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USE OF OPEN-TUBULAR COLUMNS IN LIQUID CHROMATOGRAPHY

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SUMMARY

Porous layer open-tubular columns, 25 and 105 m × 39 μm I.D., have been prepared by etching the inner wall with a solution of sodium hydroxide. Specially designed equipment with split flow, split injection and a microcell UV detector permits the use of these columns (*n*-heptane mobile phase) with moderate extra-column band broadening: the standard deviation of the equipment contribution to bandwidth is 0.10 μl. The loss of efficiency is about 15% for a compound with capacity factor, $k' = 2$ on the 25-m column, but 70% for a non-retained compound on the 105-m column. Nevertheless, efficiencies exceeding one million plates for benzene ($k' = 0$) and 0.4 million plates for anisole ($k' = 2$) have been achieved. The performance per unit length of these capillary columns for anisole is close to that of conventional columns packed with 20-μm particles.

INTRODUCTION

The potential advantages and drawbacks of using open-tubular columns (OTCs) in liquid chromatography have been discussed[1–4]. It is generally agreed that a long, very efficient, narrow-bore OTC should be developed to permit the very difficult separations encountered in some fields of the biological sciences.

OTCs of excellent quality are about 3–4 times faster than the best conventional packed columns (PCs) with particles having an average size, d_p, equal to the diameter, d_c, of the OTC and offering the same efficiency. This is due, in part, to the possibility of using shorter columns to achieve this efficiency, and to the higher velocities which can be used. Because of their much higher permeability, OTCs exhibit a much smaller pressure drop than PCs. Therefore, longer columns with $d_c < d_p$ can be operated, with higher separation powers than PC.

On the other hand, since the loading capacity of a liquid chromatographic column is proportional to the average cross-section of the stationary phase, it is

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several orders of magnitude smaller for OTCs than for PCs; the OTC columns are handicapped by the fact that the same factor controls the efficiency, the pressure drop and the loading capacity, whereas with PC, particle size controls the first two performance parameters, and column diameter independently determines the sample size which can be used. Furthermore, the use of OTCs requires the development of suitable instrumentation.

We report here preliminary results obtained with long, rather wide-bore columns.

THEORETICAL

The classical equations relating column efficiency, analysis time and pressure drop to the column length, particle size and the chromatographic parameters are summarized in Table I, and have been discussed previously[2,3].

TABLE I

CHROMATOGRAPHIC PARAMETERS IMPORTANT IN COLUMN PERFORMANCE EVALUATION

D_m = Solute diffusion coefficient in the mobile phase; d_p = average particle size for PC; H = height equivalent to a theoretical plate; h = reduced plate height; k_o = specific permeability; k' = column capacity factor; L = column length; N = number of theoretical plates; ΔP = pressure drop between column inlet and outlet; t_R = solute retention time; u = mobile phase velocity; v = reduced velocity; η = viscosity of the mobile phase.

Primary equations		*Reduced variable equations*	
$L = NH$	(1)	$h = H/d_p$ (4) $\quad v = ud_p/D_m$	(5)
$u = \frac{k_o d_p^2}{\eta} \cdot \frac{\Delta P}{L}$	(2)	$L = Nh\, d_p$	(6)
$t_R = \frac{L}{u} \cdot (1 + k')$	(3)	$t_R = N \cdot \frac{(1 + k')}{D_m} \cdot \frac{h}{v} \cdot d_p^2$	(7)
		$\Delta P = \frac{N}{d_p^2} \cdot \frac{\eta D_m}{k_o} \cdot vh$	(8)

The efficiency of an OTC is given by the Golay equation

$$h = \frac{2}{v} + \frac{1 + 6k' + k'^2}{96\,(1 + k')^2} \cdot v + \frac{2k'}{3\,(1 + k')^2} \cdot \frac{D_m}{D_s} \cdot \frac{d_f^2}{d_c^2} \cdot v \qquad (9a)$$

$$h = \frac{2}{v} + C_m v + C_s v \qquad (9b)$$

where D_s is the solute diffusion coefficient in the stationary phase, d_f is the Golay stationary film thickness and d_c is the OTC diameter. The first term in eqn. 9 accounts for band broadening by axial diffusion, the second for resistance to mass transfer in the mobile phase (due to the Poiseuille radial velocity profile) and the last one for resistance to mass transfer in the stationary phase. It has been shown that, in practice, the last term can be made small enough to be negligible compared to the second term

by using a sufficiently thin layer of stationary phase along the wall[1]. This is also in agreement with the immense amount of data now available on OTCs in gas chromatography, where the third term of eqn. 9a is almost always less than 10–20% of the second one. Compared to a PC column packed with the same material, *i.e.*, silica of similar specific surface area, pore size distribution and surface energy, the OTC will exhibit slightly weaker retention because of a lower phase ratio, *i.e.*, lower values of k', an effect which can easily be corrected for by using a slightly weaker solvent.

Accordingly, in the following, only the first two terms in eqn. 9a will be considered. The dependence of the second term on the column capacity factor, k', is very strong. Thus, the minimum reduced plate height increases from 0.29 to 0.96 when k' increases from 0 to infinity. In the following discussion, we consider as typical a compound with $k' = 3$, for which $C_m = 0.08$ and the minimum reduced plate height is 0.80 at a reduced velocity of 5. For larger reduced velocities, h can be calculated through the relationship:

$$h = 2/v + 0.08v \tag{10}$$

Eqns. 1–10 can be used to solve optimization problems. For example, we can calculate the column diameter and length permitting the achievement of the maximum plate number in a given time and with a given (maximum) pressure drop. Elimination of d_p between eqns. 7 and 8 gives

$$N = \left[\frac{k_o t_R \Delta P}{h^2 \eta (1 + k')}\right]^{1/2} \tag{11}$$

while elimination of N gives

$$d_p = \left[\frac{v^2 \eta D_m^2}{k_o (1 + k')} \cdot \frac{t_R}{\Delta P}\right]^{1/4} \tag{12}$$

and the column length is calculated using eqn. 6. The largest plate number is achieved when the plate height is minimal. A few sets of numerical values are given in Table II, which show that efficiencies well in excess of one million plates can be achieved under a variety of quite reasonable conditions. Values in excess of 10 million plates are possible but require very high pressures and long analysis times. It is not possible to achieve values of k_o below 1/32 and values of h below 0.8 for $k' = 3$. The solvent viscosity cannot be adjusted easily. A value of 1 cP can be considered as average. Some marginal gain however is possible by working at high temperature.

While the maximum plate number under the conditions of constant pressure and analysis time does not depend on the optimum velocity and the diffusion coefficient, the column diameter and length increase with the square root of these two parameters.

It is important to note that the flow velocity should be adjusted in such a way that the reduced velocity is between 5 and 30. At values lower than 5, the column is almost surely operated below the optimum velocity and we lose both efficiency and

TABLE II

OPTIMUM PERFORMANCE OF LIQUID CHROMATOGRAPHIC COLUMNS AT CONSTANT ANALYSIS TIME AND INLET PRESSURE

$D_m = 5 \cdot 10^{-6}$ cm^2/sec; η = 1 cP; $k' = 3$.

