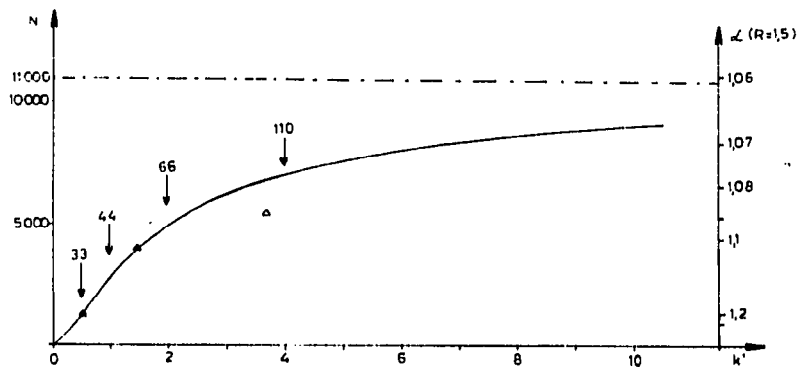


Fig. 6. Effective plate number and minimum relative retention, $\alpha$, (for resolution $R = 1.5$) as a function of the capacity ratio of the sample. The figures over the arrows give the time of analysis in seconds for given $k'$. Column length: 22 cm. $u_{opt} = 1$ cm/sec. Eluent: $n$-pentane. Samples: benzene; diphenyl; $o$-terphenyl. All other parameters as in Figs. 3 and 5.
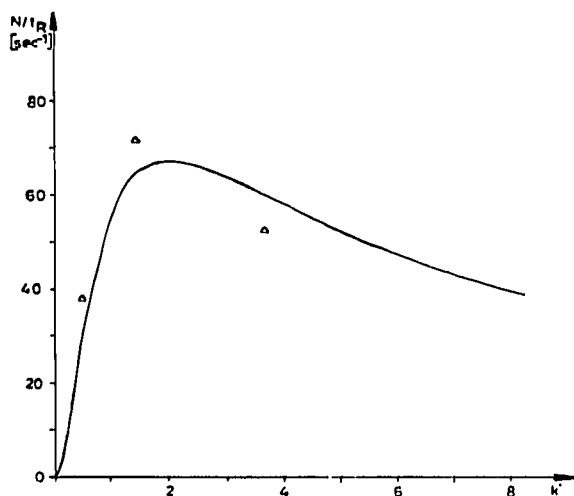
Fig. 7. Speed of analysis ($L$ = 22 cm). Eluent: $n$-pentane. $u_{opt}$ = 1 cm/sec. $h$ = 20 $\mu$m. Samples: benzene; diphenyl; $o$-terphenyl.

$N$ values are plotted as a function of $k'$ ($L$ = 22 cm) at $u_{opt}$. The number of effective plates increases with increasing $k'$ because $n$ is virtually independent of $k'$. The right-hand axis in Fig. 6 gives the minimum relative retention, $\alpha$, of two samples that could be separated if a resolution of $R$ = 1.5 (baseline separation) is required. For example, with $N$ = 7650 ($k'$ = 5), samples with $1.07 < \alpha < 1.08$ can be resolved with $R$ = 1.5. Substances with $1.04 < \alpha < 1.05$ could be separated if the required resolution is only $R$ = 1. The time of analysis, given in seconds in Fig. 6, increases, of course, with increasing capacity ratios. If $h$ is independent of $k'$, the maximum $N/t$ at a given linear velocity is generated at $k'$ = 2, as can be calculated with eqn. 6. In Fig. 7, up to 65 effective plates are achieved at $k'$ = 2. The time of analysis is short, a sample with the unusual high capacity ratio of 10 being eluted in 4 min. If a higher number of plates is necessary for a separation, the column length can be doubled. With the same pressure of 260 atm, $u_{min}$ and $h_{min}$ are achieved, i.e., the elongation is reasonable. In this situation, it is better to couple two columns with a capillary tube (I.D. = 0.25 mm) and with fittings of small dead volume. So far we have been unable to pack columns longer than 30 cm with 4–5-$\mu$m particles without a loss in efficiency.

On the other hand, short columns are sometimes wanted. The pressure drop and the time of analysis are shorter and also the concentration of the sample in the eluent increases. This is of great importance if trace analysis is to be carried out. Optimum conditions for the separation of an unknown mixture are rapidly found with a short unit, such as column III ($L$ = 7.5 cm). As demonstrated in Figs. 3 and 4, the $h$ versus $u$ curves are similar for columns with lengths of 22 and 7.5 cm, respectively. Peak broadening outside the column becomes important if the column length is decreased. The limits of our equipment were reached with the inert sample in column III. The $u_{opt}$ value in this column is also 1 cm/sec. It was, however, achieved only with $\Delta p$ = 90 atm. With $u$ = 2 cm/sec, up to 106 effective plates (or 240 theoretical plates) were generated per second with $k'$ = 2, as shown in Fig. 8. On doubling the
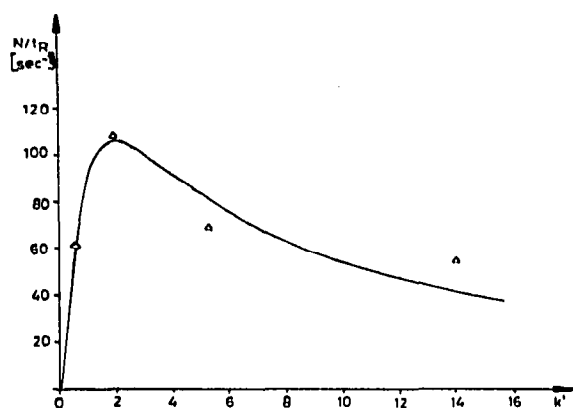
Fig. 8. Speed of analysis ($L$ = 7.5 cm). Eluent: $n$-pentane. $u$ = 2 cm/sec. $\Delta p$ = 180 atm. $h$ = 28 $\mu$m. Samples: benzene; diphenyl; $o$-terphenyl; nitrobenzene.

