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B AND *C* TERMS IN THE VAN DEEMTER EQUATION FOR LIQUID CHROMATOGRAPHY

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SUMMARY

Explicit equations are derived for the *B* and *C* terms in the Van Deemter equation for liquid chromatography, involving diffusion coefficients in the mobile zone (eluent outside the particles), the stagnant mobile phase (eluent inside the particles) and the stationary phase. Longitudinal diffusion rates (*B* term) are determined by the arrested elution method for simple aromatic solutes in methanol and methanol–water mixtures with ODS-Hypersil as packing material. When compared to diffusion rate for the same solutes in eluent, measured by the open-tube method, the rates of diffusion in the stationary ODS phase are about half those in methanol–water mixtures.

C values are obtained by measuring the band-dispersion when the same solutes are eluted from 50-μm and 540-μm ODS-bonded silica gels at high reduced velocities. After allowing for dispersion due to processes in the mobile zone, the *C* values are obtained and found to be of the magnitude predicted by the non-equilibrium theory of Giddings, taking into account the actual rates of diffusion of the solutes in the stationary zone.

It is concluded that the basic kinetic theory of dispersion in liquid chromatography accounts qualitatively and quantitatively for the experimental results, but that more experimental work is required to separate mobile-zone and stationary-zone dispersion processes unambiguously.

INTRODUCTION

It is generally accepted that band dispersion during chromatography in a uniform randomly packed column arises from three independent kinetic processes. These are: molecular diffusion along the axis of the column; inhomogeneous flow, coupled with slow transverse mass transfer in the mobile zone; and slow mass transfer within the stationary zone. In this context, the mobile zone is the flowing fluid outside the particles of the bed, while the stationary zone comprises everything within the particles, that is the particle structure itself, the stationary phase and the stagnant mobile phase or eluent held inside the pores of the particles. Each of the three kinetic processes contributes independently to the band dispersion and therefore provides additive terms in the equation for the plate height. Using dimensionless or reduced

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parameters[1] (see symbols for definitions), an equation for the reduced plate height may be derived.

$$h \equiv \frac{\sigma_z^2}{Ld_p} = \frac{B}{v} + f(v_0) + Cv_0 \tag{1}$$

The first term arises from molecular diffusion and the third from slow mass transfer in the stationary zone. Chromatographers generally agree on their respective velocity dependences. However, there is still some disagreement about the way in which the velocity dependence of the mobile-zone flow term $f(v_0)$ should be expressed. Van Deemter *et al.*[2] in their original paper and others subsequently[3,4] have split $f(v_0)$ into two sub-terms: one is a velocity-independent eddy diffusion term, originally denoted by A, and reflecting the geometry of the packing, while the other takes the form $C_m v_0$ and represents the contribution from slow mass transfer across the moving stream. Giddings[1] has argued that this formulation is erroneous and that the two mechanisms are coupled in such a way that both cooperate to *reduce* dispersion produced by velocity variations within the mobile zone. This means that the contributions must be added harmonically. In its simplest form, the Giddings theory gives

$$f(v_0) = (1/A + 1/C_m v_0)^{-1} \tag{2}$$

Unfortunately, this form is acknowledged to be much oversimplified, as has been demonstrated by experiments in which unretained solutes in glass-bead columns and retained solutes in pellicular-bead columns were used[5,6]. Kennedy and Knox[7] proposed a useful practical compromise, namely $f(v_0) = Av_0^{1/3}$. This appears to hold reasonably well over at least a 100-fold range of v_0.

In all the processes enumerated above, molecular diffusion plays a crucial role. Molecular diffusion can occur in the mobile zone, in the stagnant mobile phase and in the stationary phase. All these types of diffusion contribute to the B term, but only diffusion in the mobile zone (*i.e.* in eluent outside the particles) contributes to $f(v_0)$; diffusion in the stagnant mobile phase and stationary phase contributes to the C term. To understand these dispersion processes more fully, it is necessary to consider the rates of these different diffusion processes and how they may be combined.

We consider first overall molecular diffusion, as it relates to the B term.

The longitudinal variance, σ_z^2, arising from molecular diffusion while a band resides in a column is given, generally, by eqns. 3a to 3c.

$$\sigma_z^2 = 2 D_{eff} t_R \tag{3a}$$

$$= 2 D_{eff} (1 + k')t_m \tag{3b}$$

$$= 2 D_{eff} (1 + k'')t_0 \tag{3c}$$

where D_{eff} is the effective diffusion coefficient of the solute the column. In this context we can regard the column as a regular homogeneous unit. This concept is justifiable on the grounds that σ_z will in practice always exceed d_p by a large factor (*e.g.* 10^2 or more).

Molecular diffusion can be thought of as occurring by a random walk process[8].

Thus, if the column in which diffusion occurs is regarded as being made up of regions in which different diffusion coefficients, D_i, prevail, the effective diffusion coefficient can be written as

$$D_{\text{eff}} = \Sigma D_i t_j / \Sigma t_i \tag{4}$$

where the t_i are the mean residence times of solute molecules in the different regions. The total residence time is $t_R = \Sigma t_i$.