Column type	t_R *(sec)*	ΔP *(atm)*	N	d_p *(μm)*	L *(cm)*
OTC*	$1 \cdot 10^4$	400	$2.2 \cdot 10^6$	1.9	332
	$2 \cdot 10^3$	400	$0.99 \cdot 10^6$	1.25	99
	$1 \cdot 10^5$	$1 \cdot 10^3$	$11 \cdot 10^6$	2.65	2350
PC**	$1 \cdot 10^4$	400	$0.16 \cdot 10^6$	2.65	84
	$1 \cdot 10^5$	$1 \cdot 10^3$	$0.79 \cdot 10^6$	4.9	770

* $h = 0.8$; $v = 5.0$; $k_o = 1/32$.
** $h = 2.0$; $v = 3.0$; $k_o = 1 \cdot 10^{-3}$.

time. At reduced velocities higher than 30, both the efficiency and the analysis time are proportional to the mobile phase velocity and it would be more efficient to reduce the column length and decrease the flow-rate: a better resolution would be obtained at the same time. For a given value of the actual flow velocity, however, the reduced velocity is proportional to the particle diameter and inversely proportional to the diffusion coefficient (eqn. 5). This means that v is not the same for all components of the sample; however, it probably does not vary much, and should be optimized for the most important of them. This also means that narrower columns should be operated at higher velocities, and explains why the analysis time for a given plate number is proportional to d_p^2. These problems are illustrated in Fig. 1 which shows a plot of plate number *versus* column capacity factor for four columns with 10, 20, 40 and 60 μm I.D., all operated at the same actual flow velocity (1 cm/sec), the corresponding reduced velocities being 42, 83, 167 and 250 respectively. Whereas the plate number should be proportional to d_p (eqn. 8), as a consequence, N is divided by 35.5 and 4, for $k' = 3$, when the diameter is increased from 10 μm to 60 and 20 μm respectively, in proportion to d_p^2. On the other hand, for columns of the same length, the analysis time is unchanged. This trade-off should not be overlooked.

These performances are calculated from equations which have been proven to be valid in gas chromatography[5] and can certainly be extrapolated to liquid chromatography[6] under the assumption that suitable equipment can be designed and built. The Golay equation 9a is valid for a straight tube with a circular cross-section. Although coiling the column with a radius which is large compared to the column radius does create some degree of secondary circulation[6,7], it does not affect the validity of eqn. 9a in the reduced velocity range 5–30, in which OTCs will be operated for optimum analytical performance.

More serious are the requirements that sampling and detection do not contribute markedly to band broadening. This sets maximum limits on the sample volume which can be injected into the column, on the detector cell volume and detector time constant. As OTCs are not competitive with PC for fast analysis[1–3], the last condition is easily met.

The equipment contribution to band broadening can be expressed as the sum of the variance contributions from all the different sources. The variance, σ_a^2, of the

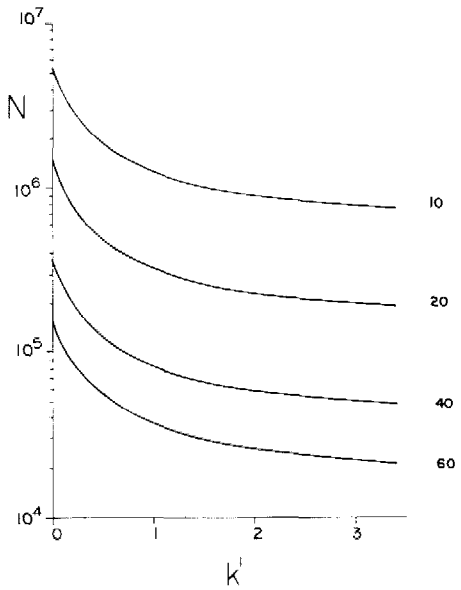

Fig. 1. Plot of theoretical column efficiency *versus* column capacity factor for open-tubular columns of different diameters. Column length 25 m, flow velocity 1 cm/sec. Diffusion coefficient $2.4 \cdot 10^{-5}$ cm^2/sec. The number on each curve gives the column diameter in μm.

chromatographic band derived from the recorded chromatogram or apparent band variance is

$$\sigma_a^2 = \sigma_c^2 + \sigma_e^2 \tag{13}$$

where σ_c^2 is the variance contributed by the column itself and σ_e^2 is the equipment contribution. From the definition of plate number, the apparent plate number, N_a, derived from the chromatogram is related to the plate number of the column, N_c, by

$$\frac{N_a}{N_c} = \frac{\sigma_c^2}{(\sigma_c^2 + \sigma_e^2)} = \frac{1}{(1 + \frac{\sigma_e^2}{\sigma_c^2})} \tag{14}$$

from which we can derive specifications for the equipment as a function of the fractional loss of efficiency we may accept. It is assumed here that the solute capacity factor is not seriously affected by the equipment extra-column contribution to the retention volume. Alternatively, comparison between the actual plate number and the plate number as derived from the Golay equation 9a gives an approximation of σ_e^2.

EXPERIMENTAL

The columns were made from soft glass tubes purchased from Alltech (Deerfield, IL, U.S.A.). The capillary tubes (I.D. 40 μm, O.D. 300 μm) were first washed

successively with methanol, acetone and methylene chloride by flowing approximately five column volumes of each solvent. The column was then dried under a stream of warm nitrogen, filled with a 1 *M* sodium hydroxide solution in water, sealed and kept for 48 h at 50°C. Afterwards, it was washed with distilled water and methanol and finally dried. A thin layer of silica was etched in the wall and served as the stationary phase. The retention of benzene with *n*-heptane saturated with water as solvent was negligible, as shown below, so this compound was used as an inert reference.

Fig. 2 shows a block diagram of the equipment. The chromatograph was used with both 1 m × 1 mm I.D. packed columns (void volume 0.79 ml) and 25 and 105 m × 40 μm I.D. OTC, columns (liquid hold-up 29.9 and 119.5 μl, respectively). A split flow with a suitable hydraulic resistance permits the use of a conventional Waters M6000A pump (Waters Assoc., Milford, MA, U.S.A.) operating in the constant pressure mode. A reproducible flow-rate of 0.1 μl/min could be obtained which, with

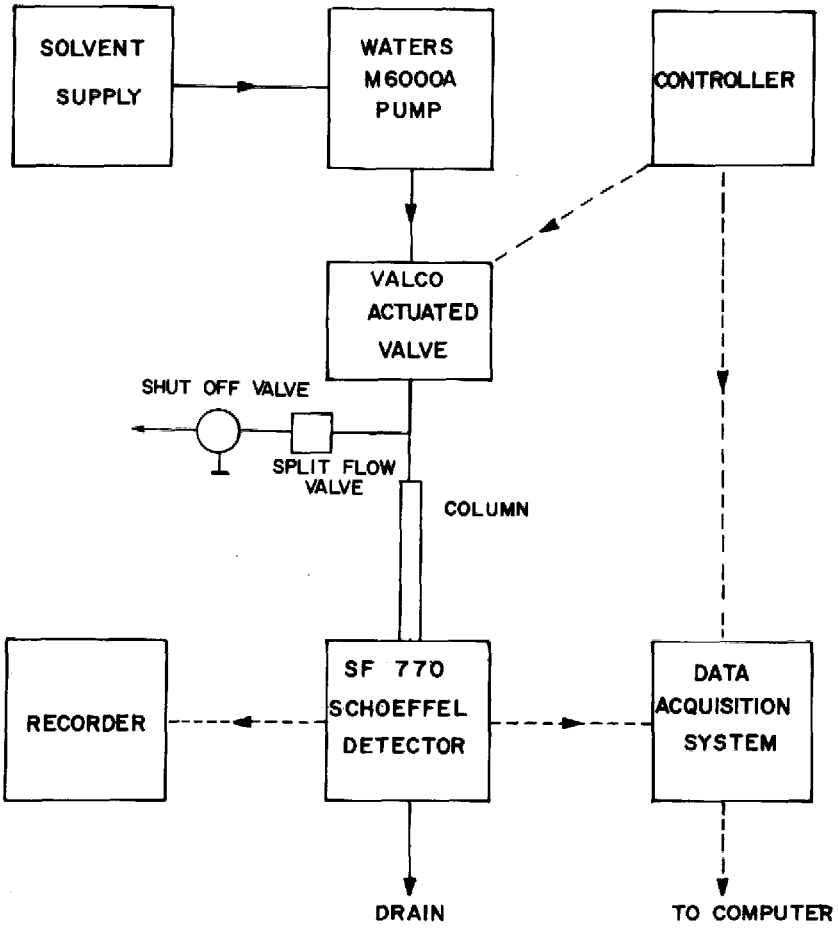

Fig. 2. Block diagram of the equipment.