linear velocity, the speed of analysis increased only by a factor of 1.6 because $h_{opt}$ = 18 $\mu$m increased here to $h$ = 28 $\mu$m.

## LIMITS IN HPLC

In the discussion above, it was demonstrated that a precise theory cannot be given for HPLC because of the temperature and other gradients inside the column. In the following discussion, we shall ignore these limitations and to a first approximation we shall handle well known equations in order to obtain a qualitative picture of the ultimate limits in HPLC. The following assumptions will be made:

(1) The Van Deemter equation (*i.e.*, eqn. 2) is valid[10].

(2) The mass transfer term in the stationary phase is negligible compared with that in the mobile phase (this is not valid, of course, for heavily loaded columns).

(3) The constants in eqn. 2 are described by the following equations[11]:

$$A = 2\lambda d_p \approx 3d_p \tag{7}$$

$$B = 2\gamma D_m \approx 2D_m \tag{8}$$

$$C = \frac{\omega d_p^2}{D_m} \approx \frac{0.047\, d_p^2}{D_m} \tag{9}$$

(4) From our experience with particles of size less than 5 $\mu$m, the values $\lambda$ = 1.5 and $\gamma$ = 1 are reasonable.

(5) The capacity ratio-dependent factor $\omega$ should be described by the Golay term; for mass transfer in the mobile phase[12]

$$\omega = \frac{1 + 6k' + 11k'^2}{96(1 + k')^2} \tag{10}$$

From our experimental work, $\omega$ is roughly independent of the capacity ratio of the sample. As a rough approximation, $\omega$ will be calculated with eqn. 8 for $k'$ = 1, *i.e.*,

$\omega$ = 0.047 or $\sqrt{\omega}$ = 0.22. This approximation is reasonable, because for $k'$ = 5 a value of $\sqrt{\omega}$ = 0.30 is calculated. The use of the theory of open tubes for packed columns is an extremely rough approximation and perhaps is not justified. Probably it is a better approach to consider $\omega$ as an empirical constant.

(6) The product of the interdiffusion coefficient and the viscosity of the eluent is constant at a given temperature, *i.e.*

$$D_m = \frac{\alpha}{\eta} \approx \frac{1}{\eta} \tag{11}$$

(7) In HPLC, the specific permeability, $K$, is defined as

$$K = \frac{u\eta L}{\Delta p} \tag{12}$$

Comparing eqns. 1 and 12:

$$K_F = K \, \varepsilon_T \tag{13}$$

where $\varepsilon_T$ is the total porosity of the column. For porous supports, the value $\varepsilon_T$ = 0.84 is a good approximation. For the sake of simplicity in the following discussion, it will be assumed that $K_F = K$.

With these assumptions, eqn. 2 becomes

$$h = 3 \, d_p + \frac{2D_m}{u} + \frac{\omega d_p^2}{D_m} \cdot u \tag{14}$$

*Efficiency as a function of the particle size*

As pointed out earlier, it is an experimental fact that with decreasing particle size the $h$ *versus* $u$ curves become independent of the capacity ratio of the sample, and the shape and position of the curves become more and more a linear function of $d_p$. In eqn. 2, the $B$ term is independent of $d_p$, $A$ decreases with $d_p$ and $C$ decreases with $d_p^2$ if the particle size decreases. Consequently, if the particle size becomes very small ($d_p < 10\,\mu$m), $A \gg (B/u + Cu)$, and with large particle sizes, the contrary is true. In gas chromatography, $d_p \approx 150\,\mu$m is typical and it is a common assumption that $h \approx d_p^2$. In liquid chromatography, the exponent $\beta$ in the equation $h \approx d_p^\beta$ as proposed by different workers[1,7,13–16] becomes increasingly smaller as the investigated particle sizes decrease ($\beta$ = 1.8–1.3). With very small particle sizes (perhaps $d_p < 3\,\mu$m), $h \approx d_p$.

*Efficiency and the speed of analysis as a function of the viscosity of the eluent*

By combining eqns. 11, 12 and 14:

$$h = 3 \, d_p + \frac{2\alpha L}{K\Delta p} + \frac{\omega d_p^2 K\Delta p}{\alpha L} \tag{15}$$

The constant $\alpha = \eta D_m$ is not a sensitive function of the viscosity of the eluent at con-

stant temperature. In this case, it can be seen from eqn. 15 that the efficiency is independent of the viscosity of the eluent. Efficiency is a function of the particle size of the support and of the pressure drop per unit length of the column.