In a packed column molecular diffusion is obstructed by the framework of the particles, which may be considered to prevent those random steps which might otherwise terminate in the solid framework[2,8–10]. Thus, for the diffusion of an unretained solute in a packed column we would write

$$D_{\text{eff}} = \gamma_{\text{m}} D_{\text{m}} \tag{5}$$

where γ_{m} is called the obstructive factor.

For a randomly packed column of impermeable spheres with a porosity of about 40%, γ_{m} is approximately 0.65[9,10] whereas for a column of porous particles, such as Chromosorb or silica gel γ_{m} is larger and may approach unity.

Combining eqns. 3 and 4 and noting that t_{m} is the time spent (on the average) by any fully permeating solute molecule in the eluent, while $k' t_{\text{m}}$ is the time spent (on the average) in the stationary phase, we obtain

$$D_{\text{eff}} = (\gamma_{\text{m}} D_{\text{m}} + k' \gamma_{\text{s}} D_{\text{s}})/(1 + k') \tag{6a}$$

or rearranged

$$(1 + k')\,(D_{\text{eff}}/D_{\text{m}}) = \gamma_{\text{m}} + k' \gamma_{\text{s}} (D_{\text{s}}/D_{\text{m}}) \tag{6b}$$

Eqn. 6b implies that if we make the reasonable assumption that $(D_{\text{s}}/D_{\text{m}})$ is more or less independent of the nature of the solute for any given eluent, we may expect a linear relationship to hold between $(1 + k')\,(D_{\text{eff}}/D_{\text{m}})$ and k'. The intercept will be γ_{m} and the gradient $\gamma_{\text{s}}(D_{\text{s}}/D_{\text{m}})$.

Combining eqn. 6a with eqns. 1 and 3 gives the contribution to h from axial diffusion as

$$h_{\text{diff}} = 2(\gamma_{\text{m}} + k' \gamma_{\text{s}}\, D_{\text{s}}/D_{\text{m}})v \tag{7}$$

where $v = (L/t_{\text{m}})\,(d_{\text{p}}/D_{\text{m}})$.

Turning now to the process of slow equilibration or mass transfer within the stationary zone, we have to consider the rate at which molecules can move around inside the particles. They will do this exclusively by diffusion. For this purpose we initially regard the particles as homogeneous and we denote the effective diffusion coefficient within the stationary zone by D_{sz}.

According to Giddings[1] the stationary-zone mass transfer coefficient, C, of eqn. 1 for a column of packed spheres is

$$C = \frac{1}{30} \frac{k''}{(1 + k'')^2} \frac{D_m}{D_{sz}} \tag{8}$$

If we assume that there is no rate-limiting barrier for transfer of solute molecules between the stationary phase and stagnant mobile phase, we can write, using eqn. 4 again

$$D_{sz} = \frac{\gamma_{sm} D_m t_{sm} + \gamma_s D_s t_s}{t_{sm} + t_s} \tag{9}$$

where the subscript "sm" refers to stagnant mobile phase. Since diffusion within the stagnant mobile phase is much like diffusion in a column of random spheres, we might expect that $\gamma_{sm} \approx 0.6$, provided that steric exclusion of the solute is insignificant —otherwise γ_{sm} may well be below 0.6. If φ is the fraction of total eluent which is stagnant and if we assume no steric exclusion, then $t_{sm}/t_m = \varphi$. Hence

$$D_{sz} = \frac{\gamma_{sm} D_m \varphi + k' \gamma_s D_s}{(\varphi + k')} \tag{10a}$$

or

$$D_{sz}/D_m = (\gamma_{sm} \varphi + k' \gamma_s D_s/D_m)/(\varphi + k') \tag{10b}$$

In handling slow equilibration or slow mass transfer in the stationary zone, as seen from eqn. 8, it is necessary to work in terms of the zone capacity ratio k'' rather than the phase capacity ratio k'. These two parameters are related by the purely geometrical relationship

$$k' = k''(1 - \varphi) - \varphi \tag{11}$$

Inserting this into eqn. 10b gives

$$D_{sz}/D_m = \frac{\gamma_{sm}\varphi + [k''(1 - \varphi) - \varphi](\gamma_s D_s/D_m)}{k''(1 - \varphi)} \tag{12}$$

which transforms eqn. 8 into eqn. 13

$$C = \frac{1}{30}\left(\frac{k''}{1 + k''}\right)^2 \frac{(1 - \varphi)}{\varphi\left(\gamma_{sm} - \frac{\gamma_s D_s}{D_m}\right) + k''(1 - \varphi)\frac{\gamma_s D_s}{D_m}} \tag{13a}$$

or

$$\frac{1}{30C}\left(\frac{k''}{1 + k''}\right)^2 = \left(\frac{\varphi}{1 - \varphi}\right)\left(\gamma_{sm} - \frac{\gamma_s D_s}{D_m}\right) + k''\frac{\gamma_s D_s}{D_m} \tag{13b}$$

Eqn. 13b shows that a plot of $[k''/(1 + k'')]^2/30C$ against k'' should be a straight line with a gradient $\gamma_s D_s/D_m$ (assuming again that D_s/D_m does not depend strongly upon the solute or eluent). It will be noted that the quantity $\gamma_s D_s/D_m$ is also obtained from measurement of D_{eff} so a direct correlation should be possible.

