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KINETIC OPTIMIZATION OF STRAIGHT OPEN-TUBULAR LIQUID CHROMATOGRAPHY

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SUMMARY

A theoretical analysis of liquid chromatography in open-tubular columns along the lines of the treatment given by Knox and Saleem for packed columns indicates that for a given pressure drop, plate number, solute and eluent, the optimal diameter of the open tubular column is about one fifth of the diameter of the particles in the optimal packed column and the analysis time is about 100 times less. However, the calculated bores of capillaries are impracticably small for operation at or near the minimum in h unless N, the number of theoretical plates required, exceeds several million. The practical limitation to capillary liquid chromatography undoubtedly arises from the dispersion produced by the detector. Accordingly, optimal conditions are derived for capillary LC in which the standard deviation of the unretained solute, σ_v^0, and pressure drop are taken as limiting conditions. It is shown that with these constraints the optimal column bore is independent of N while the analysis time increases directly with N.

Comparison of the performance of capillary columns limited in this way, with packed high-performance liquid chromatographic (HPLC) columns operated under conditions of minimum h, shows that if $\sigma_v^0 = 0.1$ mm^3 then capillaries of 30 μm bore are required and will give better performance than packed columns only if $N > 300{,}000$. However, if $\sigma_v^0 = 0.001$ mm^3 then capillaries of 10 μm bore are required and are faster than packed columns when $N > 30{,}000$. For example, the time, t_m, for a peak of $N = 10^6$ is 2 h with the capillary compared with 55 h with the packed column.

Published experimental data are examined in the light of the Golay equation and the agreement is shown to be excellent for both unretained and retained solutes. It is concluded that if detector volumes can indeed be reduced to around 0.001 mm^3 there are excellent prospects for capillary HPLC.

INTRODUCTION

Recently, attempts have been made to develop realistic open-tubular liquid chromatography[1,2], using essentially straight narrow-bore tubes with internal diameters from 50 to 200 μm. These have met with encouraging success although it is clear that there is still some way to go before capillary high-performance liquid chro-

matography (HPLC) can compare with capillary GC or even packed-column HPLC. Basically the problem is that the bore of the capillary required to give optimal performance is in the range 1–10 μm. This imposes extremely severe demands upon the injection and detection devices which no currently available commercial devices can meet. Tijssen[3] has shown that a possible way around the requirement for very narrow tubes is to employ a tightly coiled tube with very high flow velocities. Under these conditions the secondary flow brings about rapid radial mixing so that a much wider tube of say 100 to 300 μm bore can be employed. While experiment and theory are in good agreement for unretained solutes there appear to be unsolved problems with retained solutes such that the actual performance falls far short of what might be predicted theoretically. Another purely mechanical approach advocated by Desty[4] is to place flow-spoilers within the tube in order to improve radial mixing. Unfortunately this ingenious approach was unsuccessful in gas chromatography (GC) and does not seem to have any real potential in liquid chromatography (LC) where narrower tubes would certainly have to be used, bringing with them extreme problems of fabrication. By contrast, as we show below, the experimental data on straight open-tubular columns[1,2] are in excellent agreement with theory as expressed by the Golay equation[5], and we believe that such columns offer the best hope for developing very-high-performance liquid chromatography. It is furthermore our contention that the difficulties that have been envisaged are not as severe as has sometimes been thought.

Accordingly in this paper we first derive optimal conditions for capillary HPLC, taking no account of practical limitations other than the pressure available. This derivation shows that only for columns giving more than 1,000,000 plates will it be practicable to work under such optimal conditions. We then explore conditions under which capillary LC can be optimised when the dispersion produced by the detector and injector are limiting. Finally, we examine the data currently available[1,2] in the light of the theory developed.

KINETIC OPTIMIZATION OF OPEN-TUBULAR LIQUID CHROMATOGRAPHY

Knox and Saleem[6] derived basic equations for packed-column LC on the basis of which kinetic optimization could be carried out. Their key results are contained in eqns. 1, 2 and 6. Eqns. 1 and 2 present the elution times for unretained and retained solutes, respectively, in terms of the operational variables: N, the number of theoretical plates to which the column is equivalent; h, the reduced plate height; $\varnothing$, the column resistance factor; η, the eluent viscosity; Δp, the pressure drop across the column, and k' the column capacity ratio.

$$t_m = N^2h^2 \varnothing \eta/\Delta p \tag{1}$$

$$t_R = N^2h^2 \varnothing \eta(1 + k')/\Delta p \tag{2}$$

For columns packed with particles of diameter d_p, the reduced plate height and column resistance parameter are defined by:

$$h = H/d_p \tag{3}$$

and

$$\varnothing = \frac{\Delta p t_m d_p^2}{\eta L^2} \tag{4}$$

where H is the plate height and L the column length.