From eqns. 5 and 12:

$$\frac{n}{t} = \frac{K\Delta p}{\eta\, L\, h(1 + k')} \tag{16}$$

Hence the speed of analysis ($n/t$ or $N/t$) increases with decreasing viscosity of the eluent.

### Minimum of the h versus u curve

In order to obtain high resolution and/or high speed of analysis, the velocity of the eluent must be equal to or greater than $u_{min}$. Therefore, the shift of $u_{min}$ with changing particle size is of great interest. If eqns. 2 and 14 are differentiated with respect to $u$, it can be shown that

$$u_{min} = \sqrt{\frac{B}{C}} = \sqrt{\frac{2D_m^2}{\omega\, d_p^2}} = \frac{D_m}{d_p}\sqrt{2/\omega} \approx \frac{6.52 D_m}{d_p} = \frac{6.52 u}{\eta\, d_p} \tag{17}$$

Hence, with decreasing particle size, $u_{min}$ is shifted in the direction of higher velocities. If at least $u_{min}$ is required, the pressure drop needed will increase by $e^3$ if the particle size is smaller by a factor $e$, assuming that the column length, viscosity of the eluent and temperature are kept constant. A factor of $e^2$ is the consequence of the decreased permeability and a further factor $e$ is necessary because of the shift of $u_{min}$. Choosing another eluent with lower viscosity does not help to solve the problem. As shown in eqn. 17, $u_{min}$ increases linearly with decreasing viscosity of the eluent. Consequently, the pneumatic resistance of a given column at $u_{min}$ is independent of the viscosity of the eluent.

With decreasing particle size, $h_{min}$ also decreases. Replacing $u_{min} = \sqrt{B/C}$ in eqn. 2 and with eqn. 14, we obtain

$$h_{min} = A + 2\sqrt{BC} = d_p(3 + 2\sqrt{2\omega}) \approx 3.61\, d_p \tag{18}$$

The value of $h_{min}$ decreases only linearly with the particle size, and is mainly a function of $d_p$. To summarize, on decreasing the particle size, for example, by a factor of 3, the required pressure drop will increase by a factor of 27 while $h_{min}$ will decrease only by a factor of 3, if the other parameters ($L$, $\eta$) are kept constant. At the same time, $u_{min}$ increases only by a factor of 3. Consequently, the pressure drop per unit plate, $\Delta p/n$, increases with the square of the decreasing particle size. This fact and $\Delta p_{max}$ determine the smallest possible particle size in the analytical routine of HPLC.

### Pressure limits

The maximum pressure of the pump ($\Delta p_{max}$) in routine work is between 150 and 500 atm. If higher pressures are desired, the equipment will be expensive and sophisticated (consider, for example, the problem of the fittings, sampling and valves). On increasing the pressure, the compressibility of the liquid cannot be ne-

glected and the viscosity and the diffusion coefficients can become a sensitive function of the pressure. If such is the case, $h$ will be a function of the column length, as is well known from experience in working with capillary columns in gas chromatography. Finally, increasing the pressure drop over the column results in an increase in the heat of friction. It could happen that if a liquid eluent enters the column it might evaporate at the outlet since the heat of friction is great.

### Analytical conditions at the minimum of the h versus u curve

Accepting a limited $\Delta p_{max}$ (for example, 300 atm), the efficiency and the speed of analysis can be now calculated at $u_{min}$. This is the minimum velocity that is acceptable for routine work and the maximum number of plates are generated here. However, the speed of analysis is not optimal. At $\Delta p_{max}$, the column length, $L_{max}$, can be calculated from

$$L_{max} = h_{min} \, n_{max} \tag{19}$$

with eqns. 12 and 19:

$$u_{min} = \frac{K \Delta p_{max}}{\eta \, n_{max} h_{min}} \tag{20}$$

Replacing $u_{min}$ in eqn. 20 with eqn. 17 and $h_{min}$ with eqn. 18, we obtain

$$n_{max} = \frac{K \Delta p_{max}}{\eta \, D_m \, (4 + 3\sqrt{2}/\omega)} \approx \frac{K \Delta p_{max}}{23.6 \, \eta \, D_m} \tag{21}$$

Replacing $K$ in eqn. 21 by $d_p^2/10^3$ as a rough approximation, we obtain

$$n_{max} = \frac{d_p^2 \Delta p_{max}}{2.36 \cdot 10^4 \, \eta \, D_m} \tag{22}$$

An equation similar to the above was published by Giddings[11]. However, Giddings neglected the $B$ term in eqn. 2 and consequently also the minimum of the curve, and assumed that the linear velocity is extremely small ($u \rightarrow 0$). Contrary to the approach of Giddings, in eqn. 22 $u_{min}$ is assumed, i.e., a linear velocity of importance in the routine of separation. Although the assumptions are different, the equations are similar because both approximations result in the fact that $h \approx A$ in eqn. 2.

The speed of analysis at the minimum is calculated by means of eqns. 17 and 18 as

$$\frac{n_{max}}{t_R} = \frac{u_{min}}{h_{min} \, (1 + k')} = \frac{6.52 \, D_m}{3.61 \, d_p^2 \, (1 + k')} = \frac{1.81 \, D_m}{d_p^2 \, (1 + k')} \approx \frac{1.81}{\eta \, d_p^2 \, (1 + k')} \tag{23}$$

The number of (effective) plates generated per time unit increases linearly with decreasing viscosity of the eluent and increases with the square of the decreasing particle size at the minimum of the $h$ versus $u$ curve.