Two independent limiting cases of eqn. 13 exist. If the rates of obstructed diffusion in the stationary and stagnant mobile phases are the same, *i.e.* $\gamma_s D_s = \gamma_{sm} D_m$, we obtain

$$C = \frac{1}{30} \frac{k''}{(1 + k'')^2} \frac{1}{\gamma_{sm}} \tag{14}$$

In this case, C shows a maximum at $k'' = 1$ with $C = 1/120\gamma_{sm}$. This equation is equivalent to eqn. 8.

Alternatively, if the diffusion rate in the stationary phase is negligible, that is $D_s/D_m = 0$, then

$$C = \frac{1}{30} \left(\frac{k''}{1 + k''} \right)^2 \frac{(1 - \varphi)}{\gamma_{sm}\, \varphi} \tag{15}$$

Here, the entire mass transfer process occurs within the stagnant mobile phase: in this case C rises gradually to a maximum value. Fig. 1 shows how the dependence of C upon k'' varies according to the value of $\gamma_s D_s/\gamma_{sm} D_m$. As diffusion in the stationary phase becomes slower in relation to that in the stagnant mobile phase, the value of k'' at which the maximum C value occurs gets larger. A curious feature of

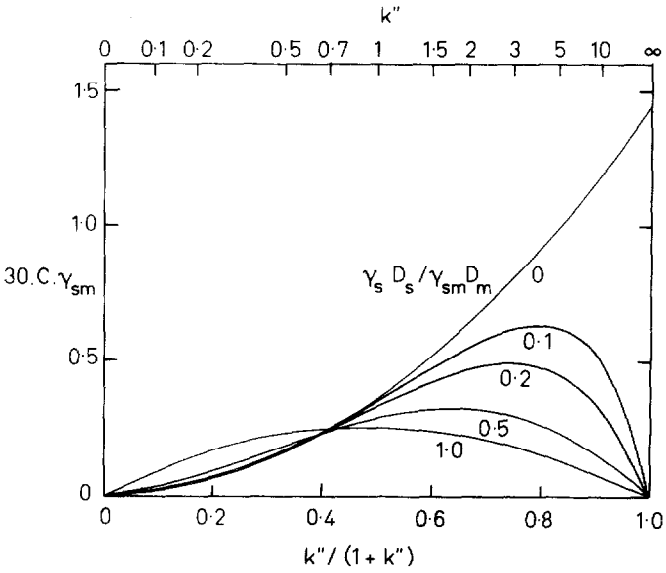

Fig. 1. Dependence of C upon k'' according to eqn. 13a for different values of $\gamma_m D_s/\gamma_{sm} D_m$ with $\varphi = 0.41$.

Fig. 1, at first sight, is that for k'' values below 0.7, C is lower when D_s/D_m is below unity. This is, in fact, an artefact of the treatment: since it has been assumed that solutes are fully permeating, the minimum value of k'' corrsponding to $k' = 0$, as seen from eqn. 11, is $\varphi/(1 - \varphi)$. Insertions of this value of k' into eqn. 13 for C gives

$$C = \frac{\varphi\,(1 - \varphi)}{30\gamma_{sm}} \tag{16}$$

This value is independent of D_s/D_m, as it must be, since the condition $k' = 0$ corresponds to solute spending no time in the stationary phase. The C value is that for a fully permeating unretained solute. Evidently, the part of Fig. 1 to the left of the intersection point has no physical significance.

k'' values below $\varphi/(1 - \varphi)$ can, of course, be achieved if solutes are excluded, either due to steric or enthalpic effects. In this case, we have $t_{sm}/t_m = K\varphi$ where K is the fraction of the pore volume into which the solute can permeate. For solutes that are excluded but not partitioned into the stationary phase, eqn. 14 remains valid, but γ_{sm} may well be low[11].

It is possible for solutes to be both excluded (for steric reasons) and retained by the stationary phase. In this case, we have to write in eqn. 9 $t_{sm}/t_m = K\varphi$ and $t_s/t_m = k' + (1 - K)\varphi$, so that the final expression for C is complicated. Nevertheless the expression will show similar features to those of Fig. 1 and, in particular, an intersection of the curves for different values of $\gamma_s D_s/\gamma_{sm} D_m$. The intersection will occur at a value of k'' for any solute which corresponds to the value it would show if unretained.

EXPERIMENTAL

All measurements were carried out using standard high-performance liquid chromatography components with minor modifications where necessary. The packing materials were fully capped octadecyl-bonded silica gels of the Hypersil type (Shandon Southern, Runcorn, U.K.). Retained solutes (detailed in Tables I and II) were simple oxygenated benzene derivatives, and polynuclear aromatic hydrocarbons

TABLE I

VALUES OF $10^9\ D_m/m^2\ sec^{-1}$ MEASURED BY THE OPEN TUBE METHOD RELEVANT TO *B*-TERM EXPERIMENTS, ADJUSTED TO 26°C

Eluents: A, methanol–water (60:40); B, methanol.