According to the theory of chromatography[3,7] amply confirmed by experiments[8–10], the reduced plate height, h, is a universal function of the reduced velocity, v, for any geometrical configuration of column (packed tube, straight open tube, coiled open tube). For a packed column the reduced velocity is defined by:

$$v = ud_p/D_m \tag{5}$$

where u is the linear eluent velocity, and D_m the diffusion coefficient of solute in eluent.

In order to achieve a particular value of h (which also implies a particular value of v) given Δp, N, $\varnothing$ and η, it is necessary to use the correct particle size d_p. This is given by:

$$d_p = \left(\frac{Nhv\varnothing\eta D_m}{\Delta p}\right)^{1/2} \tag{6}$$

To use eqn. 6 the functional relationship between h and v must be known at least for the range of h and v of interest. For example, under optimal conditions when h is a minimum we have for a slurry packed column approximately $h = 2$, $v = 4$, $\varnothing = 500$. Thus eqn. 6 can be simplified to:

$$d_{p(\mathrm{opt})} = (4000\, N\eta D_m/\Delta p)^{1/2} \tag{7}$$

Inserting some typical values of D_m and η for an aqueous eluent and taking $N = 10{,}000$ and $\Delta p = 200$ bar, we obtain $t_m = 10$ sec and $d_{p(\mathrm{opt})} = 1.4\,\mu$m. The optimal particle size is considerably less than those currently employed. However, confirmation that very small particles will provide higher performance is provided by the results of Bristow *et al.*[11] and of Unger *et al.*[10] who successfully used 3-μm and 1.8-μm particles.

A similar analysis to that given by Knox and Saleem[6] is readily developed for straight capillary LC. We define the reduced parameters h, $\varnothing$ and v for an open tube in terms of the column bore d_c, by the following equations:

$$\text{Reduced plate height } h = H/d_c \tag{8}$$

$$\text{Column resistance parameter } \varnothing = \frac{\Delta p t_m d_c^2}{\eta L^2} \tag{9}$$

$$\text{Reduced velocity } v = \frac{ud_c}{D_m} \tag{10}$$

Eqns. 1 and 2 remain unchanged, but eqn. 6 becomes

$$d_c = \left(\frac{Nhv\varnothing\eta D_m}{\Delta p}\right)^{1/2} \tag{11}$$

Whereas for a packed bed $\varnothing$ depends upon the method of packing and the particle porosity, for an open tube it is given exactly by Poiseuille's equation:

$$\varnothing_{(\text{open tube})} = 32 \tag{12}$$

Likewise the plate height for a straight open tube is given rigorously by the Golay equation first derived in 1958[5]. Using reduced parameters, this equation has the form

$$h = \frac{2}{\nu} + \frac{1 + 6k' + 11k'^2}{96\,(1 + k')^2} \cdot \nu + \frac{2}{3} \cdot \frac{k'}{(1 + k')^2} \left(\frac{d_f}{d_c}\right)^2 \left(\frac{D_m}{D_s}\right) \nu \tag{13}$$

where d_f is the depth of the stationary phase and D_s is the diffusion coefficient of solute in the stationary phase. The three terms in the Golay equation represent the contributions from axial diffusion, slow equilibration in the mobile phase and slow equilibration in the stationary phase. In GC it is generally found that the mobile phase term is substantially larger than the stationary phase term, and in LC this predominance would be expected to be even greater. Thus, to a first approximation the last term in eqn. 13 can be assumed to be negligible in LC. It is readily shown that the minimum h and optimum ν are then given by

$$h_{\min} = \frac{1}{(1 + k')} \left(\frac{1 + 6k' + 11k'^2}{12}\right)^{1/2} \tag{14}$$

$$\nu_{\text{opt}} = (1 + k') \left(\frac{192}{1 + 6k' + 11k'^2}\right)^{1/2} \tag{15}$$

and that

$$h_{\min}\, \nu_{\text{opt}} = 4 \tag{16}$$

If a stationary phase contribution were significant, then $h_{\min}$ would rise while ν_{opt} would fall but eqn. 16 would remain valid.

For the purpose of further deduction we take values of $h_{\min}$ and ν_{opt} appropriate to $k' = 3$, namely $h_{\min} = 0.8$ and $\nu_{\text{opt}} = 5$. These values are not very different from the corresponding values for a packed column.

In comparing eqns. 1 and 2 for open and packed columns it is seen that the optimal value of the key parameter $h^2\varnothing$ is reduced from about 2000 to 20. This is a measure of the very large improvement in "performance" which is obtained by going from a packed tube to an open tube and was, of course, first pointed out by Golay[5]. The improvement can manifest itself in several ways according to the operators choice. For example, if Δp, N and η are maintained the same, elution will be 100 times faster with the open tube, or if t_m, Δp, and η are maintained constant, then ten times as many plates can be achieved. Whereas our typical minimum elution time for the packed column of 10,000 plates was 10 sec, the corresponding time for the capillary would be only 100 msec, or, alternatively, 100,000 plates could be attainable in 10 sec.

