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STUDY OF THE PERTINENCY OF PRESSURE IN LIQUID CHROMATO-GRAPHY

III. A PRACTICAL METHOD FOR CHOOSING THE EXPERIMENTAL CONDITIONS IN LIQUID CHROMATOGRAPHY

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SUMMARY

A simple method is derived for selecting the experimental conditions under which a given analysis should be carried out. This method allows the choice of a compromise between speed of analysis, resolution and pressure drop and rules are given that permit the best possible use of the column packings available to be made.

Further calculations show that a large increase in pressure drop is necessary in order to reduce markedly the analysis time below that achieved when working at maximum column efficiency: a 10-fold increase in pressure reduces the analysis time by a factor of 4 at constant resolution, because the column length should be increased in order to keep the resolution constant.

Finally, it is shown that the present state of the art makes available columns with peak capacities between 200 and 300, thus allowing the analysis of fairly complicated mixtures.

INTRODUCTION

The development of liquid chromatographs during the last 6 years has been towards the use of higher pressures. However, analysts would prefer to carry out separations by liquid chromatography at zero pressure, which of course is impossible, as a force is needed to drive the mobile phase through the column. The pressure used should be as low as possible consistent with obtaining a particular separation in a reasonable time. Working at pressures lower than 20–30 atm is safer, makes possible the use of simpler, cheaper pumping devices such as one-stroke pumps or gas-pressurized reservoirs, and makes the injection easier (at least the syringe half-life becomes much longer).

In a previous paper[1], we showed that the development of fine particles and of slurry packing techniques now makes it possible to achieve fairly difficult analyses in a relatively short time, without having recourse to high-pressure technology. This, of

course, is because columns can be packed very efficiently, so that short columns can be used for most analyses. However, the basic reason of this fact, which seems paradoxical at first, is that when using fine-particle packing, the optimum velocity at which the HETP is at a minimum becomes large enough to allow reasonable transit times throughout efficient columns. Using fine particles, it then becomes possible to carry out analyses at the optimum velocity, and consequently at moderate or low pressures, while working at high velocities, which was still common recently in high-performance liquid chromatography (HPLC), would demand prohibitively high pressures.

In this paper, we show how it is possible to determine the length of the column, the particle size and the pressure drop required to achieve a given separation in a given time. The same data can be used to check the performances that can be obtained with particles of a given size or with given equipment. Finally, we show how little is really lost in terms of speed of analysis when the new optimization procedure (minimum pressure) is adopted in place of the conventional procedure (minimum analysis time).

In order to make it easier for analysts to use our results, we explain first how they can be used and then how they are obtained, and we give some further consequences of these derivations.

It should be emphasized, however, that our main aim is to show how to calculate the column parameters so that the necessary efficiency is achieved while operating the column at the optimum flow-rate. Merely operating any given column at its optimum flow-rate usually results in failure to achieve the analysis, the resolution being either too low or too high.

SIMPLE OPTIMIZATION PROCEDURE

As shown in the following sections, the optimum HETP of a liquid chromatographic (LC) column, H_m, is about 3.2 d_p, where d_p is the average particle diameter, and the optimum velocity, u_m, is about 2.3 d_p/D_m, where D_m is the diffusion coefficient in the mobile phase. These are values typical of the performances obtained for silica particles of different origins, surface area, activity and size; similar performances are achieved with other materials (alumina, polymers, porous layer coated beads, etc.). Sometimes better packing efficiencies with H_m ranging between 2 d_p and 3 d_p and u_m between 2.5 d_p/D_m and 3 d_p/D_m are obtained, but this will not change the results appreciably. Similarly, the variation of H_m and u_m with the capacity ratio (k') or from compound to compound will be neglected, although u_m is proportional to D_m.

If a column is to be used at its optimum velocity and it is necessary to achieve N plates in order to perform a given separation, then the column length, L, should be

$$L = 3.2 \, d_p \, N \tag{1}$$

where L and d_p are measured in centimetres. Fig. 1 shows a plot of the pressure gradient (atm/cm) along a column operated at optimum velocity *versus* the size of the particles used to pack the column. It is shown below that this is a straight line of equation
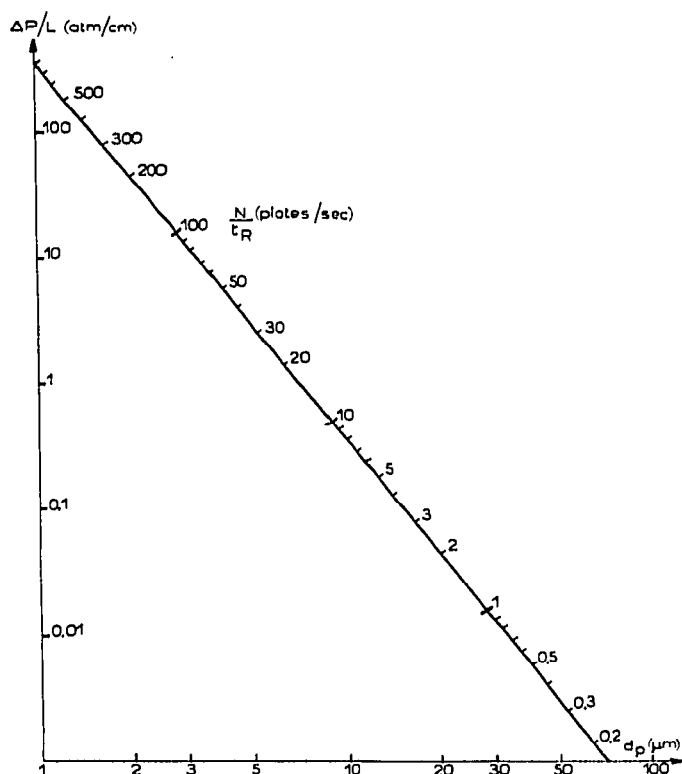
$$\frac{\Delta P}{L} = 350 \, d_p \tag{2}$$

Fig. 1. Variation of the pressure gradient along columns operated at the optimum velocity (maximum efficiency) as a function of the particle size. On the straight line is given the number of plates per unit time. $k' = 2$. Mobile phase viscosity, 0.4 cP. Diffusion coefficient of solute in mobile phase, $3.5 \cdot 10^{-2}$ cm^2/sec. Column specific permeability, $k_0 = 8.46 \cdot 10^{-4}$. Knox coefficients: $\gamma = 0.9$; $A = 1.7$; $C = 0.05$.

where L is measured in centimetres and d_p in micrometres. Combining eqns. 1 and 2 gives the pressure necessary to move the mobile phase at the optimum velocity:

$$\Delta P = 1.12 \cdot 10^{-1} \, N \, d_p^{-2} \tag{3}$$

where ΔP is measured in atmospheres and d_p in micrometres. The graduation on the straight line in Fig. 1 gives the speed of analysis in plates generated per second for a retained compound with $k' = 2$, and we have

$$\frac{N}{t_R} = 800 \, d_p^{-2} \tag{4}$$

where t_R is measured in seconds and d_p in micrometres. The analysis time will then be

$$t_R = 1.25 \cdot 10^{-3} \, N \, d_p^2 \tag{5}$$

This graph can be used in several ways, as described in the following three sections.