A		*B*	
Solute	$10^9 D_m/m^2\ sec^{-1}$	*Solute*	$10^9 D_m/m^2\ sec^{-1}$
Acetone	1.0	Naphthalene	1.83
Phenol	0.65	Anthracene	1.50
p-Cresol	0.56	Chrysene	1.45
3,4-Xylenol	0.52	Benzo[1]fluorathene	1.33
Anisole	0.75	Naphtho[2,3-*k*]fluorathene	1.22
Phenetole	0.71	Sulphanilic acid	0.92

TABLE II

VALUES OF $10^9 D_m$/m^2 sec^{-1} MEASURED BY THE OPEN-TUBE METHOD RELEVANT TO *C*-TERM EXPERIMENTS, ADJUSTED TO 20°C

Solute	*Methanol–water*			
	60:40	*55:45*	*50:50*	*45:55*
Acetone	0.79	0.72	0.71	0.62
Phenol	0.52	0.47	0.48	0.46
p-Cresol	0.45	0.42	0.43	0.40
3,4-Xylenol	0.41	0.35	0.39	0.36
Anisole	0.60	0.57	0.59	0.52
Phenetole	0.56	0.52	0.49	0.46

giving k' values up to about 20, while sulphanilic acid was used as a representative, partially excluded solute. Eluents were methanol–water mixtures: sodium nitrate was added to control the degree of exclusion for the experiments with sulphanilic acid[14]. Column packings were 5-μm ODS Hypersil, a fully capped ODS-bonded silica gel, and in-house preparations of 50-μm and 540-μm ODS bonded and capped silica gels. They were prepared by methods similar to those used in preparing ODS-Hypersil.

The experiments fell into three groups:

(a) Measurement of D_m values for the various solutes in the eluents used by the open-tube elution method[12,13].

(b) Measurement of D_{eff} values by the arrested elution method of Knox and McLaren[9] in a column packed with 5-μm ODS-Hypersil.

(c) Measurement of C values at high reduced velocities of elution (ν = 300–15,000) from columns packed with large particles (50- and 540-μm mean diameter), bonded by the same technology as used for ODS-Hypersil.

The units of equipment used for the experiments a, b and c are listed in Table III.

For columns packed with 5- and 50-μm materials, k' was determined from the normal chromatograms by using the refractive index peak to define t_m. In order to calculate k'' (see eqn. 11), it is necessary to know the fraction of mobile phase which is stagnant, φ. Knox *et al.*[14] obtained a value of φ = 0.38 when using a range of permeating and fully excluded solutes and ODS Hypersil as packing. In that work, values of φ = 0.35 for 5-μm Hypersil and φ = 0.41 for 50-μm spherical ODS silica gel were obtained from the elution time of the solvent disturbance (refractive index) peak and that of sulphanilic acid when fully excluded with methanol as eluent.

Eluent flow-rates and V_m-values were determined by weighing the eluate on an Oertling F22TD electronic balance, the output of which was fed simultaneously with that of the UV detector to a twin-pen Bryans 28200 potentiometric recorder. Knowing the density of the eluent, the volume flow-rate, f_V, and the elution volumes V_m or V_R could be found. Peak widths were measured either at half height or at the base, after drawing tangents at the points of inflection.

For the 540-μm material it was not possible to determine t_0, t_m or k' directly from the recorder trace because of the low column efficiency ($n < 20$) and the non-Gaussian peak shape. Instead V_m was obtained by weighing the column, filled first

TABLE III

UNITS OF EQUIPMENT AND PACKING MATERIALS

Suppliers: Shandon Southern Products, Runcorn, U.K., Orlita, Giessen, F.R.G., Haskel Engineering, Burbank, CA, U.S.A., Cecil Instruments, Cambridge, U.K., Bryans Southern Instruments, Mitchem, U.K., Oertling, Orpington, U.K., Rheodyne, Berkeley, CA, U.S.A., Valco Instrument, Houston, TX, U.S.A.

	(a) *Measurement of D_m*	*(b)* *Measurement of D_{eff}*	*(c)* *Measurement of C*
Pump	Gravity Feed	Orlita DMP 1515 micro-dosing pump	Haskel air pressure intensifier pump
Injector	20-μl Rheodyne valve	20-μl Valco valve	20-μl Valco Valve
Column	Open pyrex glass tube; length 10.9 m, bore 0.412 mm. Coil diameter 110 mm, 30°C. $u \approx 2$–5 mm sec^{-1}	250 × 8 mm stainless-steel Shandon pattern, 26°C; $u \approx 0.5$ mm sec^{-1}	500 × 8 mm (50 μm) stainless-steel Shandon pattern or 1000 × 8 mm (540 μm) ditto, 20°C (ambient)
Packing	none	Shandon 5-μm ODS Hypersil	50- and 540 μm spherical fully capped ODS-silica gel (Hypersil type)
Detector/thermostat	Shandon liquid chromatography detector/oven unit. Variable-wavelength UV detector 8-μl flow-cell	Water thermostat Cecil CE212 variable-wavelength UV detector, 8-μl flow-cell	Detector as for (b) or fixed-wavelength (254 nm) Du Pont UV detector type 410
Flow-rate and data recording	Bryans 28200 potentiometric recorder	Oertling F22TD recording electronic balance model. Bryans 28200 twin-pen recorder	As for (*b*)

with methanol and then with dichloromethane. The difference in weights divided by the difference in densities of the two liquids gave V_m. V_0 was assumed to be 59% of V_m, as for the 50-μm material. V_R and σ_V values for any eluted peak were obtained by digitizing the recorder output and then finding the first moment and second moment of the peak about the mean.