Regrettably many of the apparently attractive possibilities of capillary HPLC are unrealizable in practice. In going from the packed to the capillary column the

parameter $h\nu\varnothing$ will be reduced from about 4000 to 128, a decrease of about 30 times. Thus the analogue of eqn. 7 is

$$d_{c(opt)} = (128\, N\eta D_m/\Delta p)^{1/2} \tag{17}$$

If the external operating parameters N, η, D_m and Δp are to be the same for a packed column and a capillary column, the diameter of the tube has to be about 5.5 times smaller than that of the particles of the packed column. For the equivalent of the 10,000-plate column packed with 1.4-μm particles we would therefore require a capillary column with a bore of 0.26 μm and a length of 2 mm, which is clearly a completely unrealistic requirement. It will be noted, however, from eqns. 1, 2 and 17 that as N is increased, keeping other parameters unchanged, the column bore and elution times also increase, the bore in proportion to $N^{\frac{1}{2}}$ and the time in proportion to N^2. Thus capillary LC could be feasible for very high values of N.

Whether a given column can be operated in practice with a given injector–detector combination will depend upon the relationship between the volume of eluent within which the peak is eluted and the volumetric dispersion introduced by the detector. In general, with current 8-μl detection cells, it is found that the volume of the detector cell should not exceed about half the volume corresponding to the standard deviation (see ref. 12 for discussion) of the peak, σ_v. This in its turn will be given for an open tubular column by

$$\sigma_v = \frac{1}{\sqrt{N}} \frac{\pi d_c^2 L}{4}(1 + k') = \sqrt{N} \frac{\pi d_c^3 h}{4}(1 + k') \tag{18}$$

In capillary LC, as in capillary GC, there is bound to be a problem in attaining adequate retention due to the small ratio of stationary to mobile phase, a point emphasized by Purnell[12] in the context of GC and recently by Tsuda *et al.*[2] for capillary LC. It is therefore important that the detector volume should be less than $\frac{1}{2}\sigma_v$ for an unretained solute if the early eluting peaks are to be resolved. The necessary condition is therefore that

$$2V_{detector} \leqslant \sigma_v^0 = \frac{\pi d_c^2 L}{4\sqrt{N}} = \tfrac{1}{4}\sqrt{N}\pi d_c^3 h \tag{19}$$

Table I lists values for column parameters for capillary HPLC for an aqueous eluent (η and D_m fixed) with plate number requirements from 10,000 to 10,000,000. Two possible values of Δp are considered: 200 bar and 20 bar. The columns are operated under optimum conditions, giving the minimum value of $h = 0.8$.

It is evident from Table I that only for the highest values of N is there any likelihood of ever being able to operate capillary HPLC columns close to the minimum of the (h,v) curve because of the limitation on the lowest allowable value of σ_v^0. Evidently detection volumes, currently around 8 μl (mm^3), must be reduced 1000–10,000 times to the order of 1–10 nl before there is any hope of operating useful capillary HPLC systems under optimal conditions. In this context the most attractive detectors will be electrochemical, fluorescent or mass spectrometric.

TABLE I

CAPILLARY LC COLUMNS OPERATED UNDER OPTIMAL CONDITIONS

$h = 0.8$; $\varnothing = 32$; $\eta = 1.0 \times 10^{-3}$ N sec m^{-2}; $D_m = 10^{-9}$ m^2 sec^{-1};
$\Delta p = 200$ bar $= 2 \cdot 10^7$ N m$^{-2} \approx 3000$ p.s.i.

N	t_m (sec)	d_c (μm)	L (m)	σ_v^0 (mm^3)
10,000	0.10	0.25	0.002	10^{-9}
30,000	0.9	0.43	0.010	$0.8 \cdot 10^{-8}$
100,000	10.2	0.8	0.063	10^{-7}
300,000	90	1.37	0.33	$0.8 \cdot 10^{-6}$
1,000,000	1000	2.5	2.0	10^{-5}
3,000,000	9000	4.3	10	$0.8 \cdot 10^{-4}$
10,000,000*	100,000	8	63	10^{-3}

$\Delta p = 20$ bar $= 2 \cdot 10^6$ N m$^{-2} \approx 300$ p.s.i.