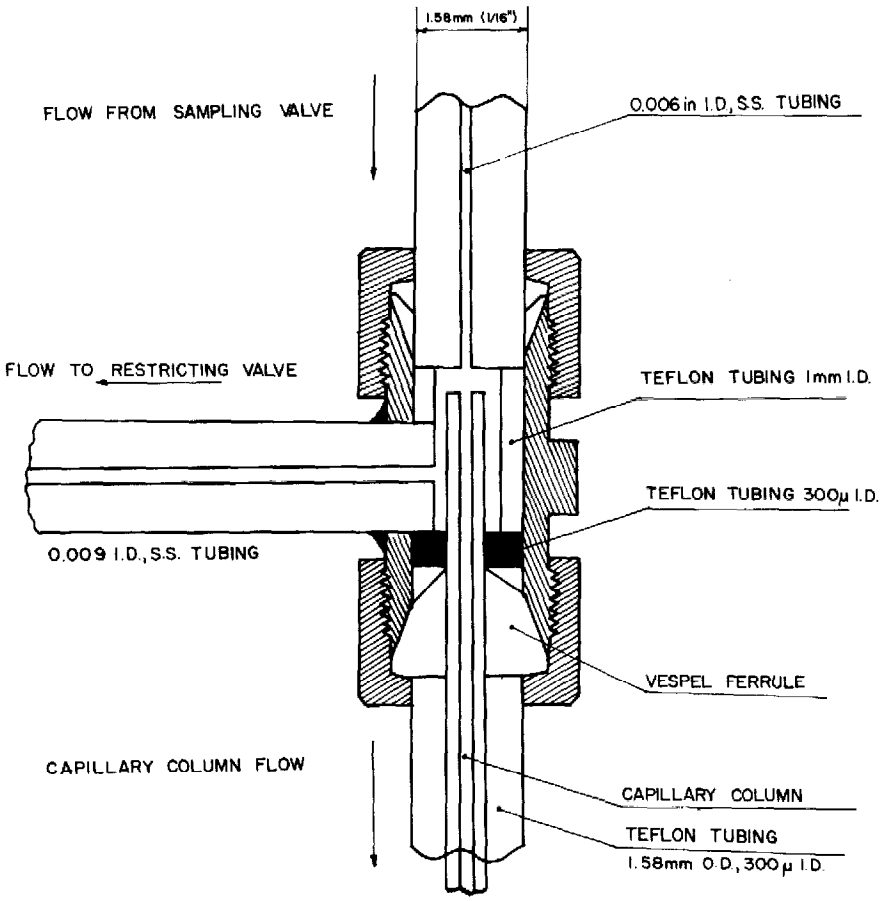

Fig. 3. Detail of the split injection system. S.S. = Stainless steel.

a 40 μm I.D. OTC and compounds with $D_m = 2.4 \cdot 10^{-5}$ cm^2/sec, corresponds to $\nu = 22$. Split injection is possible with OTC, as in gas chromatography. Fig. 3 shows the design of the split system. This experimental arrangement gave the best results. Direct injection is also possible and was used with the PC and sometimes with OTCs. A Valco sampling valve was modified to permit on-line injection onto the capillary column (Fig. 4). However, we found that it was difficult to reduce the valve contribution to band broadening to a small enough value even with the 100-m columns. With the split injector, samples of 2–100 nl could be introduced reproducibly.

The micro cell of a commercial UV detector (SF 770; Kratos Analytical Instruments, Ramsey, NY, U.S.A.) was modified to reduce its volume without changing the optical path length and the cell design by inserting a small, thin PTFE disk, 1 mm O.D., 0.25 mm I.D. (Fig. 5). The diameter was reduced from 0.8 to 0.25 mm, and the cell volume by one order of magnitude (Table III). The cell volume was estimated from geometrical calculations. Unfortunately, the noise increases and the detection limits increase also by one order of magnitude, probably because of the reduction in light flux across the cell.

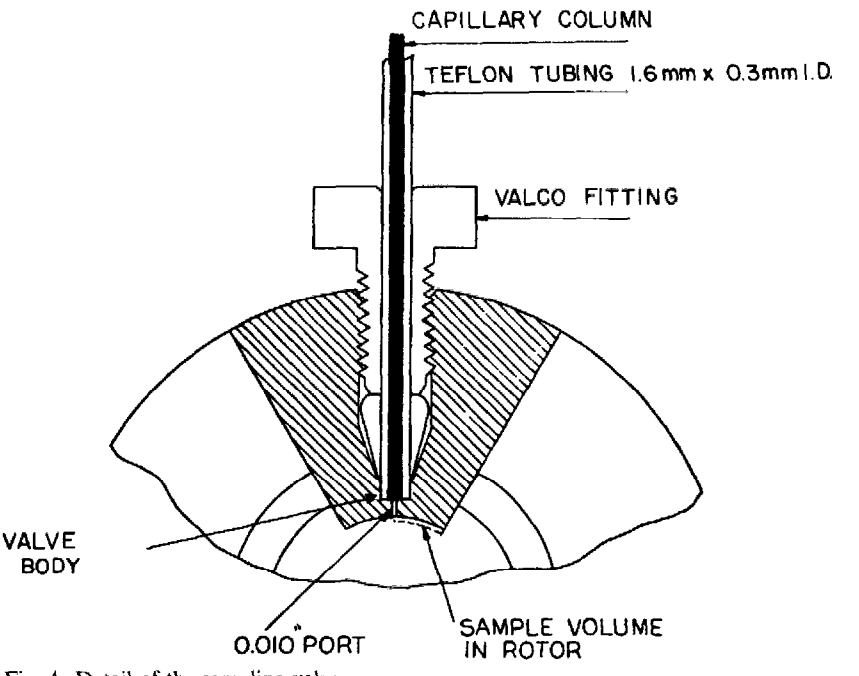

Fig. 4. Detail of the sampling valve.

This illustrates very clearly the difficulties encountered in the miniaturization of liquid chromatographic equipment. A cell volume of 50 μl is remarkable but it cannot be used with OTCs, being too large by more than one order of magnitude[1–3]. On the other hand, a detection limit of 6 ppm of benzene in *n*-heptane is not very satisfactory for a UV detector as shown in Table III. This is not sufficient for the analysis of complex mixtures because, the more complex the mixture, the lower is the concentration of minor components in which we are interested.

RESULTS AND DISCUSSION

Typical chromatograms obtained with OTCs of rather large inner diameters (*ca.* 40 μm) show good efficiency and significant retention for polar compounds in normal phase chromatography (Fig. 6). The baseline stability is very good and the column capacity factors for benzene and anisole are 0 and 2.1, respectively. Polar compounds, such as aniline derivatives, can also be eluted with good peak symmetry (Fig. 7). As shown later, the resistance to mass transfer in the stationary phase is small enough to be negligible for OTCs of 40 μm I.D.

A plot of the height equivalent to a theoretical plate (HETP) of the unretained solute benzene *versus* flow velocity is linear between 0.3 and 2.3 cm/sec (Fig. 8), as predicted by eqn. 9 since the diffusion coefficient of benzene in *n*-heptane is approximately $2.4 \cdot 10^{-5}$ cm^2/sec (Table IV). This velocity range corresponds to a reduced velocity range of 50–375, where the first term on the right hand side of eqn. 9a is certainly negligible.