The following specific points are relevant to experiments a, b and c.

(a) Determination of D_m

Samples (20 μl) of solutes in dilute (≈ 0.1 *M*) solution were injected by valve into a 10.9-m long tube coiled to a diameter of 110 mm. The tube was produced on a Shimadzu glass drawing machine (Shimadzu Seisa Kusho, Kyoto, Japan). Its diameter, 0.412 mm, was found from the weights of the tube empty and full of water. D_m was calculated from the Taylor-Aris[12] equation:

$$\sigma_z^2 = 2D_m t_m + d_c^2 L^2 / 96 t_m D_m \qquad (17)$$

in relation to which the following points are noted:

(i) At the lowest velocity used, namely $u = L/t_m = 2$ mm sec ^{1}, the first term is $\approx$ 4000 times smaller than the second, and can therefore be ignored; (ii) at the normal flow velocity used of 5 mm sec^{-1}, a typical value of σ_V, the volume standard deviation ($\sigma_V = \pi d_c^2 \sigma_z/4$), given by eqn. 17, is about 40 μl: the error in D_m arising from the extra-volume dispersion was less than 5%; (iii) other corrections, as detailed by Wakeham[13] proved to be negligible; (iv) secondary flow in helical tubes improves radial mixing at sufficiently high linear velocities[15]. Thus σ_z-values will be lower than those predicted by eqn. 17, and D_m-values derived from the equation may be expected to rise as u increases in the transition region. According to Tijssen[15], this region starts at velocities for which

$$De^2Sc \equiv u^2 d_c^3 \rho/\eta D_m d_{coil} \geqslant 30 \tag{18}$$

(De = Dean No., Sc = Schmidt No.).

For our system, with $u = 5$ mm/sec the left side of the inequality (eqn. 18) was about 10 20 and therefore below the critical value. Experimental proof that secondary flow was not, in fact, causing any error in the measurement of D_m was obtained by measuring D_m for acetone in methanol–water (60:40) at 20°C over a twofold range of u (*i.e.* a fourfold range of De^{2}Sc). Values of $10^9 D_m$/m^2 sec^{-1} and of u/mm sec^{-1} were as follows: 0.82, 5.7; 0.78, 4.8; 0.78, 3.6; 0.80, 2.7. The absence of any systematic trend confirms that secondary flow was unimportant.

D_m values were usually measured at 30°C (the lowest temperature at which the Shandon oven unit could be stabilized). Each value was the mean of six or more measurements. Correction to nearby temperatures was made by the equation

$$\eta D_m/T = \text{constant} \tag{19}$$

Viscosities were measured by a capillary viscometer.

(b) Arrested elution method

According to this method[9] a band of solute is first eluted part way along a chromatographic column; elution is then arrested for a measured time, and finally the band is eluted at a measured flow-rate. Band-spreading arising from axial diffusion is independent of whether the band is moving or static, and is given by

$$\sigma_z^2 = 2D_{eff} t_R \tag{3a),(20}$$

where t_R is the total residence time in the column. Band-spreading also arises from other effects so that the total variance can be given as:

$$\sigma_z^2 = \sigma_{z,0}^2 + 2D_{eff} t_R \tag{21}$$

where $\sigma_{z,0}^2$ is the variance arising from processes other than axial diffusion and is expected to be the same, irrespective of the delay time, provided that the flow velocity during the initial and final elution stages is kept fixed.

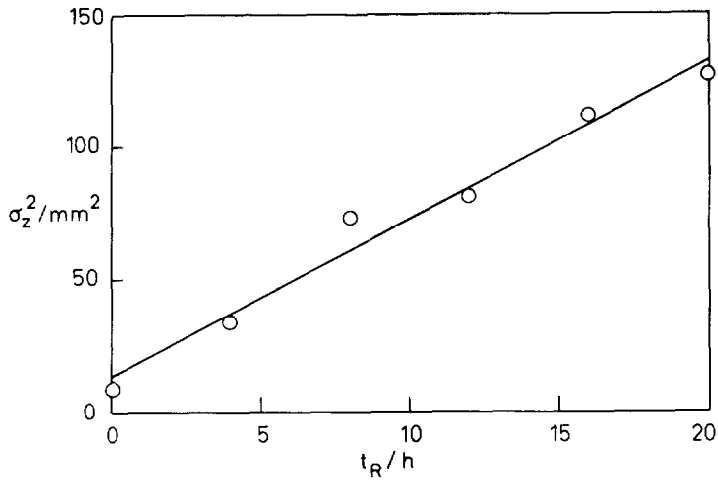

Fig. 2. Example of data from an arrested elution experiment. Packing, 5-μm ODS Hypersil; solute, acetone; eluent, methanol water (60:40); temperature, 26°C. $D_{eff} = 8.3 \cdot 10^{-4}$ mm^2/sec.