N	t_m (sec)	d_c (μm)	L (m)	σ_v^0 (mm^3)
10,000	1.0	0.8	0.006	$3.1 \cdot 10^{-8}$
30,000	9	1.4	0.033	$2.8 \cdot 10^{-7}$
100,000	100	2.5	0.2	$3.1 \cdot 10^{-6}$
300,000	900	4.3	1.0	$2.8 \cdot 10^{-5}$
1,000,000	10,000	8	6.4	$3.1 \cdot 10^{-4}$
3,000,000*	90,000	25	33	$2.8 \cdot 10^{-3}$

* Entries in these rows might just be attainable in practice.

It must therefore be concluded that operation of capillary LC systems near the theoretical optimum will be feasible only for columns with exceptionally high N values which would be unattainable using conventional packed columns. If capillary LC columns are to be of use for N values in the range 100,000–1,000,000, then columns wider than the optimum must be operated under conditions well removed from the optimum. This will turn mean that pressures and/or elution times will be much higher than could be achieved under optimum conditions.

SEARCH FOR PRACTICAL OPERATING CONDITIONS

The starting point for the derivation of practical operating conditions for the straight capillary must clearly be a statement of the limiting value of σ_v^0 rather than the desirability of working with a certain value of h. Almost certainly ν will then be very high and well above the value for minimum h. For simplicity we write eqn. 13 as

$$h = \frac{2}{\nu} + C\nu \tag{20}$$

and note that, since a typical value of C is 0.08 ($k' = 3$), a good approximation to eqn. 20 when $\nu \geqslant 20$ is

$$h = C\nu \tag{21}$$

The other relevant equations are then eqns. 1, 19 and 22.

$$d_c = \left(\frac{Nhv\varnothing\eta D_m}{\Delta p}\right)^{1/2} = \left(\frac{Nh^2\varnothing\eta D_m}{C\Delta p}\right)^{1/2} \tag{22}$$

To obtain equations for t_m, d_c and L in terms of the group of operational variables Δp, N, $\varnothing$, η, D_m, v, and C, d_c is first eliminated between eqns. 19 and 22 to give

$$h = \left(\frac{16\sigma_v^{0}\Delta p^3 C^3}{\pi^2\varnothing^3\eta^3 D_m^{\,3}}\right)^{1/8} \cdot \frac{1}{\sqrt{N}} \tag{23}$$

h is then eliminated between eqns. 1 and 23 to give

$$t_m = \left(\frac{16\varnothing\eta\sigma_v^{0\,2} C^3}{\pi^2\Delta p D_m^{\,3}}\right)^{1/4} \cdot N \tag{24}$$

and between eqns. 22 and 23 to give

$$d_c = \left(\frac{16\varnothing\eta D_m\sigma_v^{0\,2}}{\pi^2 C\Delta p}\right)^{1/8} \tag{25}$$

The column length is $L = Nhd_c$ and is given in terms of the operational variables by eqn. 26.

$$L = \left(\frac{16\sigma_v^{0\,2} C\Delta p}{\pi^2\varnothing\eta D_m}\right)^{1/4} \cdot \sqrt{N} \tag{26}$$

Eqns. 24–26 give the elution time t_m and the column dimensions d_c and L as functions of the group $\varnothing$, η, D_m, C, which are determined by the eluent and the properties of open tubes, along with σ_v^0 and Δp, which are determined by limitations of the equipment, and N, the required plate number. Essentially we are interested in minimizing t_m, and therefore eqn. 24 is the most important from the point of view of optimization.

In contrast to eqn. 1, eqn. 24 indicates that t_m is proportional to N, not N^2. Pressure drop is much less potent in decreasing t_m: a 100-fold increase in Δp only reduces t_m about 3 times. On the other hand, t_m is markedly increased by a decrease in D_m and by an increase in σ_v^0 for which the detector volume sets a lower limit. Evidently we must always work at the minimal possible σ_v^0 in order to get minimal analysis time.

An unexpected result of this analysis is that given values for the group of seven parameters, d_c is fixed and independent of N (eqn. 25). This implies that having settled on the detector volume (which sets σ_v^0) one needs only one column bore.

Indeed, we may talk of a "detector-limited column set" in which the column length is proportional to $\sqrt{N}$.

In Table II typical values for t_m, d_c, L and h are presented for representative values $\varnothing = 32, \eta = 1.0 \cdot 10^{-3}$ N sec m^{-2}, $D_m = 1.0 \cdot 10^{-9}$ m^2 sec^{-1}, $C = 0.08$ and $\Delta p = 100$ bar $= 1.0 \cdot 10^7$ N m^{-2}. Two values for σ_v^0 are used: 0.001 and 0.1 mm^3. For comparison, t_m values obtained under optimal conditions with packed columns are given assuming $\varnothing = 500$, $h_{min} = 2$ and other relevant parameters as for the capillary columns.