Performances achieved with a given packing

Here d_p is known. Suppose we have 7-μm particles, then the pressure gradient is 1 atm/cm and the speed 16 plates per second. The HETP is 22.5 μm (eqn. 1). Therefore, if we need 5000 plates, the column length should be 11.2 cm, the pressure drop is 11.2 atm and the retention time of a compound with $k' = 2$ is 312.5 sec. The retention time of an inert is then 104 sec and that of a compound with $k' = 5$ is 625 sec (*ca.* 10.5 min).

These results are further illustrated by Fig. 2, which shows the variation, with the size of the particles used, of the column length necessary to achieve 1000 plates and of the corresponding pressure and retention time. Fig. 2 is easy to use because, as the compressibility of liquids can be neglected below a few hundred atmospheres, the column length, pressure and retention time are proportional to the efficiency (*cf.*, eqns. 1, 3 and 5), so that in order to achieve 3000 plates, a column three times longer is necessary, with a pressure three times higher, and the analysis time is three times longer.

Fig. 2 confirms that 1000 plates are generated in 1 min ($k' = 2$) with a 2.2-cm long column and a pressure of 2.2 atm. Consequently, 60,000 plates could be generated in 1 h by using a 132-cm long column and a pressure of 132 atm, which shows that high performances can be achieved when using moderately high pressures.
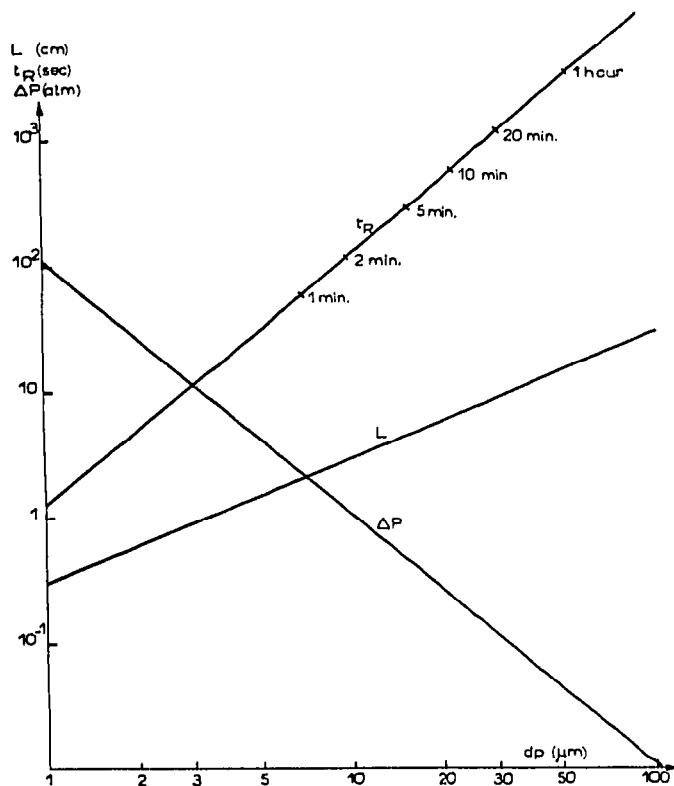

Fig. 2. Variation, with the particle diameter, of the length of the column that gives 1000 plates at the optimum flow velocity, of the retention time of a compound with $k' = 2$, and of the pressure.

Column design to achieve a given analysis

        An analysis is characterized by the analysis time and by the efficiency needed in order to separate the compounds of interest. From the analysis time and the value of k' for these compounds, t_R for a compound with $k' = 2$ is derived [by multiplying the analysis time by $3/(1 + k')$]. Then the value of N/t_R is calculated. From Fig. 1, the corresponding particle size and the pressure gradient are derived, and hence the column length (eqn. 1) and the pressure. For example, in order to separate two compounds with $k' = 2$, 900 plates are generated in 30 min (0.5 plates per second), by using an 11.5-cm long column packed with 40-μm particles and a pressure of 0.064 atm. The velocity is, of course, low and it is not difficult to improve these performances; 7200 plates can be generated in 15 min by using a 23-cm long column packed with 10-μm particles and a pressure of 8.2 atm.

Maximum plate number achievable with given equipment

        This number depends, of course, on the time available. If one can work at 300 atm and require elution of the compound with $k' = 2$ in less than 1 h, the number of plates that can be generated, obtained by multiplying eqns. 3 and 5, is:

$$N = 84.5 \ \sqrt{\Delta P \, t_R} \tag{6}$$

or 90,000 in the case in question. The particle size is 5.7 μm and the column length 160 cm (eqn. 1).

        These examples illustrate how Figs. 1 and 2 as well as eqns. 1–6 can be used in practice in order to derive easily and rapidly the solutions of analytical problems. It should be pointed out that in many instances particles of the correct size will not be available; however, this is not critical and there is little to gain by very careful optimization of all parameters. The use of particles up to 40% larger than the optimum usually results in a pressure of less than 20% greater than the optimum, although the column length varies roughly in proportion to the particle size.