A plot of σ_z^2 against t_R will give D_{eff}. Such a plot is shown in Fig. 2, from which it may be calculated that $D_{eff} = 2.96$ mm^2 h^{-1} $= 8.2 \cdot 10^{-10}$ m^2 sec^{-1}. In order to obtain good values for D_{eff}, it is desirable to make $\sigma_{z,o}^2$ as small as possible. This is achieved by using small particles of packing material. In the present experiment, 5-μm ODS Hypersil (Shandon Southern) was used. This is a fully-capped ODS-bonded material with a negligible content of exposed silanol groups.

In practice, σ_z, being the standard deviation of the band measured as a length within the column, cannot be directly determined. Accordingly, the solute band was eluted through the detector and the eluate weighed on the recording electronic balance. The signals from detector and balance were fed to a twin-pen recorder. σ_z was then obtained from the following formula

$$\sigma_z = \sigma_t \left(\frac{dm}{dt}\right) \frac{L}{\rho\,(1 + k')\,V_m}$$

$$= \sigma_m \frac{L}{\rho\,(1 + k')V_m} \tag{22}$$

where σ_t and σ_m are standard deviations measured in time and mass units, dm/dt is the rate of increase of the mass of collected eluate, and ρ is the density of the eluate.

(c) Determination of C values

According to basic chromatographic theory, the reduced plate height, h, depends upon reduced velocity according to an equation of the form

$$h = \frac{B}{v} + f(v_0) + Cv_0 \qquad (1),(23)$$

To isolate the C term, associated with slow mass transfer in the stationary zone, it is desirable to make the other two terms negligible. The B term can be made negligible compared to the C term by working at $v \geqslant 100$, but it is not so simple to reduce $f(v_0)$ to negligible proportions. Unfortunately, the form of $f(v_0)$ and its dependence upon k'' is not well established, especially for retained solutes[1–7]. Almost certainly, its dependence upon both v_0 and k'' is weaker than that of the C term, and, by working at sufficiently high reduced velocities (*e.g.* around 1000), its effect can be much reduced. Since values of $v_0 > 100$ cannot readily be attained with 5-μm particles (*e.g.* for $v_0 = 100$, an aqueous eluent, and a 100-mm column, $u_0 \approx 20$ mm sec^{-1}, $\Delta p \approx 1000$ bar), we have used 50- and 540-μm particles. These were prepared and subsequently ODS-bonded and capped by proprietary procedures, similar to those used in the manufacture of ODS-Hypersil (Shandon Southern). With such particles, reduced velocities in the ranges 600–4000 (50-μm particles) and 1500–15,000 (540-μm particles) were easily attained. Fig. 3 shows a typical plot of h versus v_0 for the 50-μm material. The best straight line through the data, fitting $h = A_0 + C_1 v_0$, gives $A_0 = 21$, $C_1 = 0.0165$. The best curve (also shown), fitting $h = A v_0^{1/3} + C v_0$, gives $A = 2.5$ and $C = 0.0125$. The first approximation is not theoretically acceptable: the Van Deemter A cannot be as high as 20, as this would imply a column the minimum plate height of which could never fall below 20 d_p. The second approximation is more realistic, since $A = 2.5$, while not indicating a particularly well packed column, is at least within an acceptable range. However, within the limits of accuracy of the data, the term $2.5 v_0^{1/3}$ is well approximated over the range $600 < v_0 < 4000$

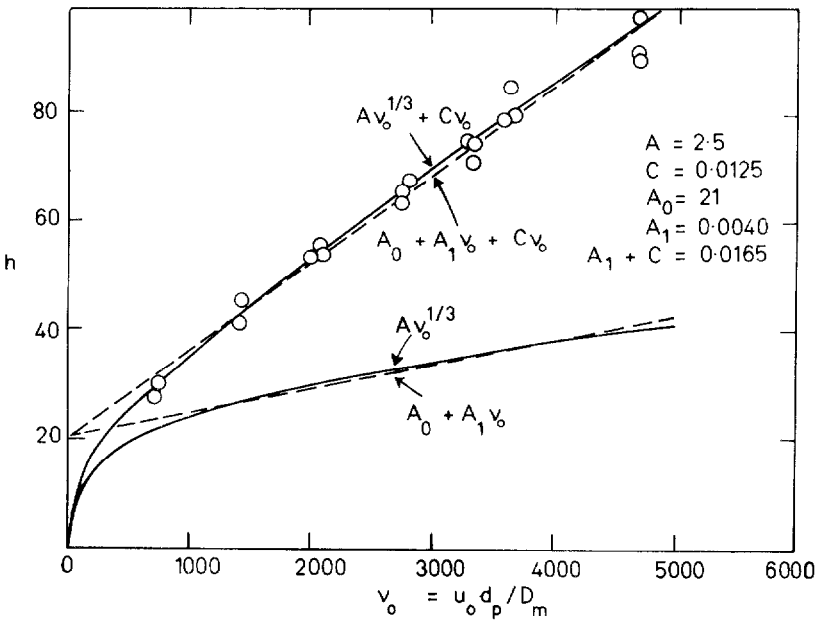

Fig. 3. Example of data for determination of C. Packing, 50-μm ODS bonded silica gel; solute, phenol k'' = 1.86; eluent, methanol water (60:40); temperature, 20°C.