TABLE II

CAPILLARY HPLC COLUMNS OPERATED UNDER CONDITIONS OF CONSTANT σ_v^0 AND Δp

$\varnothing = 32; \eta = 1.0 \cdot 10^{-3}$ N sec m^{-2}; $D_m = 10^{-9}$ m^2 sec^{-1}; $C = 0.08$; $\Delta p = 100$ bar $= 1.0 \cdot 10^7$ N m^{-2}.

(a) $\sigma_v^0 = 10^{-3}$ mm^3 $= 10^{-12}$ m^3.

N	*HPLC capillary*					*Packed column:*
	t_m *(sec)* *(0.00716 N)*	d_c *(μm)*	L *(m)* *(0.0141 √N)*	h $\left(\frac{1500}{\sqrt{N}}\right)$	v $\left(\frac{h}{0.08}\right)$	t_m *(sec)* $(2 \cdot 10^{-7} N^2)$
10,000	72	9.5	1.4	15	190	20
30,000	215	9.5	2.4	8.7	110	180
100,000	720	9.5	4.5	4.7	60	2000
300,000	2150	9.5	7.7	2.7	35	18,000
1,000,000	7200	9.5	14	1.5	18	200,000

(b) $\sigma_v^0 = 0.1$ mm^3 $= 10^{-10}$ m^3.

N	*HPLC capillary*					*Packed column:*
	t_m *(sec)* *(0.0716 N)*	d_c *(μm)*	L *(m)* *(0.141 √N)*	h $\left(\frac{4730}{\sqrt{N}}\right)$	v $\left(\frac{h}{0.08}\right)$	t_m *(sec)* $(2 \cdot 10^{-7} N^2)$
10,000	720	30	14	47	600	20
30,000	2150	30	24	27	350	180
100,000	7200	30	45	15	180	2000
300,000	21,500	30	77	8.6	110	18,000
1,000,000	72,000	30	140	4.7	60	200,000

It is clear from Table II that when $\sigma_v^0 = 0.001$ mm^3, HPLC capillary columns will give a performance which is superior to that of packed columns when $N > 30{,}000$, with the superiority being greater when $N = 1{,}000{,}000$. However, if $\sigma_v^0 = 0.1$ mm^3 the packed column gives the better performance until $N = 300{,}000$ and the capillary column becomes superior only when N exceeds this value. It may therefore be concluded that capillary HPLC will become a viable proposition only when a detector with an effective volume of the order of 1 nl (10^{-3} mm^3) can be devised. One such device, recently reported by Hershberger *et al.*[13], is a laser fluorimeter employing a sheathed flow which gives a minimum detection volume of below 6 nl. Electrochemical systems using very fine wires also offer the possibility of extremely small detection volumes.

EVALUATION OF RECENT WORK ON CAPILLARY LIQUID CHROMATOGRAPHY

Taylor[14] was the first to demonstrate the dispersion of bands of solutes when passed along tubular columns in a flow of liquid. With fairly wide bore tubes he demonstrated the correctness of his dispersion equation for unsorbed solutes. This equation in chromatographic terminology has the form

$$H = \frac{2D_m}{u} + \frac{d_c^2 u}{96 D_m} \quad \text{or} \quad h = \frac{2}{v} + \frac{v}{96} \tag{27}$$

This is a special case of the more general Golay equation when $k' = 0$. Knox and Parcher[15] used this equation in order to determine the diffusion coefficient of acetone in water by passing it along a 1-m long tube 1.00-mm in bore. By obtaining a value in close agreement with the accepted value they confirmed the accuracy of the Taylor equation.

As has been shown above, for capillary HPLC to become a practically useful technique, it will be necessary to employ very narrow tubes with bores in the region of 10 μm. The narrowest tubes that have been employed to date are 50 or 60 μm[1,2]. Tsuda and Novotny[1] examined the dispersion of unsorbed benzene when eluted by hexane from open-tubular glass columns 50, 82 and 195 μm in diameter. To minimize extra-column dispersion they used a 1000:1 or 2500:1 inlet splitter, and an additional flow to the detector to reduce its effective volume. Fig. 1 shows their results plotted logarithmically. The full line shows the (h, v) relationship predicted by the Taylor equation. The best values of C for the three capillaries are shown in Table III.