THEORETICAL

Plate height equation

        One of the critical factors to consider is the variation of the column performance with particle size. The work of Snyder[2], Halász and Naefe[3] and Majors[4] showed that at large flow velocities the HETP is given by an empirical equation:

$$H = \lambda \, d_p^{1.8} \, u^{0.4} \tag{7}$$

This equation is valid only in a limited velocity range, in which molecular diffusion and convection jointly accelerate mass transfer in the mobile phase[5]. Although this equation permits some interesting calculations and optimization of some parameters using the time–resolution equation[6], it is better to refer to the Knox equation, which relates the reduced plate height, h, to the reduced velocity, v. Now,

$$h = H/d_p \tag{8a}$$

and

$$v = u d_p / D_m \tag{8b}$$

and the Knox equation is

$$h = \frac{2\gamma}{\nu} + A\nu^{0.33} + C\nu \qquad (9)$$

where γ is the tortuosity of the packing, A and C are coefficients that account for the resistance to mass transfer in the mobile phase flowing through the particle bed (A) and inside the particles (C).
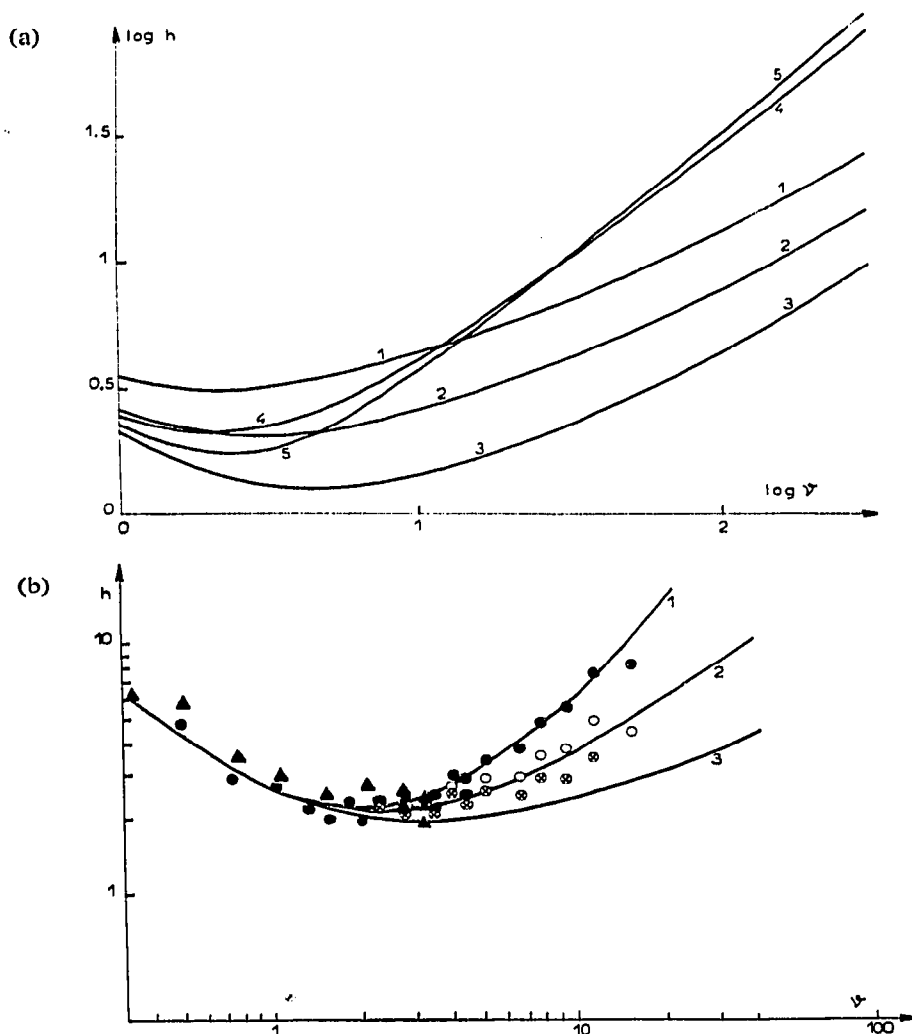

Fig. 3. Variation of the reduced plate height with the reduced velocity. (a) Curves 1–5 correspond to different sets of Knox coefficients (cf., Tables I and II). (b) Experimental points. ●, 6-cm long column, 4 mm I.D., packed with Reeve Angel Partisil 5 ($d_p = ca.\ 5\ \mu$m); solvent, n-heptane; solute, anthracene ($k' = 2.6$). ▲, 50-cm long column, 1 mm I.D., same packing, solvent and solute. ○, Experimental points for the 6-cm long column, corrected for a detector response time of 0.4 sec and an injection time of 0.2 sec; ⊗, same correction except response time = 0.5 sec. Curves: 1, $h = 1.8/\nu + 0.7\nu^{0.33} + 0.2\nu + 0.023\nu^2$; 2, $h = 1.8/\nu + 0.7\nu^{0.33} + 0.2\nu$; 3, $h = 1.8/\nu + 0.85\nu^{0.33} + 0.04\nu$.

Fig. 3a shows plots of log H *versus* log v, covering the range of reduced velocities from 1 to 300 in which are found the conventional conditions of high-pressure liquid chromatography (v = 50–200) and the optimum efficiency range (v = 2–4). Several curves are given that summarize typical performances obtained either in our laboratory or by other workers.

TABLE I

OPTIMUM CONDITIONS OF THE HETP CURVES IN FIG. 3

Curve No.	γ	A	C	h_{opt}	v_{opt}
1	0.9	1.7	0.05	3.14	2.15
2	0.8	1.0	0.03	2.06	2.86
3	0.8	0.5	0.02	1.27	4.42
4	0.8	0.63	0.25	2.09	2.05
5	0.9	0.20	0.30	1.74	2.31

The optimum reduced plate height is generally between 2 and 3, which is also the case in gas chromatography, and the optimum reduced velocity is between 3 and 2, the larger values of v being associated with the smaller values of h.

It follows that the optimum plate height is proportional to the particle size while the optimum velocity is inversely proportional to that size. At larger values of v, the variation of H with d_p is more complicated because C is proportional to d_p^2/D_m, hence the empirical eqn. 7, which fits reasonably well the experimental results between v = 10 and 300.

In the numerical calculations made to derive Figs. 1 and 2, optimum values of 3.2 and 2.1 were selected for h and u, respectively. They are rather conservative at present, as shown by the data in Fig. 3a.