An efficiency slightly in excess of 10^6 theoretical plates was obtained for the

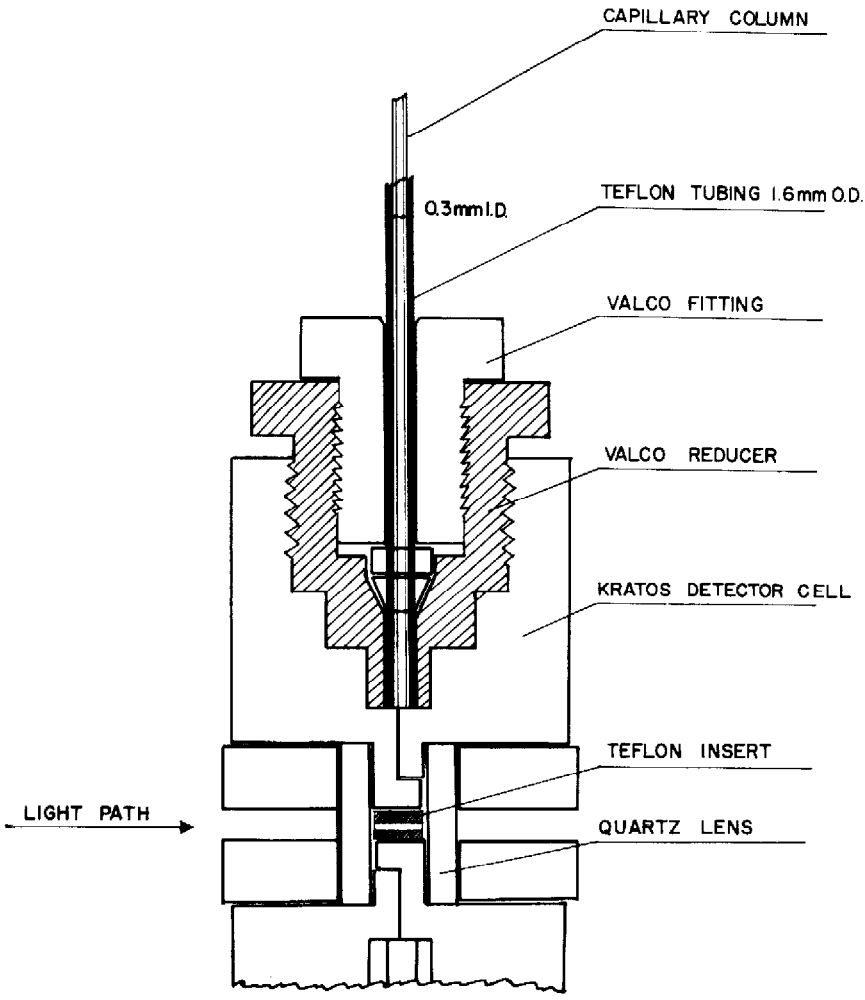

Fig. 5. Detail of the UV detector cell for use with open-tubular columns.

non-retained peak at a reduced velocity of 120 on a 105 m × 39 μm I.D. column ($H = 10.4 \cdot 10^{-3}$ cm). In this case, eqn. 9a reduces to

$$h = \frac{2}{v} + \frac{v}{96} \tag{15}$$

and we should have obtained $h = 1.25$, $H = 4.9 \cdot 10^{-3}$ cm and $2.15 \cdot 10^{6}$ plates. The difference corresponds to the efficiency lost because of imperfect equipment. Similarly, at a velocity of 2.35 cm/sec ($v = 380$) we obtain $H = 0.023$ cm instead of a theoretical value of 0.016 cm ($h = 4$). The straight line in Fig. 8 obeys the equation

$$H = (6.67 + 6.72\,u) \times 10^{-3} \tag{16a}$$

TABLE III

EVALUATION OF UV/VIS DETECTORS USED WITH MICROCOLUMNS

Model	*Cell pathlength (mm)*	*Cell diameter (mm)*	*Cell volume (μl)*	*Noise (μV)*	*Time constant (msec)*	*Detection limit (g/ml)*
SF 770 (Regular cell)	10	1.0	7.85	170	100	$2.23 \cdot 10^{-7}$
SF 770 (Microcell)	1	0.8	0.5	160	100	$6.75 \cdot 10^{-7}$
SF 770 (Modified)	1	0.25	0.05	338	100	$6.2 \cdot 10^{-6}$
SF 773 (Regular cell)	8	1.5	12.0	18	100	$3.2 \cdot 10^{-8}$
SF 773 (Microcell)	3	0.46	0.5	18	100	$1.0 \cdot 10^{-7}$

instead of:

$$H(k' = 0) = 6.60 \cdot 10^{-3}\, u \qquad (16b)$$

We can certainly attribute the 2% difference in the velocity coefficient to experimental error. The constant term arises from the equipment contribution. The volume variance of this contribution is $H_e L S^2$ where H_e is the equipment contribution, (*i.e.*, $6.67 \cdot 10^{-3}$ cm), L the column length (105 m) and S the column cross-sectional area. Here, the variance of the equipment contribution is $1 \cdot 10^4$ $(\text{nl})^2$, *i.e.*, a standard deviation of 100 nl, about twice the estimate of the UV cell detector volume, which is reasonable

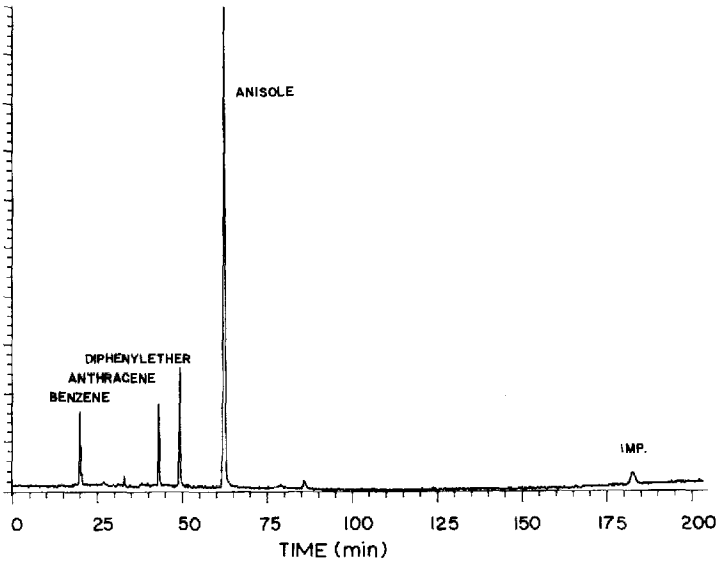

Fig. 6. Separation of a mixture of standard compounds on a capillary column. Column: 25 m × 39 μm I.D. Flow-rate: 1.5 μl/min (u = 2.1 cm/sec, v = 450). Benzene is unretained. Solvent: *n*-heptane. IMP. is impurity.