by $21 + 0.040v_0$. Thus, the equation which we believe best represents the dynamic processes within the column, namely

$$h = Av_0^{1/3} + Cv_0 \tag{24}$$

where $A = 2.5$, $C = 0.0125$ can be adequately approximated by eqn. 25 over this particular range of v_0 by

$$h = A_0 + A_1v_0 + Cv_0 = A_0 + C_1v_0 \tag{25}$$

where $A_0 = 21$, $A_1 = 0.0040$ and $C = 0.0125$ or $A_0 = 21$ and $C_1 = 0.165$.

The simplest way to allow for the dispersion due to processes in the mobile zone is, therefore, to subtract 0.0040 from the value C_1, which is obtained by fitting the best straight line to the (h,v_0) data. This procedure does, of course, assume that the A term has no k' dependence, an assumption which could be in error but can only be checked by further, more accurate experiments. These ought to involve a comparison of porous and pellicular materials of well-defined structure.

RESULTS AND DISCUSSION

Measurement of D_m

Tables I and II list the values of D_m obtained for the solutes used in experiments b and c. They were measured at 30°C and corrected by eqn. 19 to 26°C (for experiments b) and 20°C (for experiments c).

Measurements of D_{eff}

D_{eff} was measured as described above by the arrested elution method. These values, combined with those of D_m listed in Table I, provide D_{eff}/D_m. Fig. 4 plots D_{eff}/D_m against k', while Fig. 5 plots $(1 + k')\ D_{eff}/D_m$ (see eqn. 6b) against k'.

For the oxygenated benzene derivatives and acetone, D_{eff}/D_m falls gently with k', whereas $(1 + k')\ D_{eff}/D_m$ rises with k'. The gradient of the plot in Fig. 5 is $\gamma_s D_s/D_m \approx 0.55 \pm 0.05$ while the intercept γ_m is about 0.9. No such regularity is observed for the polynuclear aromatic hydrocarbons. D_{eff}/D_m appears to fall quite steeply with increase of k' while $(1 + k')\ D_{eff}/D_m$ shows a weak rise. However, the data are too scattered to allow the derivation of a value of $(\gamma_s D_s/D_m)$. However, if we assume an intercept γ_m of 0.9, the maximum reasonable value of $\gamma_s D_s/D_m$ is about 0.3 and the minimum zero. The scatter of the data does not seem to be due to experimental error, since the standard errors of the values (calculated from the σ_z^2 *versus* t plots) are relatively small.

Bearing in mind that D_m values in methanol (Table I) are about three times those in methanol water, it is not unreasonable that $\gamma_s D_s/D_m$ should be at least three times smaller than in water–methanol mixtures.

We conclude, therefore, that the $\gamma_s D_s$ values for solutes in the bonded ODS layer are about half of what they are in a relatively viscous eluent, such as methanol–water (60:40).

For sulphanilic acid which, being ionized, is partially excluded from the nega-

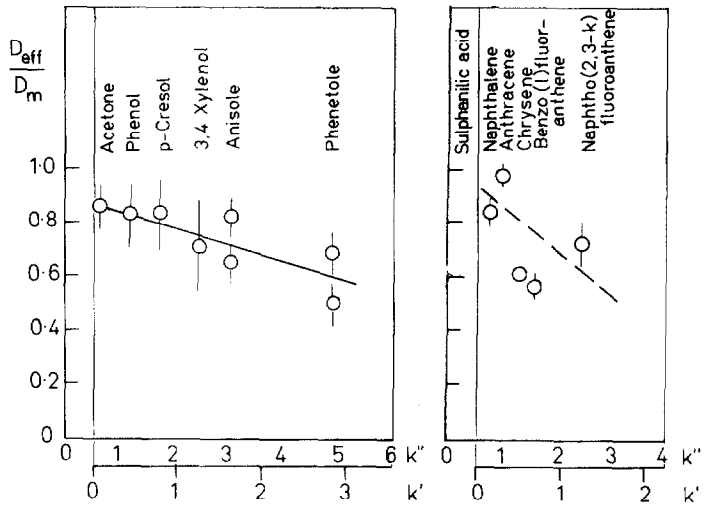

Fig. 4. Dependence of D_{eff} upon k' for simple aromatic substances. Packing, 5-μm ODS-Hypersil; eluent, methanol–water (60:40); temperature, 26°C.

tively charged ODS-bonded materials[14] D_{eff}/D_m values are constant at 0.65 for a range k'' of zero to 0.26 (the last corresponding to about 50% permeation of the pore volume of the packing). The value of the obstructive factor $\gamma_m = 0.65$, is very much in agreement with that expected for a completely excluded solute the diffusion region of which would be similar to that in a column of impermeable particles[9,10].