Derivation of equations 1–5

This derivation could be made by using the optimization approach developed previously using the reduced values of the pressure, column length and particle size introduced here[1]. It is more convenient to start from the fact that if the necessary analysis (defined by N and t_R) is achieved with the minimum pressure drop, the corresponding column is also operating at the minimum HETP. By differentiation of eqn. 9, it can be shown that the corresponding flow velocity is

$$v_{opt} = \left(\frac{A}{9C}\right)^{3/2} \left[2 \cos\left[\frac{1}{3}\arccos\left(\frac{729\gamma C^2}{A^3} - 1\right)\right] - 1\right]^{3/2} \tag{10}$$

if

$$729\,\gamma\,C^2 < 2\,A^3 \tag{11}$$

If not, then

$$v_{opt} = \left\{\sqrt[3]{\frac{\gamma}{C}\left(1 - \frac{A^3}{729\gamma C^2}\right)}\left[\sqrt[3]{1 + \sqrt{1 - \frac{1}{\left(1 - \frac{729\gamma C^2}{A^3}\right)^2}}} + \right. \right.$$

$$\sqrt[3]{1 - \sqrt{1 - \frac{1}{\left(1 - \frac{729\gamma C^2}{A^3}\right)^2}}} - \frac{A}{9C}\Bigg\}^{3/2} \tag{12}$$

In both instances, h_{opt} is derived from eqn. 9. Finally, if

$$729 \, \gamma \, C^2 = 2 \, A^3$$

we have

$$v_{opt} = \left(\frac{A}{9C}\right)^{3/2} \text{ and } h_{opt} = \frac{14}{27} \cdot \frac{A^{3/2}}{C^{1/2}} \tag{13}$$

which corresponds to the limits of eqns. 10 and 12. From the classical equations relating the analysis time, t_R, the column length, L, the flow velocity, u, the column capacity factor, k', the plate number, N, and the particle size, d_p, we have

$$t_R = \frac{L}{u} (1 + k') \tag{14}$$

$$L = N \, h \, d_p \tag{15}$$

and combining with eqn. 8, we obtain

$$t_R = \frac{N \, h \, d_p^2 (1 + k')}{v \, D_m} \tag{16}$$

This equation is also valid under the optimum conditions, so that

$$d_{p_{opt}} = \sqrt{\frac{v_{opt}}{h_{opt}} \cdot \frac{D_m}{1 + k'} \cdot \frac{t_R}{N}} \tag{17}$$

and

$$L_{opt} = \sqrt{h_{opt} \, v_{opt} \left(\frac{D_m}{1 + k'}\right) t_R N} \tag{18}$$

As the pressure drop is given by the equation

$$\Delta P = \frac{\eta \, u \, L}{k_0 \, d_p^2} \tag{19}$$

where η is the solvent viscosity, we have similarly

$$\Delta P_{opt} = h_{opt}^2 (1 + k') \frac{\eta}{k_0} \cdot \frac{N^2}{t_R} \tag{20}$$

Eqn. 18 is in agreement with eqn. 4, the numerical values for the constants h_{opt}, v_{opt}, D_m and k' resulting from the choice of the experimental conditions, which in

turn determine the Knox coefficients, and may change from system to system. Combination of eqns. 1, 3 and 4 gives

$$\Delta P = 1.40 \cdot 10^{-4} \cdot \frac{N^2}{t_R} \tag{21}$$

and

$$L = 90.5 \ \sqrt{t_R} \ N \tag{22}$$

These two equations are in agreement with eqns. 18 and 20, which demonstrates the validity of eqns. 1–5 and of Figs. 1 and 2. In each practical case, the correct values derived from the Knox coefficients should be used. Calculations using these equations, especially eqn. 10 or 12, may seem tedious and complex. However, the use of pocket calculators such as the Hewlett-Packard Model HP 35 or 65 makes them easy to carry out.

Numerical results obtained in various cases are given in Table II. It can be seen that when a different set of values of A and C result in very similar values of h_{opt} and v_{opt}, the characteristics of the corresponding optimum column are not very different.

TABLE II

OPTIMUM CONDITIONS FOR A PARTICULAR ANALYSIS

$t_R = 300$ sec; $N = 5000$ plates; $k' = 2$.

Curve No.	ΔP_{opt} (atm)	L_{opt} (cm)	$d_{p_{opt}}$ (μm)	H (μm)	u (cm/sec)
1	11.6	10.9	6.9	21.7	0.109
2	5.04	10.2	9.8	20.3	0.102
3	1.91	9.9	15.6	19.8	0.099
4	5.18	8.7	8.3	17.3	0.087
5	3.56	8.4	9.6	16.8	0.084

Finally, it is worth noting that by combining eqns. 8, 17 and 18 we obtain

$$H_{opt} = \sqrt{h_{opt} \ v_{opt} \cdot \frac{D_m t_R}{(1 + k') \ N}} \tag{23}$$

and

$$u_{opt} = \sqrt{h_{opt} \ v_{opt} \ D_m (1 + k) \frac{N}{t_R}} \tag{24}$$

The more difficult the analytical problem, the smaller is the value of t_R/N and the more efficient the column has to be, and so the smaller the particle size and the HETP and the higher the flow velocity.

Further comparison between performances of columns operated at low and high reduced velocities

It is obvious from Fig. 3 that for any given column the plate height increases more slowly than the reduced velocity. Consequently, the operation of a column longer

than the optimum at a velocity higher than the optimum will always enable a more rapid analysis to be achieved. However, a great disadvantage may arise when achieving this reduction, as shown in Figs. 4 and 5. Fig. 4 shows the variation, with the reduced velocity, of the length of the column packed with 10-μm particles that is it necessary
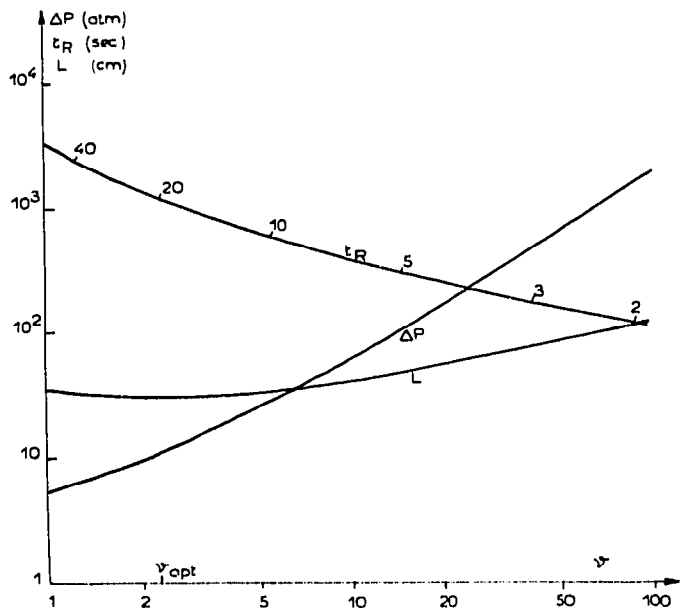