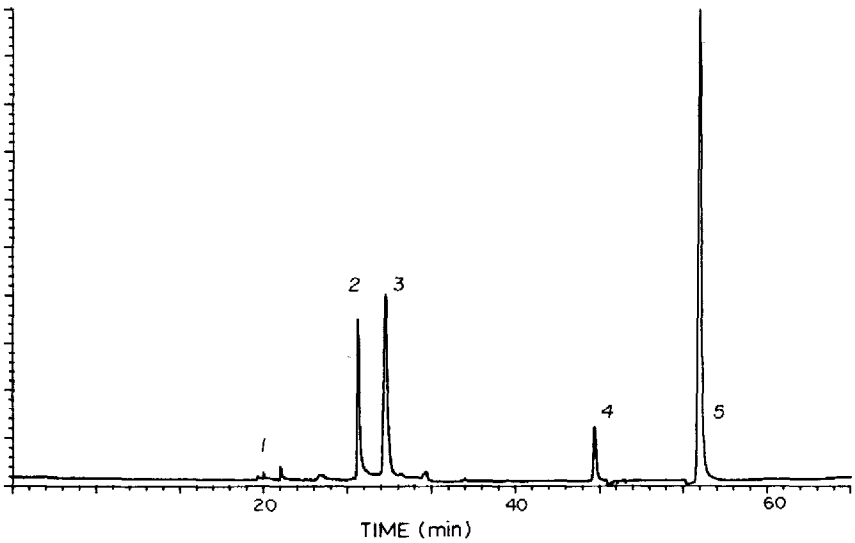

Fig. 7. Analysis of polar compounds on an open-tubular column. Conditions as in Fig. 6. Peaks: 1 = unretained solvent; 2 = N-ethylaniline; 3 = *m*-chloroaniline; 4 = 2,3-dimethylaniline; 5 = 2,3-dimethyl-5-nitroaniline.

since this is the only equipment contribution which remains significant with split injection, but its estimate from geometrical considerations is approximate and neglects the contribution of the connecting tubes. A similar estimate (σ_v = 97 nl) for the standard deviation of the equipment contribution to band broadening is derived from the chromatogram (Fig. 9) showing the benzene peak obtained with a 100-m column at a velocity of 0.38 cm/sec. The efficiency obtained is 3.5 times smaller than the theoretical value, because of this equipment contribution. The effect on a retained peak is much smaller since such a peak is both broader (because of retention) and

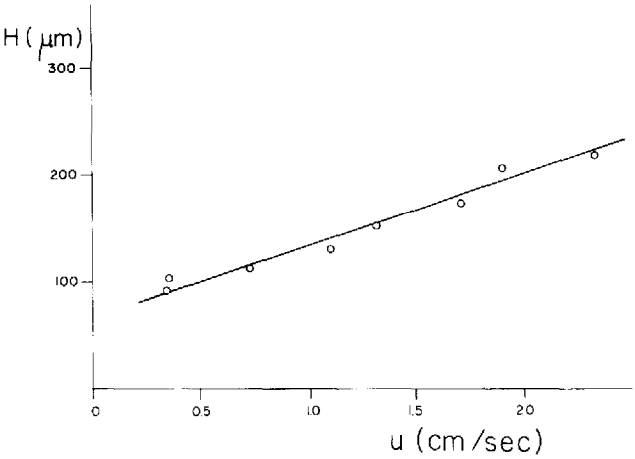

Fig. 8. Plot of the height equivalent to a theoretical plate of an OTC *versus* flow velocity. Column: 105 m × 39 μm I.D. Unretained benzene in *n*-heptane. Column volume: V_0 = 125 μl. The equation of the straight line is given in the text (eqn. 16a).

TABLE IV

DIFFUSION COEFFICIENT OF BENZENE IN *n*-HEPTANE

Wilke-Chang equation[8] (error ± 50%)	$3.39 \cdot 10^{-5}$ cm²/sec
Handbook[9] (error ± 1%)	$2.40 \cdot 10^{-5}$ cm²/sec
This work, determined from H/u slope	
column 25 m long	$2.35 \cdot 10^{-5}$ cm²/sec
column 105 m long	$2.29 \cdot 10^{-5}$ cm²/sec

less efficient (because of the dependence of h on k', *cf.*, eqn. 9). This is also in agreement with the value derived from eqn. 14.

The slope of the straight line in Fig. 8 (eqn. 16b) permits the calculation of the diffusion coefficient of benzene in *n*-heptane, which appears to be in excellent agreement with other experimental values found in the literature (Table IV) and, as expected, only in fair agreement with the value derived from the Wilke and Chang equation[8].

Further data on column efficiency are given in Fig. 10, for narrow-bore PCs and OTCs. The data, corresponding to non-retained compounds on OTCs lie on a line slightly above the one predicted by the Golay equation 9 as explained above. Due to the very strong increase in plate height with increasing k', also predicted by the Golay equation, the equipment contribution becomes of moderate importance

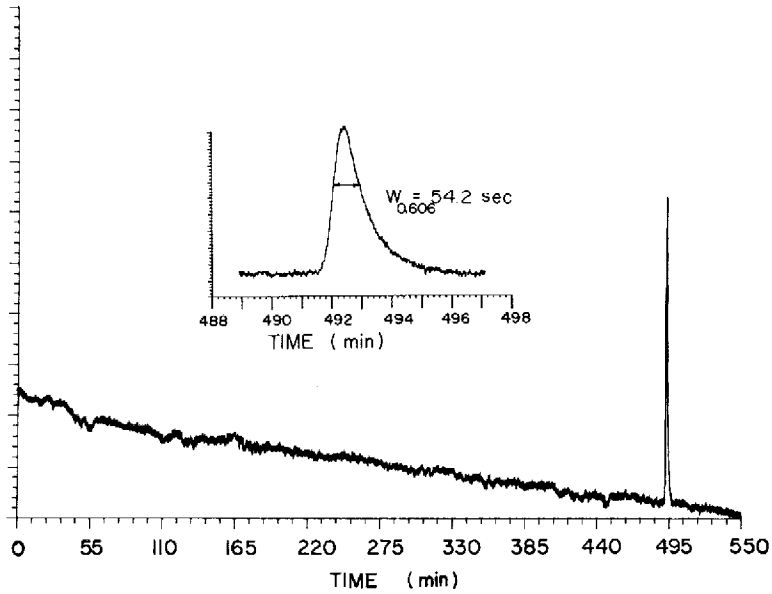

Fig. 9. Chromatogram obtained on a 105 m × 39 μm I.D. OTC for benzene ($k' = 0$) in *n*-heptane. Flow-rate 0.27 μl/min ($u = 0.38$ cm/sec, $v = 61.7$). Sample size: 5 nl. Eficiency: 1,166,700 plates. Analysis time: 8 h 15 min. The t_0 calculated from the column volume and flow-rate would be 463 min. The excess 30 min corresponds to the extra-column volumes and to the weak retention of benzene ($k' < 0.07$).

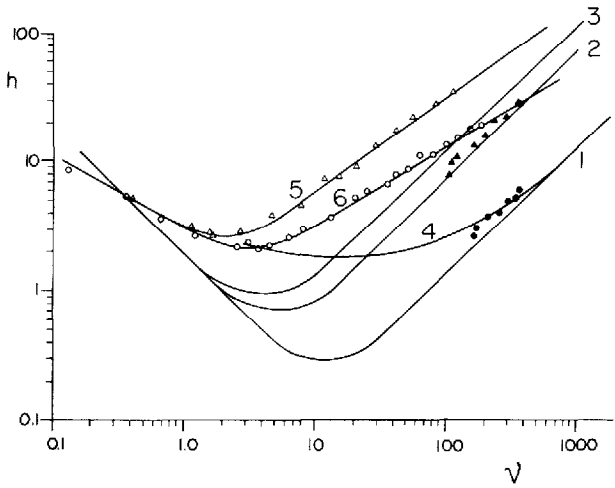

Fig. 10. Plot of reduced efficiency *versus* reduced velocity (logarithmic scales). Curves 1–3 corresponding to OTCs are derived from the Golay equation (eqn. 9) with $C_m = 0$ and $k' = 0$ (curve 1), $k' = 2$ (curve 2) and $k' =$ infinite (curve 3), respectively. Curve 4 is derived from eqn. 16a

$$h = 1.71 + 0.0106\, v + \frac{2}{v}$$

and fits reasonably well the experimental data. Curves 5 and 6 are the best fits of eqn. 17 to the experimental data. Experimental data: capillary column (105 m × 39 μm I.D.), benzene ($k' = 0$); ○; capillary column (25 m × 39 μm I.D.), benzene ($k' = 0$), +; anisole ($k' = 2$), △; narrow-bore column (50 cm × 1 mm I.D.), packed with 20-μm silica particles (Partisil 20), benzene ($k' = 0.1$), O; anisole ($k' = 2$), △.