With the equations given above, a rough picture of the ultimate limits as a function of the particle size in HPLC can be made. It is always assumed that the

TABLE IV

CALCULATED EFFICIENCY AS A FUNCTION OF $d_p$ AT $u_{min}$

Eluent: $n$-pentane. $D_m = 2.33 \cdot 10^{-5}$ cm²/sec (ref. 17). $\eta = 0.23$ cP. Sample: benzene ($k' = 1$). $\Delta p_{max} = 300$ atm.

| Parameter | $d_p$ ($\mu m$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $n_{max}$ | 2362 | 9449 | 21 259 | 37 795 | 59 055 | 85 300 |
| $L_{max}$ (cm) | 0.85 | 6.8 | 23.0 | 55.0 | 107.0 | 185.0 |
| $h_{min}$ ($\mu m$) | 3.6 | 7.2 | 10.8 | 14.6 | 18.1 | 21.7 |
| $u_{min}$ (mm/sec) | 15.2 | 7.6 | 5.1 | 3.8 | 3.0 | 2.5 |
| $t_0$ (sec) | 0.56 | 8.95 | 45.0 | 145 | 357 | 730 |
| $n/t_R$ (sec⁻¹) | 2111 | 528 | 236 | 130 | 83.0 | 58.4 |

maximum available pressure is 300 atm and the capacity ratio of the sample is $k' = 1$. The number of effective plates per second, $N/t$, can be calculated by dividing $n/t$ by the factor 4, because $[(1 + k')/k']^2 = 4$ for $k' = 1$. The maximum $N/t$ is calculated, of course, for $k' = 2$ if $h_{min}$ is independent of $k'$. In Table IV, the calculated maximum number of plates, $n_{max}$, the maximum column length, $L_{max}$, the minimum relative peak broadening, $h_{min}$, the linear velocity of the eluent at the minimum of the $h$ versus $u$ curve, $u_{min}$, the hold-up time of the eluent, $t_0$, and the number of theoretical plates generated per unit time, $n/t_R$ (for $k' = 1$) are shown as a function of the particle size, $d_p$. The specific permeabilities are not tabulated, because they are defined by eqn. 1. The plate number, $n_{max}$, is indeed the maximum because the values in Table IV are calculated for the minimum of the $h$ versus $u$ curve. For the same reason, the speed of analysis could be optimised by increasing $u_{min}$ by a factor of about 2 in order to achieve $u_{opt}$. If doing so, $n_{opt}$ will be smaller than $n_{max}$. With decreasing particle size, it becomes increasingly difficult to generate higher velocities than $u_{min}$ because of the limits of the inlet pressure.

In Table IV, $D_m = 2.33 \cdot 10^{-5}$ cm²/sec was assumed, as given in the literature[17]. It should be mentioned that the interdiffusion coefficients as given in the literature are often contradictory and most of the values of interest in HPLC were not determined by experiment.

As demonstrated in Table IV, if the particle size of the support is decreased by a factor $e$:

(1) $n_{max}$ decreases with $e^2$, because $\Delta p_{max}$ is constant and the pressure drop per unit plate decreases with $e^2$;

(2) $L_{max}$ decreases by $e^3$, because of the decreasing permeability ($e^2$) and because of the increase in $u_{min}$ ($e$);

(3) $h_{min}$ decreases only by a factor $e$ (eqn. 18);

(4) the retention time of the inert peak decreases with $e^4$, because the column is shorter ($e^3$) and the velocity is increased ($e$);

(5) $u_{min}$ increases by a factor $e$ (eqn. 17); and

(6) $n/t_R$ increases with $e^2$ (eqn. 23).

At the minimum of the $h$ versus $u$ curve, $L_{max}$ ($e^3$) and $t_0$ ($e^4$) are sensitive functions of the particle size.

TABLE V

CALCULATED EFFICIENCY AS A FUNCTION OF $d_p$ AT $u_{min}$

Eluent: $n$-pentane. $D_m$ = 5.2·10⁻⁵ cm²/sec (Wilke–Chang equation). $\eta$ = 0.23 cP. Sample: benzene. $\Delta p_{max}$ = 300 atm.

| Parameter | $d_p$ ($\mu m$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $n_{max}$ | 1064 | 4257 | 9578 | 17 028 | 26 601 | 38 312 |
| $L_{max}$ (cm) | 0.38 | 3.1 | 10.3 | 24.9 | 48.1 | 83.1 |
| $h_{min}$ ($\mu m$) | 3.6 | 7.2 | 10.8 | 14.6 | 18.1 | 21.7 |
| $u_{min}$ (mm/sec) | 33.9 | 17.0 | 11.3 | 8.5 | 6.8 | 5.7 |
| $t_0$ (sec) | 0.11 | 1.82 | 9.11 | 29.29 | 70.74 | 145.8 |
| $n/t_R$ (sec⁻¹) | 4708 | 1181 | 523 | 291 | 188 | 131 |