Fig. 4. Variation, with reduced velocity, of the length of a column packed with 10-μm particles and giving 10000 plates, of the pressure, and of the retention time of a compound with $k' = 2$. $h(v)$ is given by curve 1, Fig. 3.
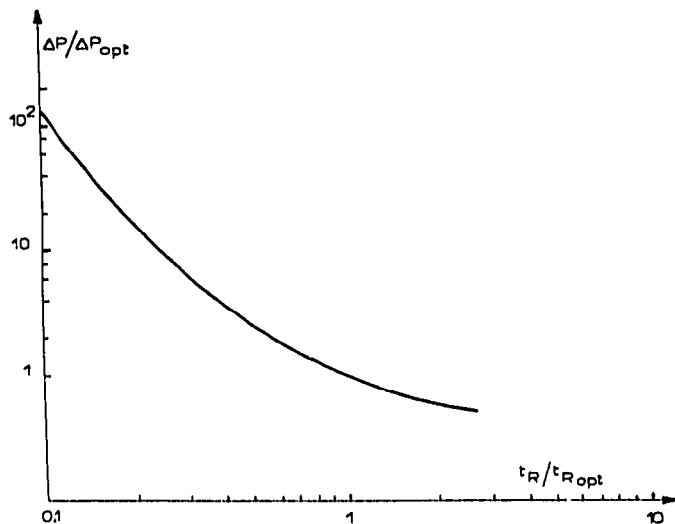

Fig. 5. Variation of the necessary relative increase in pressure *versus* the decrease in analysis time.

to build in order to obtain 10^4 theoretical plates (cf., Fig. 3), of the pressure required in order to operate it at the corresponding velocity and of the retention time of a retained compound ($k' = 2$). It is observed that a 10-fold reduction in the analysis time from the minimum plate height conditions requires the use of a column 3.5 times longer operated at a pressure 150 times higher. Fig. 5 shows for the same type of column ($d_p = 10 \mu$m) the variation of the factor by which the pressure has to be multiplied in order to effect a given reduction in analysis time. This illustrates the fact that a moderate reduction in the analysis time is obtained at the cost of an increase in the pressure that is slightly more than proportional while a significant reduction in the analysis time becomes prohibitively costly.

Table III lists the experimental conditions that are necessary in order to achieve different performances, easy or difficult, at different reduced velocities, ranging from the optimum velocity ($v = 2.1$) to the high velocities (50–150) at which HPLC was conventionally carried out until recently in order to take advantage of the coupling effect[5,7,8]. The column length and the particle size vary considerably with v for any set of performances. Fig. 6 shows the variation, with the reduced velocity, of the pressure for different column performances.

TABLE III

COLUMN LENGTH, PARTICLE DIAMETER AND PRESSURE DROP NECESSARY TO OBTAIN N PLATES IN TIME t_R ($k' = 2$) AT A REDUCED VELOCITY v

| N and t_R (min) required	Parameter	v			
		2.15	8.5	50	150
$N = 10000$,	ΔP (atm)	232.8	368.8	1800	6400
$t_R = 1$	L (cm)	6.6	15.12	53.9	128.15
	d_p (μm)	2.3	4	6.6	8.35
	$\Delta P/L$ (atm/cm)	35.27	24.26	33.4	49.94
$N = 5000$,	ΔP (atm)	58.2	92.2	450	1600
$t_R = 1$	L (cm)	4.8	11.0	39.2	93.2
	d_p (μm)	3.1	5.4	8.9	11.25
	$\Delta P/L$ (atm/cm)	12.02	8.35	11.5	17.16
$N = 5000$,	ΔP (atm)	11.6	18.4	92	320
$t_R = 5$	L (cm)	10.9	24.7	87.7	208
	d_p (μm)	6.9	12	20.5	25.1
	$\Delta P/L$ (atm/cm)	1.06	0.74	1.04	1.5
$N = 5000$,	ΔP (atm)	5.80	9.3	45.9	160
$t_R = 10$	L (cm)	15.4	35	124	295
	d_p (μm)	9.8	17	28	35.6
	$\Delta P/L$ (atm/cm)	0.38	0.26	0.37	0.54
$N = 5000$,	ΔP (atm)	3.9	6.2	30	107
$t_R = 15$	L (cm)	18.80	42.7	152	360.5
	d_p (μm)	12	21	34.5	43.5
	$\Delta P/L$ (atm/cm)	0.2	0.14	0.2	0.3
$N = 2500$,	ΔP (atm)	1.46	2.3	11.5	40.5
$t_R = 10$	L (cm)	10.84	24.65	87.7	208.3
	d_p (μm)	13.8	24.0	40	50.4
	$\Delta P/L$ (atm/cm)	0.13	0.09	0.13	0.194
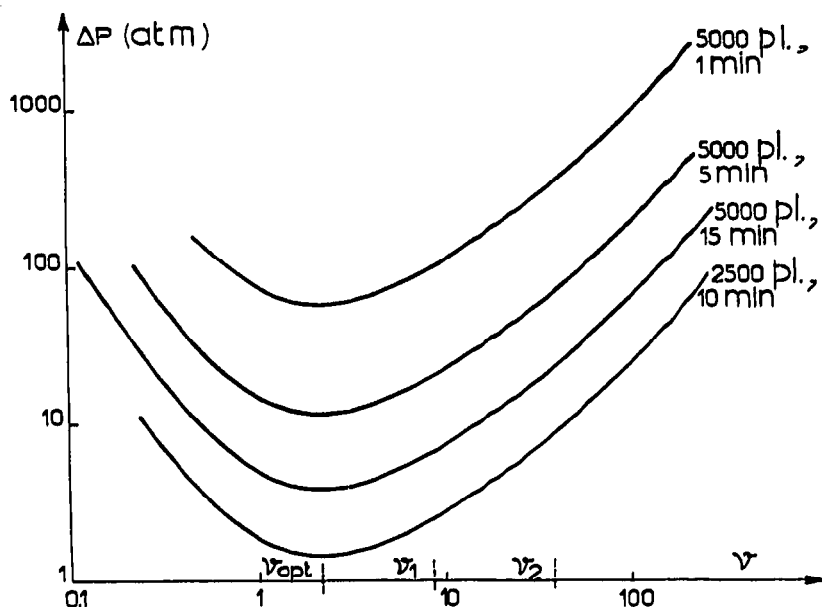
Fig. 6. Variation of the pressure that is necessary to achieve a given set of performances (N, t_R) with the reduced velocity. The column length and particle size change along these curves. v_1, minimum of the pressure gradient; v_2, transition point (*cf.*, ref. 1, eqn. 13).