for compounds with $k' = 2$. At high velocities the column plate height becomes 6.3 times larger for $k' = 2$ than for $k' = 0$ and eqn. 16b becomes:

$$H\,(k' = 2) = 42 \times 10^{-3}\, u \qquad (16c)$$

Under these conditions, the equipment contribution to band dispersion is only 16% of the column contribution, which is almost negligible. It is interesting to note that the results for the narrow-bore PC show a smaller resistance to mass transfer term in the particles (mass transfer in the stationary phase and diffusion in the stagnant mobile phase inside the pores of the particles) for a non-retained solute than for retained compounds. The influence of this variation of the coefficient C with k' on the minimum plate height is small, however. At a reduced velocity of *ca.* 2, the reduced efficiency of packed columns is practically independent of k' (Fig. 11). The data in Fig. 10 can also be fitted to the Knox equation:

$$h = \frac{B}{v} + Av^{1/3} + Cv \qquad (17)$$

The values of the coefficients are given in Table V.

Fig. 12 shows an example of the problems which may arise from a poor choice of experimental conditions. A flow velocity of 6.5 cm/sec with a 60 μm I.D. column

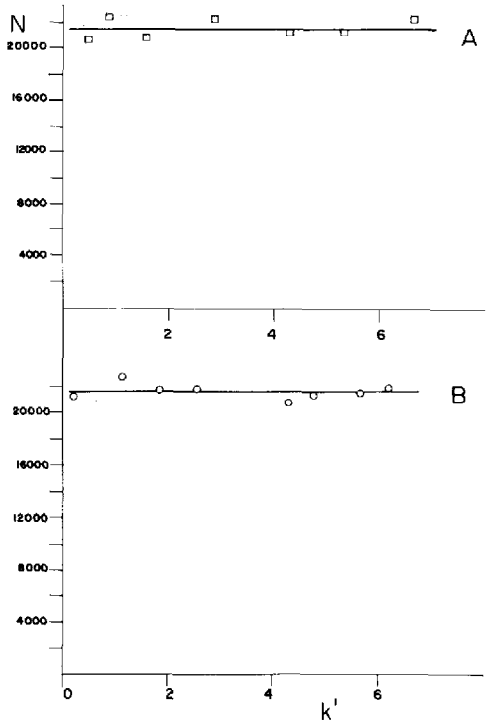

Fig. 11. Plots of column efficiency *versus* column capacity factor. Narrow-bore (1 mm I.D.) packed column, L = 50 cm. A, Silica gel 10 μm, solvent *n*-heptane, flow-rate 10 μl/min, solutes aromatic hydrocarbons; B, silica gel RP-18 (10 μm), solvent methanol–water (85:15), flow-rate 10 μl/min, solutes aromatic hydrocarbons.

and compounds which have a diffusion coefficient *ca.* $1.2 \cdot 10^{-5}$ cm^2/sec, as those used in this experiment, corresponds to a reduced velocity of about 3250, a very large value which should never be used in chromatography but has been too often in

TABLE V

COMPARISON OF THE HETP COEFFICIENTS

Column	*B*	*C*		*A*		*C*	
		k' = 0	*k' = 2*	*k' = 0*	*k' = 2*	*k' = 0.1*	*k' = 2*
OTC, theory*	2	0.0104	0.066	–	–	–	–
OTC, experiment	2	0.0106	0.080**	–	–	–	–
Narrow bore***	1.5	–	–	1.43	1.48	0.047	0.261

* As predicted by eqn. 9a.

** The terms C_m and C_s in eqn. 9b cannot be separated in this work. In gas chromatography a ratio C (experimental)/C_m = 0.83 is considered as good. This result justifies neglecting C_m in the theoretical derivation. The discrepancy could be explained with D_m/D_s = 10 and d_f/d_c = 0.20, but both values seem large, probably because of lack of homogeneity of the column.

*** OTC inner diameter 39 μm, PC average particle size 21 μm. The experimental value of C incorporates temperature and pressure effects.

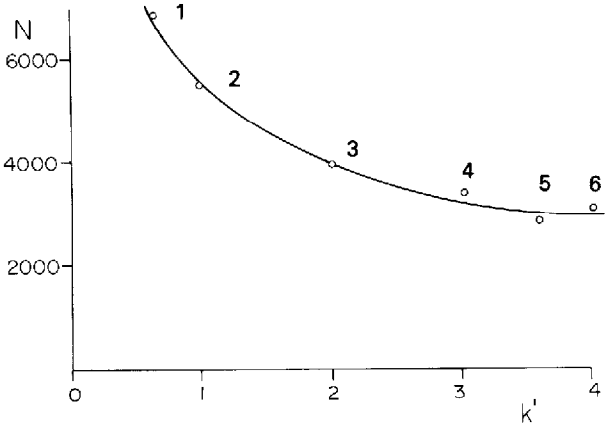

Fig. 12. Plot of column efficiency *versus* capacity factor. OTC, L = 1018 cm, d_c = 60 μm, flow velocity u = 6.5 cm/sec. Solutes (increasing k'): 1 = N-ethylaniline; 2 = 2,3-dimethyl-5-nitroaniline; 3 = 3,4-dimethylbenzyl alcohol; 4 = fluorobenzyl alcohol; 5 = anisyl alcohol; 6 = *m*-nitrobenzyl alcohol.

similar work. The corresponding reduced plate heights are 34 for k' = 0, 102 for k' = 0.5 and 250 for k' = 3 (680 plates for a 10-m column), neglecting the resistance to mass transfer in the stationary phase which is significant in this case. It is not surprising that low efficiencies are achieved in spite of a relatively long analysis time; the inert peak is eluted in 2 min 37 sec. It is easy to do much better with a PC. Fig. 12 also illustrates the rapid drop in efficiency with increasing retention which is experienced by OTCs at high flow velocities.

The use of high flow velocities and long columns is a way to increase the column bandwidth and operate narrow-bore OTCs while achieving reasonable effi-

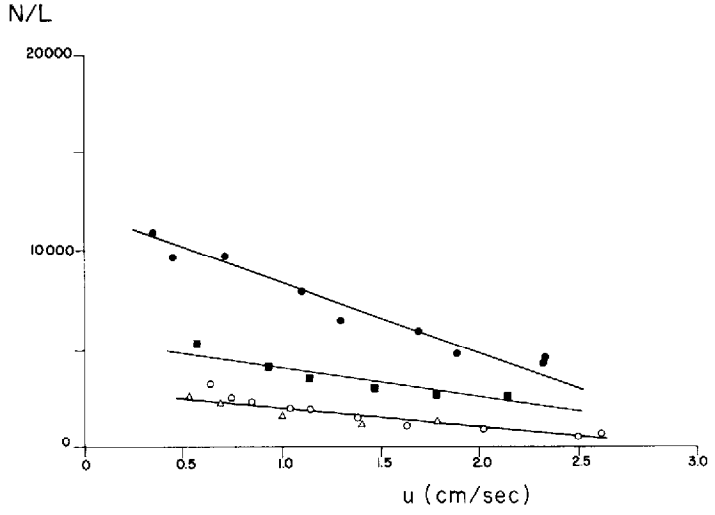

Fig. 13. Plot of theoretical plate number *versus* flow velocity for different columns. Solvent: *n*-heptane. ●, OTC, L = 105 m, d_c = 39 μm, benzene (k' = 0); ○, OTC, L = 25 m, d_c = 39 μm, anisole (k' = 2); ■, PC, L = 50 cm, d_p = 20 μm, benzene (k' = 0.1); △, PC, L = 50 cm, d_p = 20 μm, anisole (k' = 2).