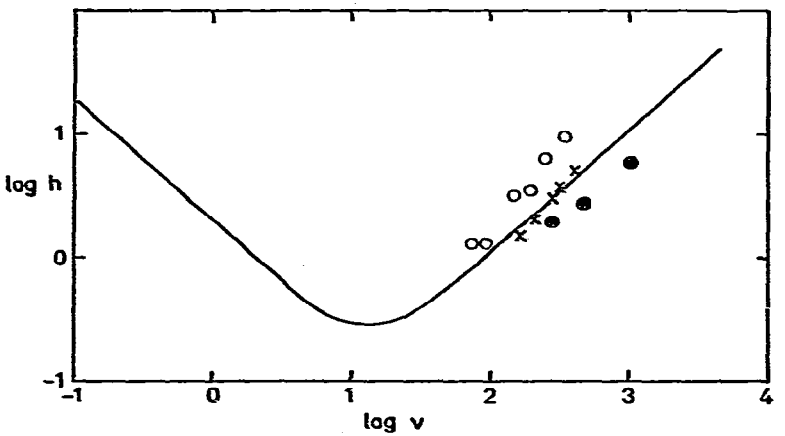

Fig. 1. Plots of log h against log v for data of Tsuda and Novotny[1] for open-tubular columns compared with the Taylor equation (eqn. 27). ●, $d_c = 195\,\mu$m; ×, $d_c = 82\,\mu$m; ○, $d_c = 50\,\mu$m.

It is evident that for the widest and longest column the value of C is below that predicted theoretically. As suggested by Tsuda and Novotny[1], this is probably caused by additional mixing due to secondary flow in their coiled capillary tube, the theory of which has been discussed in detail by Tijssen[3]. With the narrower tubes this effect is less important. Thus the results for the 82-μm capillary are in good accord

TABLE III

DATA FOR UNRETAINED SOLUTES (TSUDA AND NOVOTNY[1])

Benzene in hexane: $D_m = 3 \cdot 10^{-9}$ m^2 sec^{-1}.

d_c (μm)	L (m)	C_{exp}	C_{Golay}	C_{exp}/C_{Golay}
50	11	0.020	0.0104	2.0
82	7.5	0.010		1.0
195	102	0.006		0.6

with the Taylor equation while the high results for the 50-μm column may be an indication that the effects of detector and injection dead volumes were not completely eliminated.

Tsuda *et al.*[2] investigated the dispersion of solutes retained by a unimolecular layer of octadecyl groups bonded to the etched inner surface of a glass capillary 3330 mm long and 60 μm in bore. This capillary was directly connected to a 300-μm bore tube which was integral with the detection cell, the detection point being 15 mm downstream of the end of the 60-μm bore column. Samples of 0.023 mm^3 volume were injected directly into the column by a special capillary injector. The authors reported measurements of plate height, H, for three solutes, benzene, naphthalene and biphenyl, eluted by a variety of eluents at a variety of eluent velocities and sample loadings. The plate height data are re-plotted as a function of velocity on a reduced basis in Fig. 2 and compared with plots of the Golay equation for k' values of 0, 1 and ∞. The diffusion coefficient for the solutes in the eluent, methanol–water (52:48) was taken as $6 \cdot 10^{-10}$ m^2 sec^{-1} (ref. 16) and the derived values of C are listed in Table IV. Unfortunately, Tsuda *et al.*[2] fail to give values of k' for all three solutes for the conditions under which H was measured. If, as

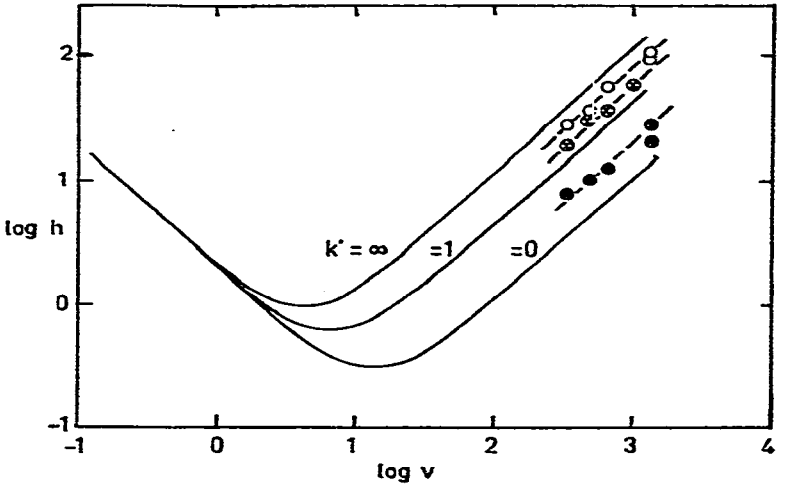

Fig. 2. Plots of log h against log v for data of Tsuda *et al.*[2] for solutes retained on an ODS-capillary ($d_c = 60$ μm). Lines according to Golay equation for the marked values of k': points are experimental data taken from original paper. ●, Benzene ($k' = 0.11$); ×, naphthalene ($k' = 0.56$); ○, biphenyl ($k' = 1.22$).

they assume, benzene is unretained, a value of $k' = 1.0$ for biphenyl in methanol–water (52:48) can be read from their Fig. 2. k' for naphthalene cannot be deduced directly but can be estimated to be about 0.4 using data from one of the chromatograms in which acetonitrile–water was used as eluent. Another important parameter which was not given is the phase ratio, that is, the mass or volume of the stationary phase divided by the mass or volume of the eluent.