From the data in Table IV, the optimum particle size seems to be $2\,\mu m$. The speed of analysis is high enough, i.e., $n/t_R$ = 528/sec or $Nt_R$ = 132/sec. More than 9000 theoretical plates are generated, usually sufficient for routine separations. The column length can be hardly shorter than 6.8 cm because (1) it is difficult to pack shorter columns, (2) with decreasing column length (i.e., decreasing particle size) the influence of the inlet and outlet turbulances increases, (3) the small volume of the column results in the peak broadenings outside the column becoming dominating compared with the broadening inside the column. Finally, the time of analysis with $d_p$ = $2\,\mu m$ is very short. Consequently, in this efficient and short column, the peak widths are so small that UV galvanometers are required so as not to falsify the peak shape if columns with smaller particle sizes should be used. At the same time, the peak broadening due to the injection of the sample and that in the connecting pipes disturb the efficiency of the column.

If the available pressure drop is greater than 300 atm, the speed of analysis can be increased in the same column. At the same time, of course, the number of the generated plates decreases. The efficiencies in Table V are also calculated for $n$-pentane eluent; however, $D_m$ = 5.20·10⁻⁵ cm²/sec, as calculated by the Wilke–Chang equation, is used as the diffusion coefficient. Tables IV and V show that the value of the diffusion coefficient does not influence the minimum particle size that is reasonable

TABLE VI

CALCULATED EFFICIENCY AS A FUNCTION OF $d_p$ AT $u_{min}$

Eluent: $n$-heptane. $D_m$ = 3.75·10⁻⁵ cm²/sec (Wilke–Chang equation). $\eta$ = 0.41 cP. Sample: benzene ($k'$ = 1). $\Delta p_{max}$ = 300 atm.

| Parameter | $d_p$ ($\mu m$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $n_{max}$ | 828 | 3311 | 7451 | 13 246 | 20 696 | 29 802 |
| $L_{max}$ (cm) | 0.30 | 2.4 | 8.0 | 19.3 | 37.5 | 64.7 |
| $h_{min}$ ($\mu m$) | 3.6 | 7.2 | 10.8 | 14.6 | 18.1 | 21.7 |
| $u_{min}$ (mm/sec) | 24.5 | 12.2 | 8.2 | 6.1 | 4.9 | 4.1 |
| $t_0$ (sec) | 0.12 | 1.97 | 9.76 | 31.64 | 76.53 | 157.8 |
| $n/t_R$ (sec⁻¹) | 3403 | 847 | 380 | 209 | 135 | 95 |

in routine work. Of course, if $D_m \ll 10^{-6}$ cm²/sec (*i.e.* the molecular weight of the sample or eluent becomes large), the optimum particle size decreases. This can be important in gel permeation (exclusion) chromatography.

Because of its low boiling point, *n*-pentane is not suitable for use as an eluent in routine work. If the boiling point of the eluent at atmospheric pressure is near the column temperature (*i.e.*, $\Delta T < 30°$), the probability of bubbles forming in the detector increases. Further, under such conditions, the viscosity and the diffusion coefficient change rapidly with pressure. In this region, the density and the viscosity can change up to 3–5% per 100 atm and can result, among other things, in a rapid decrease in the flow-rate of the pump with increasing pressure.

Typical eluents for routine work are, for example, *n*-heptane and water. The same calculations as above are shown for *n*-heptane in Table VI with $D_m = 3.75 \cdot 10^{-5}$ cm²/sec, as calculated with the Wilke–Chang equation. In Table VII, the sample is α-alanine and the eluent is water with a relatively high viscosity ($\eta = 1$ cP) and with $D_m = 0.91 \cdot 10^{-5}$ cm²/sec (ref. 17).

If Tables IV–VII are compared, it can be seen that the different parameters have different values with $d_p = $ constant. As shown in the equations above, $n_{max} \approx (\eta D_m)^{-1}$, $L_{max} \approx (\eta D_m)^{-1}$, $u_{min} \approx D_m$, $t_0 \approx (\eta D_m^2)^{-1}$ and $n/t_R \approx D_m$. If, at a given temperature, the constant $a$ in eqn. 11 is independent of the type of the eluent–sample pair, $n_{max}$ and $L_{max}$ will be independent of this pair and $t_0$ will be proportional to $D_m^{-1}$. As can be seen from the tables, this assumption is only a rough approximation.

It is surprising, however, that the optimum particle size, as determined by $L_{max}$ and $t_0$, is about 2 μm in all of the tables. In routine work, the optimum particle size will be about 2 μm, independent of the type of the usual eluents and samples, if the assumptions in the calculations are reasonable. It is possible that the estimations made in the derivation of our equations are too rough and that the optimum particle size is up to three times larger. However, the optimum (minimum) particle size is limited in HPLC as long as the heat of friction is not negligible (better heat exchange inside the column) and/or the inlet pressure of the pump is limited. This optimum particle size is about 1 μm and it is questionable whether, with the instrumentation used today and with the known packing methods, these limits can be approached.

With decreasing particle size, the speed of analysis increases; however, the column length also becomes shorter and, consequently, the number of plates generated

TABLE VII

CALCULATED EFFICIENCY AS A FUNCTION OF $d_p$ AT $u_{min}$

Eluent: water. $D_m = 0.91 \cdot 10^{-5}$ cm²/sec (ref. 17). $\eta = 1.0$ cP. Sample: α-alanine ($k' = 1$). $\Delta p_{max} = 300$ atm.