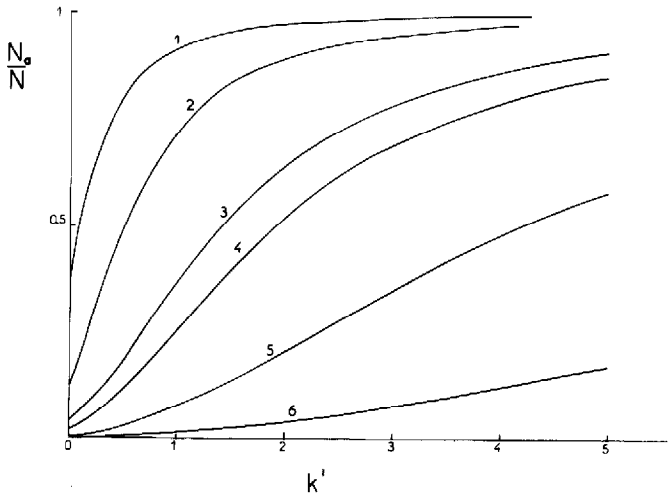

Fig. 14. Plot of the ratio of the apparent efficiency (as measured on the chromatogram) to the column theoretical efficiency (as predicted by the Golay equation) *versus* column capacity factor. Curves: 1, column length L = 105 m, d_c = 39 μm, v = 100; 2, L = 25 m, d_p = 39 μm, v = 100; 3, L = 25 m, d_p = 39 μm, v = 20; 4, L = 105 m, d_c = 20 μm, v = 100; 5, L = 25 m, d_c = 39 μm, v = 100; 6, L = 105 m, d_c = 10 μm, v = 100. For effect of column diameter compare curves 1, 4 and 6; for the effect of column length compare curves 1 and 2 or 4 and 5; for the effect of velocity compare curves 2 and 3. At constant reduced flow velocity and column length, the actual column efficiency is proportional to $1/d_c$, so the apparent plate number of column 1 will be larger than that of column 6 in all practical conditions (up to k' = 6.5) and larger than that of column 4 up to k' = 1.8.

ciencies using equipment which has a significant contribution to bandwidth. Similar or better results would be obtained with a shorter column at a lower flow-rate using perfect equipment, but the bandwidth would be smaller (Fig. 14, curves 2 and 3). It is easy to calculate the apparent efficiency, knowing the column characteristics and the equipment contribution.

The length variance of a band at the column outlet is defined as

$$\sigma_l^2 = LH \tag{18}$$

and as the solute is eluted from the column the variance increases by a factor of $(1 + k')^2$. Hence, the volume variance of the band is:

$$\sigma_v^2 = LHS^2(1 + k')^2 \tag{19}$$

H is given by eqn. 9a as:

$$\sigma_c^2 = \sigma_v^2 = L\,S^2\left[\frac{2}{v} + \frac{(1 + 6k' + 11k'^2)}{96\,(1 + k')^2} \cdot v\right](1 + k')^2 d_c \tag{20}$$

Combination with eqn. 14 permits the calculation of the apparent efficiency of the column as a function of k' or of the performance ratio, N_a/N_c, which represents the

fraction of potential column efficiency actually achieved. This is illustrated in Fig. 14. At large flow velocities ($v > 40$), eqn. 20 reduces to:

$$\sigma_c^2 = 6.43 \cdot 10^{-3} L\, d_c^5\, (1 + 6k' + 11k'^2)v \tag{21}$$

The equipment we have built ($\sigma_e = 0.10$ μl) is quite satisfactory at $v = 100$, for a 105 m × 39 μm I.D. column and still acceptable for a 25-m column. Nevertheless, the effect of the extra-column band broadening is very significant on the "short" 25-m column and more than offsets the drop in efficiency at low k' values, resulting in an almost constant value of the apparent column efficiency (Fig. 15). With a lower reduced velocity the results would still be acceptable, although barely, with a 25 m × 39 μm I.D. column operated at $v = 20$ (Figs. 14 and 15). The efficiency increases very rapidly, at first, but this is quite satisfactory for $k' \geqslant 2$. The dependence of σ_c^2 on the fifth power of the column diameter (eqn. 21) is tremendous, however, thus a mere reduction of column diameter by a factor of 2 decreases the column variance of the zone by 32 and practically sends the equipment designer back to the drawing board (Fig. 14). The corresponding curve for a 10 μm I.D. column hardly shifts above the abscissa axis (σ_c^2 is reduced by three orders of magnitude).

We can calculate the optimum diameter to use with given equipment in order to achieve the largest plate number with some constraints of analysis time and pressure drop. The column volume variance is given by eqn. 19, so the plate number is given by:

$$N = \frac{V_R^2}{(\sigma_c^2 + \sigma_e^2)} = \frac{[SL\,(1 + k')]^2}{[\sigma_e^2 + LS^2H\,(1 + k')^2]} \tag{22}$$

where V_R is retention volume.

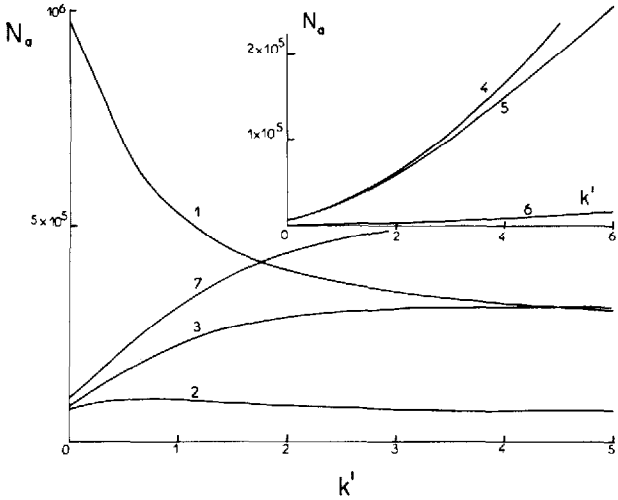

Fig. 15. Variation of actual and apparent column efficiency with column capacity factor. Curves: 1, L = 105 m, d_p = 39 μm, v = 100; 2, L = 25 m, d_p = 39 μm, v = 100; 3, L = 25 m, d_p = 39 μm, v = 20; analysis times for k' = 5 are respectively 34.1, 8.1 and 40.6 h for curves 1, 2 and 3 ($D_m = 2 \cdot 10^{-5}$ cm^2/sec; u = 0.51 cm/sec); 4, L = 105 m, d_p = 10 μm, v = 20; 5, L = 105 m, d_p = 10 μm, v = 100; 6, L = 25 m, d_p = 10 μm, v = 100; analysis times for k' = 5 are respectively 44, 8.8 and 2.2 h; 7, L = 105 m, d_p = 20 μm, v = 100, t_A = 17.5 h.