TABLE IV

k' VALUES OF SOLUTES ELUTED FROM ODS HYPERSIL AND AN ODS-COATED CAPILLARY[2]

Eluent: methanol–water (52:48).

Solute	k'			$k'_{ODS\text{-}Hypersil} / k'_{ODS\text{-}capillary}$	C_{exp}	C_{Golay}***
	ODS-Hypersil	*ODS-capillary*				
		*(a)**	*(b)***			
Benzene	4.0	0.0	0.11	36	0.020	0.015
Naphthalene	18	0.4	0.56	32	0.056	0.033
Biphenyl	45	1.0	1.22	37	0.079	0.052
				Mean: 35		

* Estimated values assuming k' for benzene = 0.

** Corrected values calculated to give best fit to values found with ODS Hypersil.

*** Value of C calculated from $C = (1 + 6k' + 11k'^2)/[96(1 + k')^2]$ using "corrected" values of k'.

As accurate values of k' are essential for any correlation of the experimental data with theory, and as the phase ratio is essential when comparing the data on column overloading with that for packed columns[17,18], we have chromatographed a mixture of benzene, naphthalene and biphenyl on a 100 × 5 mm column packed with ODS-Hypersil, using the same eluent as employed by Tsuda *et al.*[2]. The k' values relative to the solvent disturbance peak are given in Table IV. It is notable that on ODS-Hypersil benzene is clearly retained with $k' = 4.0$ and it is therefore almost certainly retained to some extent on the ODS-capillary column. If we assume that the distribution coefficients for the three solutes between the eluent and the ODS layer are the same for ODS-Hypersil and the ODS-capillary, corrected values of k' are readily derived for the capillary as they will fall in the same ratio as those for the packed column. In this way the corrected values for the three solutes are found to be 0.11, 0.56 and 1.22, rather than 0.0, 0.40 and 1.00. The ratio of the k' values on ODS-Hypersil and the ODS-Capillary then give the relative phase ratio, that is —

$$\text{Relative phase ratio} = \frac{\text{Phase ratio for ODS-Hypersil}}{\text{Phase ratio for ODS-capillary}} = \frac{k'_{ODS\text{-}Hypersil}}{k'_{ODS\text{-}capillary}} \quad (28)$$

From Table IV it is seen that the relative phase ratio is about 35. As the phase ratio of ODS-Hypersil can readily be found from established data, we can easily obtain the phase ratio for the ODS-capillary. The necessary data are presented in Table V, and the phase ratio for the ODS-capillary is found to be $2.7 \cdot 10^{-3}$ g cm^{-3}. As the

ODS groups (taken to have the formula $C_{18}H_{37}$) are bonded as a unimolecular layer on to the etched silica surface we calculate, assuming the same surface density as for ODS-Hypersil, that the underlying etched surface of the capillary has an area about 60 times larger than the internal surface area of a smooth 60-μm bore tube. It is also noted that the actual weight of ODS in the 3.3-m column is only 25 μg compared with 125 mg in the packed column. Table IV and Fig. 2 show that the C values found experimentally are between 1.3 and 1.7 times the values predicted by the Golay equation on the assumption that the stationary phase mass transfer term is negligible. However, bearing in mind the great experimental difficulties in this work, the agreement is excellent. In particular the closeness between the experimental and predicted values of C for benzene is excellent evidence that little band spreading arose from the injection and detection systems. The somewhat larger discrepancies for the retained solutes probably indicate that there was a significant contribution from stationary phase mass transfer.

TABLE V

PHASE COMPOSITION OF ODS-HYPERSIL AND ODS-CAPILLARY[2]

Properties	*Value*
Properties of ODS-Hypersil (manufacturers)	
Weight-% C	9.6%
Surface area of Hypersil	190 $m^2 g^{-1}$
Specific volume of Hypersil	0.45 $cm^3 g^{-1}$
Specific pore volume	0.72 $cm^3 g^{-1}$
Interparticle volume (for 40% porosity)	0.78 $cm^3 g^{-1}$
Derived properties of ODS-Hypersil	
Weight ratio of ODS to silica	0.124
Volume occupied by ODS (assumed density 780 kg m^{-3})	0.16 $cm^3 g^{-1}$
Volume occupied by eluent in ODS-Hypersil column	1.34 $cm^3 g^{-1}$
Area of silica gel per cm^3 of eluent	142 $m^2 cm^{-3}$
Phase ratio for ODS-Hypersil	0.093 g cm^{-3}
Weight of ODS in 5 × 100 mm column packed with ODS-Hypersil	125 mg
Chromatographic property	
Phase ratio ODS-Hypersil / Phase ratio ODS-capillary	35
Derived properties of ODS-capillary	
Phase ratio for ODS capillary	$2.7 \cdot 10^{-3}$ g cm^{-3}
Area of silica gel per cm^3	4.0 $m^2 cm^{-3}$
Internal area of smooth 60-μm bore tube	0.067 $m^2 cm^{-3}$
Surface roughness factor	60
Weight of ODS in 3.3-m long capillary	25 μg