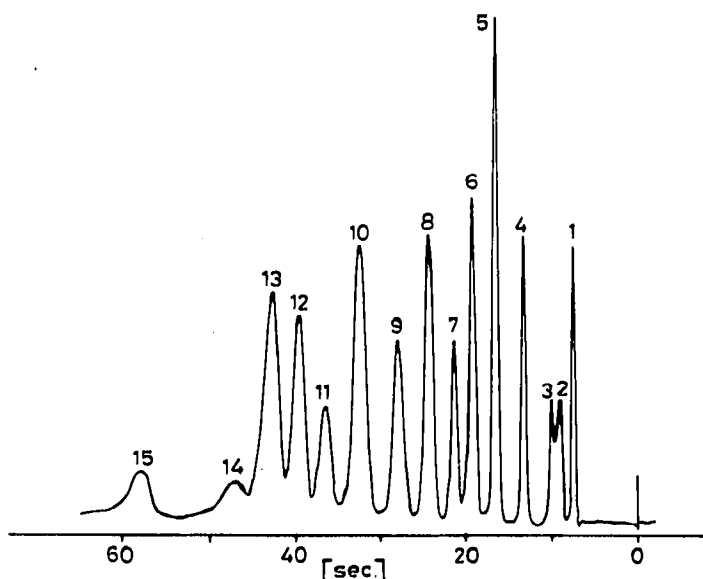| Parameter | $d_p$ (μm) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $n_{max}$ | 1399 | 5595 | 12 588 | 22 379 | 34 967 | 50 353 |
| $L_{max}$ (cm) | 0.50 | 4.0 | 13.6 | 32.7 | 63.3 | 109.3 |
| $h_{min}$ (μm) | 3.6 | 7.2 | 10.8 | 14.6 | 18.1 | 21.7 |
| $u_{min}$ (mm/sec) | 5.9 | 3.0 | 2.0 | 1.5 | 1.2 | 1.0 |
| $t_0$ (sec) | 0.85 | 13.3 | 68 | 221 | 525 | 1093 |
| $n/t_R$ (sec⁻¹) | 825 | 206 | 92 | 51 | 33 | 23 |

Fig. 9. Separation of condensed aromatics. Stationary phase: spherical silica ($d_p$ = 4.4 $\mu$m). $K_F$ = 1.93·10⁻¹⁰ cm². Column: 6.5 cm long, 4.0 mm I.D. $T$ = 25°. Eluent: $n$-pentane. $lp$ = 72 atm. $u$ = 9.3 mm/sec. Sample size = 3–50 $\mu$g per compound. $h$ = 20–25 $\mu$m. $n$ = 2600–3250. UV detector. Peaks: 1, methylene chloride (inert); 2, chloroform (solvent); 3, benzene; 4, naphthalene; 5, diphenyl; 6, anthracene; 7, pyrene; 8, fluoranthene; 9, $o$-terphenyl; 10, 1,2-dibenzanthracene; 11, 3,4-benzpyrene; 12, perylene; 13, 1,12-benzperylene; 14, coronene; 15, 1,2,5,6-dibenzanthracene.

decreases. The number of plates required is determined by the relative retentions of neighbouring samples. From experience, for most routine analyses less than 1500 effective plates are required.

*Separations*

In Fig. 9 the separating power and the speed of analysis are demonstrated for the analysis of an artificial mixture of condensed aromatics (methylene chloride as the inert sample). The column was 6.5 cm in length and was packed with spherical silica ($d_p$ = 4.2 $\mu$m). The pressure drop over the column was only 72 atm. For a linear velocity of 9.3 mm/sec with $n$-pentane as eluent, $h$ values were between 20 and 25 $\mu$m.

When $\varDelta p$ was increased to 250 atm, the separation of the same mixture became poorer, but the time of analysis decreased to less than 20 sec, as shown in Fig. 10. With a linear velocity of $u$ = 28.8 mm/sec, $h$ increased to 30–40 $\mu$m, i.e., the mass transfer term, $Cu$, was not negligible. This type of separation could be of interest if exhaust gases of cars are to be analyzed. Because the column is short and efficient, the peak heights are large and consequently "trace" analysis can also be carried out. The minimum detectable concentration of a compound increases with increasing linear velocity and efficiency and with decreasing length of the column.