The conditions are:

$$\frac{L}{u}(1 + k') = t_R \tag{23}$$

$$32L\eta\, u/d_c^2 = \Delta P \tag{24}$$

Elimination of L between eqns. 22 and 23 gives

$$N = \frac{(Sut_R)^2}{[\sigma_e^2 + t_R u S^2 H(1 + k')]} \tag{25}$$

where u is a function of the particle diameter (eqn. 5). Derivation of the right hand side in eqn. 25 with respect to d_c and v gives relationships permitting derivation of the optimum conditions. The derivative dN/dd_c becomes zero for:

$$d_c^4 = \frac{16\sigma_e^2}{\pi^2 D_m t_A (1 + k')hv} \tag{26}$$

The derivative dN/dv is always positive. The largest v value will be chosen, fulfilling the condition 24. Elimination of L and u between eqns. 5, 23 and 24 gives:

$$d_c^4 = \frac{32 t_A \eta D_m^2 v^2}{\Delta P (1 + k')} \tag{27}$$

Elimination of v or d_c between eqns. 26 and 27 and assuming that v is large enough to limit the HETP equation to the second term of eqn. 9 ($h \approx Cv$) gives the optimum conditions:

$$d_c^4 = \frac{32}{(1 + k')\pi}\sqrt{\frac{\eta D_m \sigma_e^2}{2\Delta P C}} \tag{28}$$

$$v^4 = \frac{\sigma_e^2 \Delta P}{2\pi^2 \eta D_m^3 t_A^2 C} \tag{29}$$

$$L = \frac{t_A D_m}{(1 + k')} \cdot \frac{v}{d_c} \tag{30}$$

$$u = D_m \cdot \frac{v}{d_c} \tag{31}$$

$$N = \frac{t_A D_m}{(1 + k') C\, d_c^2} \tag{32}$$

For example if we want to limit the analysis time to one day for $k' = 5$ ($C = 0.089$, eqn. 9), with compounds having a diffusion coefficient of $1 \cdot 10^{-5}$ cm^2/sec and a mobile phase with $\eta = 1$ cP, using the present equipment ($\sigma_e^2 = 1 \cdot 10^{-8}$ cm^6,

ΔP_{max} = 300 atm) we would obtain d_c = 26 μm, v = 70, L = 28 m, u = 0.27 cm/sec and N = 240,000 plates. Better efficiencies are shown in Fig. 15 but the analysis time is longer.

It has been shown[2,3] that the performance of OTCs, in terms of efficiency and analysis time, should be comparable to those of PCs if the OTC diameter is equal to twice the particle size of the PC. The data in Fig. 13 confirm the validity of this statement. The efficiency per unit length of a 39 μm I.D. OTC is almost identical to that of a conventional narrow-bore PC packed with 20-μm silica particles. The standard deviation of the anisole band on the OTC is predicted to be 0.60 μl, which is large, compared to the standard deviation of the equipment contribution to band broadening, *i.e.*, 0.1 μl (see above), thus eqn. 14 predicts that the apparent efficiency is only 15% less than the column efficiency in this case.

This relationship is of course valid only in some range of flow velocities. Plotting N/L *versus* u is equivalent to plotting $1/H$ *versus* u, and the relationship between H and u is different for OTCs and PCs (Fig. 10), as shown by eqns. 9 and 17.

Finally, as expected, measurements of the linear velocity of *n*-heptane as a function of the pressure drop applied to the column give a specific permeability of 1/32 (Fig. 16) as predicted by the Poiseuille equation, which confirms that, in the range of velocities studied, the coil radius of the column is large enough not to promote excessive secondary circulation[6]. In fact, eqn. 11 in ref. 6 shows that, with a curvature radius of 1 cm, the effect would still be small. The specific permeability of the narrow-bore packed column is 733, somewhat less than the value usually accepted for conventional packed columns, which seems to be partly due to the narrow diameter of the column and the use of spherical particles.

CONCLUSIONS

This report can be considered as both optimistic and pessimistic. Open-tubular columns with performances close to those predicted by the Golay equation have been prepared. This has been possible only by using long columns with rather large diameters, operated at very high velocities. Using large values for these three parameters

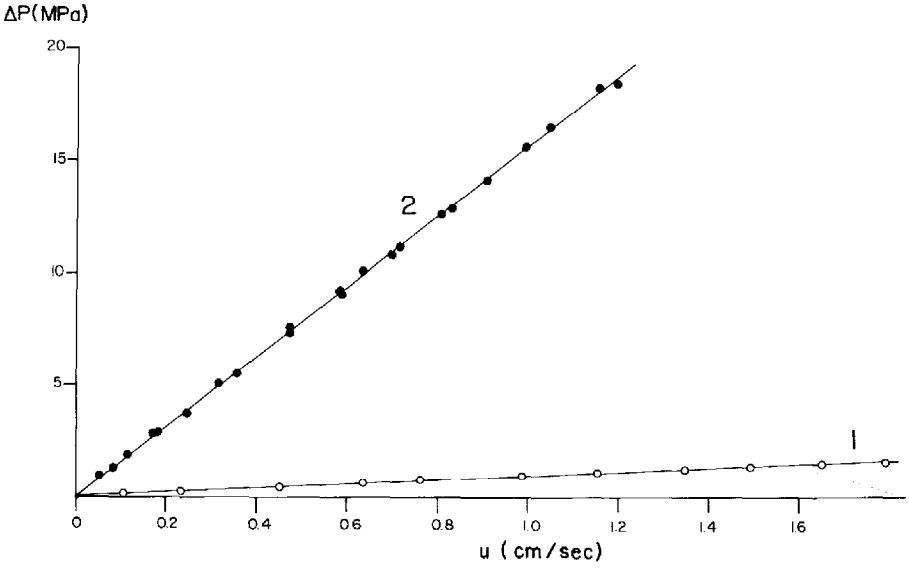

Fig. 16. Plot of column pressure drop *versus* flow velocity. Solvent: *n*-heptane. Curves: 1, OTC, L = 105 m, d_c = 39 μm, slope 1.01 · 10^7 dyne sec cm^{-3}, specific permeability 1/32. 2, PC, L = 50 cm, 1 mm I.D., d_p = 10 μm, slope 15.72 · 10^7 (dyne sec cm^{-3}, specific permeability 1.36 · 10^{-3}.

(L, d_c, v) result in large zone volumes and reduces the relative importance of the instrument contribution. This contribution reduces by about 5% the efficiency of retained peaks and by a factor of 3–4 that of unretained peaks.

Nevertheless, efficiencies in excess of one million plates for unretained peaks and several thousand plattes for anisole ($k' = 2$) have been achieved ($N = 270{,}000$; $t_R = 17.5$ h). These performances for retained peaks are difficult but possible to exceed using narrow-bore conventional packed columns[7]. It should be emphasized that an OTC column is much easier to prepare than a PC column of comparable effficiency (10 m with $d_p = 20$ μm). Thus, this paper demonstrates that current OTCs are competitive with conventional columns made with 20-μm particles.

On the other hand, this result is not yet sufficient. Even the OTCs prepared here could not be used at their optimum performance level. This would be achieved at a reduced velocity double that for which the plate height is minimal (here $v = 10$ and $h = 1.0$ for $k' = 3$). The efficiency would then be 2.7 million plates for a compound with $k' = 3$ and the analysis time 9.5 days (with $N = 8.8$ million plates for the unretained peak) for a 105-m column. The standard deviation of the zone would be 305 μl ($k' = 3$) and 42 μl ($k' = 0$) respectively. With the present equipment, the apparent efficiency would be respectively 90% and 15% of the theoretical efficiency. Excellent analytical results would be obtained, but the analysis time would be totally impractical, even if the reliability of the equipment were good enough and a constant flow-rate of 37 nl/min could be maintained.

The only practical solution to the problem is in the use of still narrower columns, a few μm to 10 μm in diameter. They would require an equipment contribution to band broadening 10–100 times smaller than that of our equipment. Considering the fact that every conceivable effort has been made to design and build an instrument dedicated to the operation of narrow OTCs, this conclusion is somewhat disappointing. Another approach to circumvent severe extra-column instrumental band dispersion with OTCs could be to use the so-called multiple capillaries or "capillary bundles" where the total dead volumes of all capillaries[11] would be so large that a conventional detector and injector system could be used. The formidable difficulties here would lie in the adjustment of the capillaries, so that they all give the same retention time for each of the components of the mixture, under the same mobile phase pressure drop.

Otherwise, it seems to us that further progress requires a major breakthrough in detector design, for example, the use of a focused laser beam-induced fluorescence detector. But the time when open-tubular columns will be available to solve extremely difficult separation problems does not seem very distant.

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