It is useful for this discussion and for the solution of more involved optimization problems, where various compromises are made between gain in analysis time and loss in operating costs, to have direct relationships between the pressure, ΔP, and the column length, L, or the particle size, d_p. These relationships $\Delta P = f(L, t_R, N)$ and $\Delta P = f(d_p, t_R, N)$ depend on the analysis time and column efficiency, which determine the difficulty of the analytical problems. They are the analytical expressions of the curves published previously (ref. 1, Figs. 1 and 2). Once t_R and N have been selected, together with the chromatographic system, there is only one degree of freedom. In practice, however, it is easier to choose the reduced velocity as a parameter and to derive the three relationships between ΔP, L or d_p and v, which for all purposes are similar.

As we are comparing the operating parameters of different columns that give identical analyses (same t_R, N, k'), and these columns are no longer operated under optimum conditions, let us define relative parameters:

$$\pi = \frac{\Delta P}{\Delta P_{opt}}; \quad \lambda = \frac{L}{L_{opt}}; \quad \delta = \frac{d_p}{d_{p_{opt}}}; \quad \mu = \frac{u}{u_{opt}}; \quad v = \frac{v}{v_{opt}} \tag{25}$$

all parameters being reported to their values for the column that is operating at maximum efficiency (minimum plate height). These parameters are not independent. From the classical equation

$$t_R = \frac{L}{u}(1 + k') \tag{26}$$

it results that, as t_R and k' are constant for all columns, then

$$\lambda = \mu \tag{27}$$

By combining eqns. 8, 19 and 27, we obtain

$$\lambda = \delta \sqrt{\pi} \tag{28}$$

and

$$v = \frac{\lambda^2}{\sqrt{\pi}} \tag{29}$$

From eqn. 28 and the definition of the plate height

$$\sqrt{\pi} = \frac{\lambda}{\delta} = N \cdot \frac{d_{p_{opt}}}{L_{opt}} \cdot h \tag{30}$$

where h is given by eqn. 9 as a function of v.

Now, the choice of any value of v determines v, v_{opt} being given by eqn. 10 or 12, and hence π (eqn. 30) and ΔP (eqn. 25) as $d_{p_{opt}}$, L_{opt} and ΔP_{opt} are given by eqns. 17, 18 and 20, respectively. Knowing π and v, λ (eqn. 29) and L (eqn. 25) can be found. Similarly, eqns. 30 and 25 give d_p.

It is thus possible to construct Figs. 7 and 8, which give λ and π, respectively, as functions of v. Figs. 7 and 8 permit the calculations of the characteristics of col-
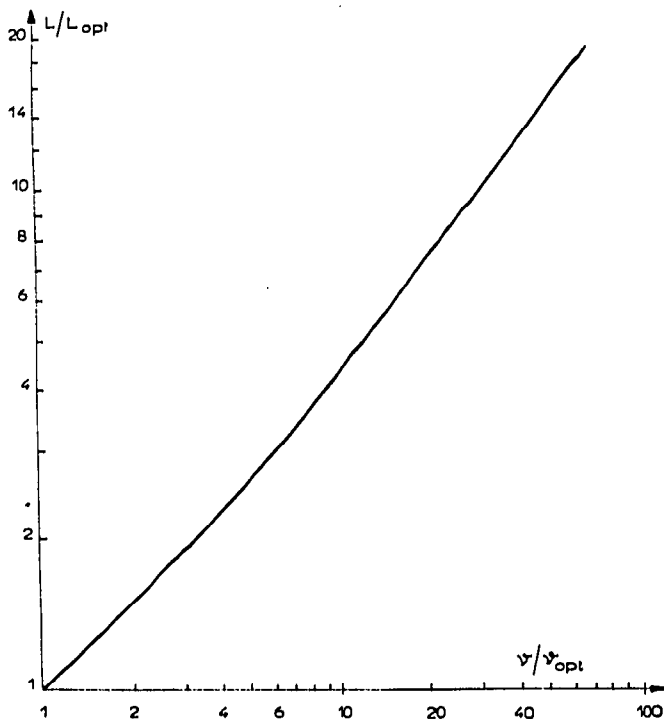

Fig. 7. Relative variation of column length *versus* the relative variation in reduced velocity. L/L ($v = 2.15$) *versus* $v/2.15$.

umns that give any performances at any velocity. They are used to derive the data
in Table III.

These calculations are possible because the relative parameters defined by eqn.
25 are independent of t_R and N. This is so because it has been shown earlier[1] that the
relative parameters such as the relative pressure, $p = \Delta P/\Delta P_0$, are independent of t_R
and N. Although the reference pressure, ΔP_0, in this work is not the optimum pressure,
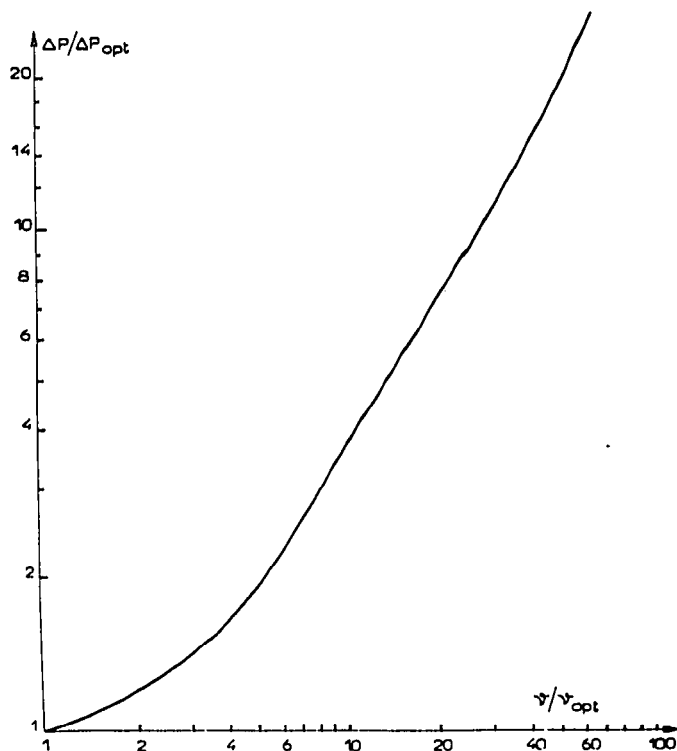ΔP_{opt}, obviously $\pi = p/p_{opt}$ and π is independent of t_R and N also.

Fig. 8. Relative variation in pressure *versus* the relative variation in reduced velocity. $\Delta P/\Delta P$ ($v =$
2.15) *versus* $v/2.15$.