The stationary phase contribution to C is given by the last term of eqn. 13. In calculating values of C_{Golay} this term was ignored on the grounds that a unimolecular ODS layer would be so thin as to provide no resistance to mass transfer. However, we have now shown that the etched surface must have about 60 times the area of the smooth walled tube. It is therefore likely that the etched region will contain pores of significant depth, so that the root mean square thickness of the stationary

zone d_f may well no longer be negligible. The thickness of this zone may be calculated if we assume that the difference C_{exp}—C_{Golay} is due entirely to the stationary zone mass transfer term given by

$$C_{stat.zone} = \frac{2}{3} \cdot \frac{k'}{(1+k')^2} \cdot \frac{D_m}{D_s} \left(\frac{d_f}{d_c}\right)^2 \quad (29)$$

We assume for this purpose that $D_m/D_s = 1.7$. Table VI then gives the relevant values of d_f/d_c and d_f.

TABLE VI

STATIONARY ZONE MASS TRANSFER AND STATIONARY ZONE THICKNESS, d_f

Solute	k'	C_{exp}	C_{Golay}	$C_{stat.zone}$	d_f/d_c	d_f (μm)
Benzene	0.11	0.020	0.015	0.005	0.22	13
Naphthalene	0.56	0.056	0.033	0.023	0.30	18
Biphenyl	1.22	0.079	0.052	0.027	0.31	19
						Mean: 17

The remarkable result of this calculation is that nearly identical values of d_f are obtained for the two well retained solutes. Consistency here suggests that the additional contribution to C does indeed arise from the stationary zone which is of significant depth. The calculated value for d_f is, of course, the root mean square depth of the etched pores and it may well be that their actual volume is very low. To provide the most efficient capillary columns the etching should be very uniform and confined to a very thin layer at the surface of the column.

The data of Tsuda *et al.*[2] on the effect of sample load on H may be compared with the data of Done[17] using Spherisorb ODS. Tsuda *et al.*[2] found that H was increased by about 10% for loads of 2.5 μg of benzene ($k' = 0.11$), 250 ng naphthalene ($k' = 0.56$) and 50 ng of biphenyl ($k' = 1.22$). Done[17] likewise found that the load which gave a 10% increase in H decreased sharply as k' increased. In relation to the weight of stationary phase, which for the ODS-capillary was 25 μg, a 10% overload occurred at weight fractions of 0.1 for benzene, 0.01 for naphthalene and 0.002 for biphenyl. In Done's experiments a 10% overload occurred at a weight fraction of about 10^{-4} for a sample with $k' = 2.2$. Done showed, however, that the absolute increase in H with load was independent of the magnitude of H and therefore that the higher is u (which gives higher H) the larger is the load required to give a specified percentage increase in H. Done's experiments were carried out near the minimum of the (h, ν) curve while those of Tsuda *et al.*[2] were carried out at values of ν around 1000, where h was 10–20 times the value at the minimum. By analogy drawn from Done's data, it would be expected that an ODS-capillary column operated near the minimal plate height would show a 10% increase in h at a load of around 10^{-4}, in good agreement with Done's findings for the packed column.

CONCLUSIONS

The comparison of existing data on capillary HPLC with the Golay theory of chromatography in open tubes shows excellent agreement and proves the general

feasibility of capillary HPLC tubes whose internal diameters are around 50 μm. Comparison of data obtained with an etched 60-μm capillary treated with ODS and data obtained with a column packed with ODS-Hypersil indicates that the etched surface of the capillary had 60 times the area of a smooth walled tube of the same bore (60 μm) and probably had a root mean square depth of about 30 μm. The loading characteristics of the capillary column appear to be similar to those of packed columns, when allowance is made for the different amount of stationary phase in the two types of column.

The theoretical analysis of capillary HPLC confirms that the real practical limitation will undoubtedly be the dispersion caused by the detector. If this can be reduced to 0.001 mm^3, capillary HPLC will become a powerful technique which will be faster than packed column HPLC when $N > 30{,}000$. Indeed, when $N = 1{,}000{,}000$ capillary HPLC is 27 times faster than packed column HPLC. The column bore required for this situation (when the pressure drop is 100 bar and the eluent is aqueous methanol) is about 10 μm. As 50–60 μm capillaries have been successfully operated there seems no reason to suppose that 10-μm capillaries will not be equally successful if the detection problem can be overcome and a sufficiently thin stationary zone can be achieved by careful etching of the internal wall of the tube.

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