*Optimum particle size for trace analysis*

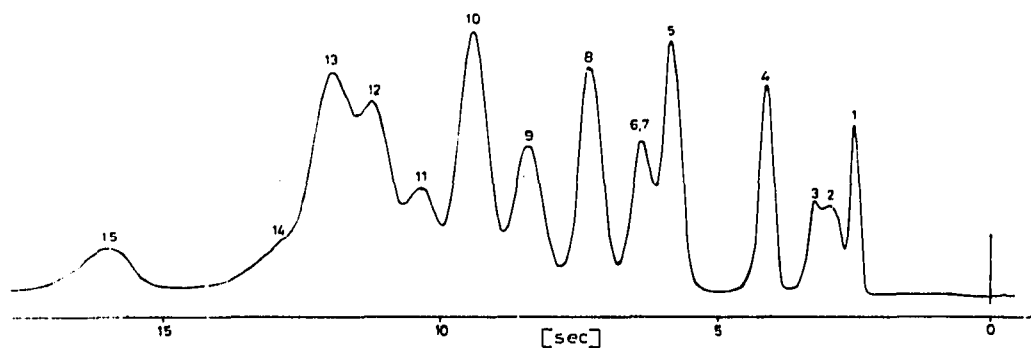In trace analysis, two different problems exist:

Fig. 10. Separation of condensed aromatics. Conditions as in Fig. 9 except for the following: $\Delta p$ = 250 atm; $u$ = 28.8 mm/sec; $h$ = 30–40 $\mu$m; $n$ = 1625–2170; sample size = 1–15 $\mu$g per compound.

(1) the concentration of the trace compound in the original mixture is small and there is no practical limit to the volume; or

(2) the weight of the sample is extremely small.

Both problems can exist in the same analysis.

In HPLC, concentration-sensitive detectors (UV, refractive index) are used. Trace amounts of compounds can be detected if their concentration in the eluent inside the detector is high.

The minimum number of plates for a given separation is determined by the relative retention of the two neighbouring compounds to be resolved. With decreasing particle size of the support, the peak broadening and consequently the column length required for a given plate number decrease. Therefore, the concentration of the sample in the detector increases, if all other parameters are kept constant. For trace analysis, short columns packed with particles of very small size are required. The minimum particle size (i.e., the shortest possible column) is limited by the peak broadening outside the column and by the time constants of the detector, amplifier and recorder.

On increasing the sample size over a given limit (loadability), the efficiency and the resolving power of the column decrease. In our experience, the loadability can be given in grams of sample per gram of stationary phase, provided that the inner diameter of the column is larger than 3 mm and smaller than 8 mm. In this region, the sample size can be increased by a factor of four if the inner diameter can be doubled without a loss in efficiency (i.e., $h$ versus $u$ curve). So far it has been assumed that with the plate number required for the separation the loadability is also defined because the resolving power of a column decreases if the column is overloaded. It is always assumed that the maximum sample size is injected without loss in resolving power.

Provided that the concentration of a compound in the mixture is small but the amount of sample size is unlimited, the inner diameter of the column (between the limits given above) is unimportant. The amount of sample is often also limited. If so, columns with small diameters are required. In our experience, the efficiency of a column decreases rapidly if its inner diameter (packed by conventional methods) is smaller than about 3 mm.

CONCLUSIONS

In conventional column chromatography and in HPLC, small particle sizes are required in order to increase the efficiency and to decrease the time of analysis. The minimum particle size, however, is limited. If the particle size, $d_p$, is decreased, then the permeability, $K_F$, also decreases (or the hydrodynamic resistance increases). To define and to determine $d_p$ by experiment is difficult and it is therefore reasonable to define $d_p$ with $K_F$, as in eqn. 1. In 1951, Gregg[18] pointed out that in column chromatography 1–10 $\mu$m seems to be the minimum particle size, otherwise the time of analysis becomes intolerably long. It was believed that in HPLC the minimum $d_p$ could be smaller, because a much higher pressure is available. In this paper, we have tried to demonstrate that the minimum $d_p$ in HPLC is also about 1 $\mu$m. The arguments that result in similar minimum particle sizes in conventional and high-pressure liquid chromatography are, however, different.

The heat of friction increases with the pressure drop, the column is not isothermal and exact theoretical considerations are not possible. With rough empirical assumptions, it can be shown that with decreasing particle size $u_{min}$ increases. In routine work, $u_{min}$ is the lowest velocity of practical interest. It was shown that if the pressure is limited, at $u_{min}$ the maximum column length increases with $d_p^3$ while the time of analysis increases with $d_p^4$. Therefore, the minimum particle size in HPLC is virtually independent of $\Delta p_{max}$ (i.e., 200 or 500 atm), of the viscosity of the eluent and of the diffusion coefficient of the sample. The optimum particle size of the support seems to be about 1 $\mu$m because (1) of the position of the minimum of the $h$ versus $u$ curve, (2) of the limited inlet pressure due to the heat of friction, (3) of the number of plates available at $u_{min}$ ($n_{max} \approx d_p^2$), (4) peak broadening outside increases compared with that inside the column with decreasing particle size, (5) of the problems with the sampling system and the fittings at high pressures, (6) of the difficulties in packing columns with $d_p < 3$ $\mu$m because of the increasing surface energy of the support ("particle bridging"), and (7) $h_{min} \approx d_p$ while the permeability $K \approx d_p^2$ and the pressure drop per unit plate increases with the square of the decreasing particle size. The advantage gained with decreasing particle size is, however, the shorter time of analysis. For orientation purposes only, we can oversimplify the problems by assuming that $\eta = 3.16 \cdot 10^{-3}$ P ($3.16 = \sqrt{10}$) and $D_m = 3.16 \cdot 10^{-5}$ cm$^2$/sec. With these and other approximations made above, the equations below are valid, with the following units: $d_p$, $\mu$m; $u$, mm/sec; $L$, cm; $\Delta p$, atm; $n/t_0$ or $N/t_R$, sec$^{-1}$; $t_0$, sec, if the velocity of the eluent is $u_{min}$ and if the particle size is about or less than 5 $\mu$m:

$$\frac{n}{t_0} = \frac{5700}{d_p^2} \tag{24}$$

$$\left(\frac{N}{t_R}\right)_{k'=2} = \frac{850}{d_p^2} \tag{25}$$

$$u_{min} = \frac{20}{d_p} \tag{26}$$

$$h_{min} = 3.6\, d_p \tag{27}$$

$$n_{max} = 4\, \Delta p\, d_p^2 \tag{28}$$

$$L_{max} = 1.5 \cdot 10^{-3}\, \Delta p\, d_p^3 \tag{29}$$

$$t_0 = 7.5 \cdot 10^{-4}\, \Delta p\, d_p^4 \tag{30}$$

Most of routine analyses can be carried out if 5000 theoretical plates (*i.e.*, with $k' = 2$, about 2200 effective plates) are generated. With such a column, baseline separation ($R = 1.5$) is achieved if $\alpha < 1.15$ or $\alpha$ can be as small as 1.09 if a resolution of only $R = 1$ is required. With these assumptions, the parameters are shown in Table VIII as calculated by eqns. 24–30. As demonstrated in Table VIII, if the particle size is less than 3 $\mu$m, the column length becomes too small (problems with broadening outside the column) and the time of analysis too short (problems with the recorder, etc.). If $d_p > 3\,\mu$m, the separation can be achieved at $u_{opt} = 2\,\mu_{min}$ by doubling the pressure. In our experience $h_{opt}$ (at $u_{opt}$) will be only about 33% higher than $u_{min}$.

TABLE VIII

PARAMETERS FOR $n = 5000$ AT $u_{min}$

$\eta = 0.316$ cP. $D_m = 3.16 \cdot 10^{-5}$ cm$^2$/sec.

| Parameter | $d_p$ ($\mu$m) | | | | |
|---|---|---|---|---|---|
| | 5 | 4 | 3 | 2 | 1 |
| $\Delta p$ (atm) | 50 | 78 | 139 | 312 | 1250 |
| $L_{max}$ (cm) | 9.4 | 7.5 | 5.6 | 3.7 | 1.9 |
| $u_{min}$ (mm/sec) | 4 | 5 | 6.7 | 10 | 20 |
| $h_{min}$ ($\mu$m) | 18 | 14.4 | 10.8 | 7.2 | 3.6 |
| $t_0$ (sec) | 24 | 15 | 8.4 | 3.7 | 0.9 |
| $n/t_0$ (sec$^{-1}$) | 228 | 356 | 633 | 1425 | 5700 |
| $(N/t_R)_{k'=2}$ (sec$^{-1}$) | 34 | 53 | 94 | 213 | 850 |

The aim of achieving a higher speed of analysis also has limits. In our experience, a time of analysis shorter than 5 min is usually of no interest in routine work, because more time is required to prepare for the next analysis and to evaluate the previous one.

Very small particles are required for trace analysis because here the columns are short, the linear velocity is high and, if the sample size is large enough, the peak height will be large.

LIST OF SYMBOLS

| | |
|---|---|
| $c$ (cal/g) | specific heat of the eluent |
| $d_p$ ($\mu$m) | average particle size as defined in eqn. 1 |
| $h$ ($\mu$m) | height equivalent to a theoretical plate |

$k' = \dfrac{t_R - t_0}{t_0}$      capacity ratio

$m$ (g)      mass of the eluent in eqn. 3

$n$      number of theoretical plates

$\Delta p$ ($10^{-6}$ atm $\approx$ dynes/cm$^2$)      pressure drop over the column

$r$ (cm)      radius of the column

$t$ or $t_R$ (sec)      time of analysis

$t_0$      hold-up time of the inert peak

$u$ (mm/sec)      linear velocity of the eluent

$v$ (cm$^3$)      volume of the eluent in eqn. 3

$\bar{w}$ (sec)      average peak width

$A$ ($\mu$m)      "packing" term in eqn. 2

$B$ ($10^5$ cm$^2$/sec)      longitudinal diffusion term in eqn. 2

$C$ (msec)      mass transfer term in eqn. 2

$D_m$ ($10^5$ cm$^2$/sec)      interdiffusion coefficient in the mobile phase

$E$ (°C/atm)      constant in eqn. 4

$F$ (cm$^3$/sec)      flow-rate

$K$ (cm$^2$)      permeability defined with the linear velocity in eqn. 12

$K_F$ (cm$^2$)      permeability defined with the superficial velocity in eqn. 1

$L$ (cm)      column length

$N$      number of effective plates

$Q$ (cal)      adiabatic heat of friction of the eluent

$R = \dfrac{\Delta t_R}{\bar{w}}$      resolution

$T$ (°C)      temperature

$\alpha = \dfrac{k_2'}{k_1'}$      relative retention

$a = \eta\, D_m$      constant in eqn. 11

$\gamma$      constant in eqn. 8

$\eta$ (P)      viscosity of the eluent

$\lambda$      constant in eqn. 7

$\varrho$ (g/cm$^3$)      density of the eluent

$\varepsilon_T$      total porosity defined in eqn. 13

$\omega$      constant in eqn. 9

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