Heat effect

As shown by Endele *et al.*[9], the energy required to pump the mobile phase
through the column is used to work against the frictional forces that occur in the
packing and is transformed into heat inside the column. Provided that the velocity
of the liquid is small, the temperature increase resulting from this heat production
can be neglected, but the double temperature gradient, longitudinal and radial (be-
cause of heat loss to the column wall), may eventually become detrimental to the
column performance.

Assuming that the column is adiabatic, it follows from the results of Sabersky
and Acosta[10] that the temperature change resulting from the work against frictional
forces in a steady flow is

$$\Delta T = - \frac{\Delta P}{C_v} \tag{31}$$

where C_v is the heat capacity of the liquid at constant volume. Under steady-state conditions, the temperature of each particle of packing is constant, while the temperature of the liquid increases while it flows from inlet to outlet. Therefore, the heat capacity of the packing itself has no effect. In practice, values of the heat capacities of liquids at constant volume or pressure are very similar. Table IV lists C_p values for some common mobile phases. The striking conclusion is that for most liquids, except water, the temperature increase is approximately 0.1°/atm; for water it is 0.025°/atm. This explains why *n*-pentane (b.p. 36°) is so difficult to use in HPLC.

TABLE IV

HEAT CAPACITIES OF TYPICAL SOLVENTS USED IN LIQUID CHROMATOGRAPHY[11]

Solvent	C_p (erg/°/cm³)
n-Pentane	$1.055 \cdot 10^7$
n-Heptane	$1.16 \cdot 10^7$
Benzene	$0.921 \cdot 10^7$
Ethanol	$1.25 \cdot 10^7$
Methanol	$1.21 \cdot 10^7$
Water	$4.184 \cdot 10^7$

The heat effect is obviously much more important when the column is operated at large reduced velocities (*cf.*, Fig. 8 and Table III). This is another reason for working with short columns at moderate pressures.

In fact, the column is not adiabatic and there is a heat loss along the column. The heat balance then becomes

$$dT = - \frac{dP}{C_v} - k(T - T_0)\,dt \tag{32}$$

T_0 being the temperature along the column wall and k the coefficient of heat loss. The Darcy law:

$$u = \frac{dx}{dt} = - \frac{k_0 d_p^2}{\eta} \cdot \frac{dP}{dx} \tag{33}$$

can be written as

$$\left(\frac{dx}{dt}\right)^2 dt = u^2\,dt = - \frac{k_0 d_p^2}{\eta} \cdot dP \tag{34}$$

Eqn. 34 becomes

$$\frac{d(T - T_0)}{dP} - k\frac{k_0}{\eta} \cdot \frac{d_p^2}{u^2}(T - T_0) + \frac{1}{C_v} = 0 \tag{35}$$

and we assume that the velocity and the heat capacity are constant along the column. Owing to the boundary conditions ($P = P_i$, $T = T_0$), the solution of this equation is

$$\varDelta T = \frac{-\varDelta P}{C_v\,kt_m}\,[1 - e^{-kt_m}] \qquad (36)$$

where t_m is the retention time of an inert compound ($t_m = L/u$), the minus sign on the right-hand side denoting that the pressure and the temperature gradients inside the column vary in opposite directions. Eqn. 36 reduces to eqn. 29 when k becomes very small. As we can write

$$\varDelta T \approx -\frac{\varDelta P}{C_v}\,(1 - kt_m/2) \qquad (37)$$

it follows that $\varDelta T$ depends on the inlet pressure and the retention time but not on the column length. Consequently, the smallest temperature difference will be observed when the column is operated at the minimum pressure, but this does not mean that such conditions offer the minimum temperature gradient. In fact, it can be shown that the minimum pressure gradient, and hence the minimum temperature gradient, is observed for a reduced velocity of about 8.5.

Eqn. 36 gives the difference between the cross-section averaged temperatures at the column inlet and outlet. It does not indicate the radial distribution of temperature, which might be more effective in adversely affecting column performance, as the viscosity of the liquid varies by 10-20% for a temperature change of 5°.

Eqn. 36. places some very drastic limitations on the use of modern liquid chromatography for measurements in physical chemistry. Pressure gradients no greater than a few atmospheres per 10 cm would have to be used, together with specially designed column walls, in order to promote heat exchange.

Much larger gradients can be accepted in analysis, where the limitations arise only from convenience (the solvent should not boil inside the column or the detector) or from the necessity to achieve reproducible results, and it is not yet clear what the practical pressure limits will be.

Ultimate performances

The results achieved up to now set by no means an ultimate limit to the performances that can be achieved in LC. The numerical values of the Knox parameters corresponding to our experimental data for LC columns (*cf.*, Table I and Fig. 3b) show that the packing method is very good ($A \ll 1$) but the mass transfer term is very large. It should be 5-6 times smaller and we have no good explanation for this discrepancy, although we are of the opinion that the C term derived from the data in Fig. 3b does not arise from resistance to mass transfer in the column but most probably from the contribution of the apparatus to band broadening, related to the introduction of the sample and to its detection. For example, for $v = 20$, the reduced plate height of a well packed column is certainly less than 6.3 (*cf.*, Fig. 3a), which for 5-μm particles corresponds to $u = 1.2$ cm/sec and $H = 38\,\mu$m. As the column is 6 cm long (*cf.*, Fig. 3b), the anthracene peak ($k' = 2.6$, $N = 1580$ plates) is eluted in 18 sec and its width is 1.8 sec. In order to decrease the plate number by less than 1%, the injection time and the detector time constant should be less than 100 and 50

msec, respectively[12], which is far smaller than the experimental values: our detector time constant (τ) is between 0.4 and 0.5 sec and the sampling time is estimated to be about 0.2 sec. The errors in these values are certainly large. Anyway, if we make a correction to the experimental points (cf., Fig. 3b) for $\tau = 0.4$ sec we obtain the lower points. No correction was made for ν less than 2, as it is negligible. These points are certainly more correct as far as peak broadening in the column is concerned, and the contribution of the equipment to plate height, proportional to ν^2, is almost completely accounted for. A correction for $\tau = 0.5$ sec results in some erratic points. It is, however, impossible to derive any accurate information from these data on the value of the mass transfer coefficient, C.

The results in Fig. 3b certainly show that presently available equipment is unable to provide the experimental results that would be needed in order to make use of HETP plots or even to derive satisfactory analytical data at large velocities from the columns that we can prepare. At velocities around the optimum, on the other hand, very good results can be obtained.

A 50-cm long column packed with the same particles gives the results shown in Fig. 3b. Unfortunately, owing to the high flow resistance of this column, it was not possible to make measurements much above the optimum velocity. The trend, however, is in agreement with the results for the shorter column and confirms that injection and detection are critical when short columns are used. It also shows that efficiencies in the $5 \cdot 10^4$ to 10^5 range can be achieved by using the high pressures available and peak capacities well above 100 in the useful range ($k' = 0-10$) are obtained, allowing the analysis of complex mixtures without recycling, as illustrated in Fig. 9, which shows the separation of a steam-cracking effluent cut, a complex mixture of polynuclear fused-ring aromatic hydrocarbons.

As shown by Scott[13], the peak capacity of a column is limited because the sample size is limited by either (a) a chromatographic or (b) a thermodynamic condition. Condition (a) is that the sample size should not result in a significant peak broadening; the earliest peaks are the most sensitive to that source of broadening. Condition (b) is that the column should not be overloaded, i.e., the concentrations should be small enough and the isotherm should remain linear. Although there has been some controversy regarding the choice of the numerical values[14,15], there is no doubt about the validity of these general conclusions. A good estimate of the peak capacity, n, which is much simpler than that given by Scott and about as exact, has been derived by Grushka[16]:

$$n = 1 + \frac{\sqrt{N}}{4} \ln (1 + k'_M) \tag{36}$$

This equation gives the maximum number of peaks, with a resolution equal to 1, between $k' = 0$ and a maximum value of $k' = k_M$.

The development of very efficient columns enables larger peak capacities to be obtained, apparently on two counts. Firstly, the plate number can be made much larger, roughly by a factor 50 over Scott's most optimistic estimate, which provides for an approximately seven times higher peak capacity. Secondly, the dilution is slower, which means that the maximum value of k' at which a peak can be detected will also be larger. Unfortunately the sample size that can be injected decreases in
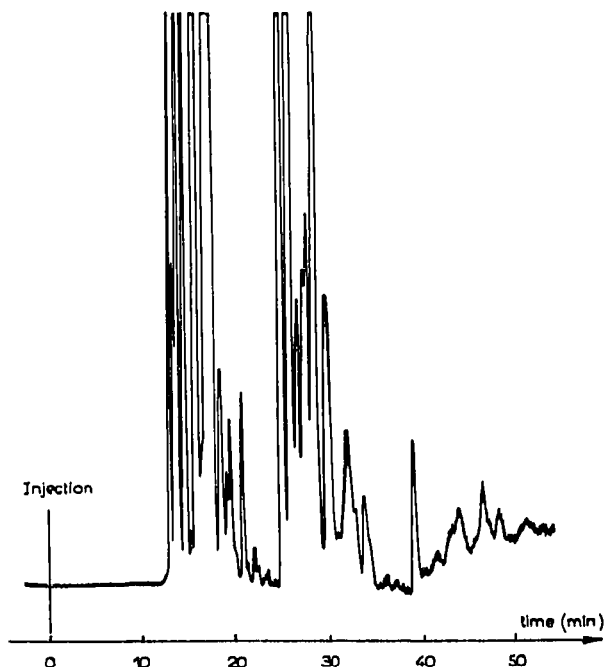
Fig. 9. Separation of a mixture of polynuclear aromatic hydrocarbons. Column 50 cm long, 10 mm I.D., packed with Partisil 5 (Reeve Angel), particle diameter *ca.* 5 μm. Mobile phase, *n*-heptane ; pressure, 340 atm; flow-rate, v = 3.2. Sample: 150–350° boiling range, steam-cracking condensate.

the same proportion, which nullifies the last advantage[17]. In agreement with the qualitative prediction of Scott (eqn. 8, ref. 11), the maximum k' is independent of the column efficiency, provided that the sample size is such that the efficiency of the inert peak is 10% smaller than for a very small sample size.

The improvement in peak capacity by a factor of seven may seem small, as indeed it is. Nevertheless, it means that experimental conditions in which peak capacities between 150 and 300 could be achieved are now possible, and these are conservative estimates[14,15]. The corresponding values of k' are approximately 5 and 45, and so the analysis times will be 50 min and 7 h, respectively (u = 0.2 cm/sec, L = 100 cm). As the increase in peak capacity is very slow for $k' > 10$, it is probably better to speed up the analysis of complex mixtures of widely different compounds using gradient elution[14], which is a completely different problem.

EXPERIMENTAL

All of the experiments were carried out by using home-made equipment. The basic components are an Orlita MS 4 or DMP 1515 pump (Orlita, Giessen, G.F.R.), a pressure controller[18] and an injection port[17], designed in such a way that the syringe needle tip can touch the upper layer of stationary phase or even penetrate it, a column and a UV detector (LKB, Stockholm, Sweden). The detector cell was replaced with a flow-through cell of about 6 μl capacity just at the top of the column outlet.

Packings were made by using the balanced gravity slurry method. Silica particles are equilibrated in benzene–dibromoethane solution so that there is no separation visible after centrifugation for 30 sec at 2000 g. The exact composition of the solution depends on the surface area, activity and origin of the silica. The slurry is then forced through the column under a pressure of 400 atm with an Orlita S 600 pump. The tube is previously cleaned by drilling using a twist-drill about 0.1 mm larger than the internal diameter of the tube[19]. A lathe should be used and the drill introduced into the tube carefully and slowly.

The only difference observed so far between irregular packing materials such as Partisil 5 (Reeve Angel, Ferrières, France) and spherical particles such as Spherosil (Rhone Progil, Antony, France) is the permeability, which, for the same average particle size, is about twice as small for irregular particles. The efficiencies are about the same. The figures given above relate to Spherosil.

CONCLUSION

There is considerable room for differences of opinion and creative expression in the design of a chromatographic system to perform a given analysis, and different workers will always weigh differently the required characteristics and find different compromises; some will place the emphasis on short analysis times, others on high sensitivity, and others on low pressure or practicability or ease in building the chromatograph from scratch. There is therefore rarely a single solution to an analytical problem, all others being inferior.

Keeping this in mind, we have shown how the analyst can select a practical system to fulfil his needs, in order to achieve a given separation in a certain time. This, of course, is only part of the problem, but it should be emphasized that the necessary selectivity can be achieved in most instances by changing the composition of the liquid phase or the degree of hydration of the adsorbent surface, so that one silica gel column, one alumina column and one column for reversed phase operation would be enough to solve probably over 95 % of the analytical problems, which is a situation very different from that encountered in gas chromatography. Then there would be few objections to manufacturing the column together with the detector cell and sampling system in one factory-assembled unit, thus providing a much easier solution to many technological and design problems.

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