Measurements of C values

C values were measured for the solutes and eluents listed in Table II. These were obtained from *h vs.* v_0 plots for each solute in each eluent. As described above, the gradient of the best straight line through the *h vs.* v_0 data was first found. The

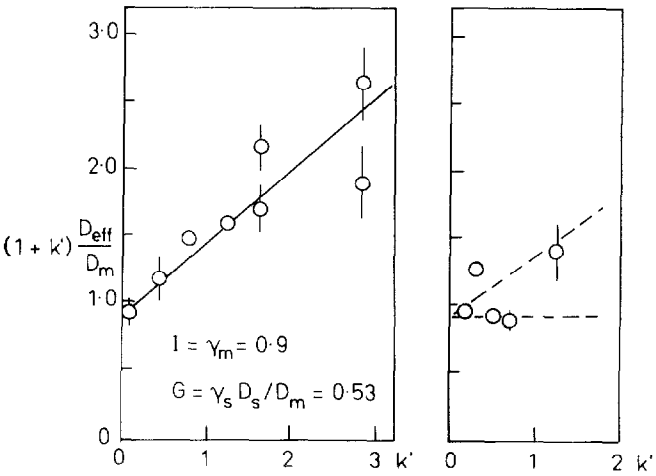

Fig. 5. Data for D_{eff} from Fig. 4 plotted according to eqn. 6b.

assumption of $A = 2.5$ then allowed true C values to be obtained from the gradient of these lines by subtracting 0.004 for the 50-μm particles and 0.002 for the 540-μm particles. The values of C so obtained are plotted against k'' in Fig. 6. The same data are plotted according to eqn. 13b in Fig. 7.

In both figures it is clear that there is a fairly wide scatter of points. This is largely due to the difficulty of obtaining accurate values of C with the experimental system used. There is no evidence of any systematic variation in C from one component to another; there is as much variation in the C values within the data for a single solute as across the different solutes. Fig. 6 shows that, broadly speaking, C has a maximum value at low k'' and decreases as k'' increases to high values. This is in accord with moderately rapid diffusion in the stationary phase.

The curves drawn in Fig. 6 follow eqn. 13a with parameters derived from Fig. 7. In Fig. 7 it is clear that $(1/C)\,[k''/(1 + k'')]^2$ rises rapidly with k', implying that $\gamma_s D_s/D_m$ has a significant value. The gradient of the median line is, in fact $\gamma_s D_s/D_m = 0.5 \pm 0.1$. This value is close to 0.55, derived from measurements of D_{eff} reported above. The intercept in Fig. 7 gives a value of $[\varphi/(1 - \varphi)]\left(\gamma_{sm} - \dfrac{\gamma_s D_s}{D_m}\right) \approx 0.35$. Since $\varphi/(1 - \varphi) = 0.70$ we obtained $\left(\gamma_{sm} - \dfrac{\gamma_s D_s}{D_m}\right) \approx 0.5$. Use of the value established for $\gamma_s D_s/D_m$ then gives $\gamma_{sm} \approx 1.0$. This is an unexpectedly high value compared with the expected value of 0.6. This is probably due to an accumulation of factors: in particular the treatment of the data is very sensitive to the value of both A and φ.

The data obtained with the 540-μm particles show distinctly higher C values than those from the 50-μm material after assuming the same A value of 2.5. The data do, however, confirm that the reduced-parameter approach is valid. Had mass trans-

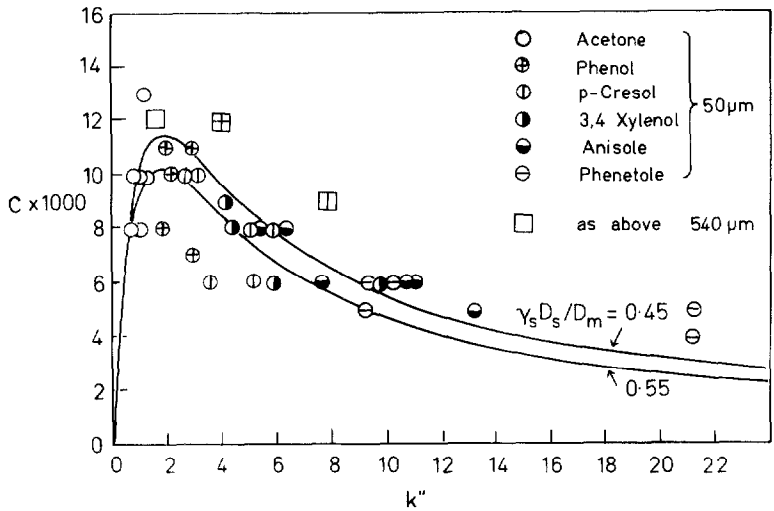

Fig. 6. Dependence of C upon k''. Packings, 50- and 540-μm ODS-bonded silica gels; solutes and eluents listed in Table III; temperature, 20°C. Lines drawn according to eqn. 13a with $(\gamma_{sm} - \gamma_s D_s/D_m)\,\varphi/(1 - \varphi) = 0.35$ and $\gamma_s D_s/D_m$ values as shown on lines.

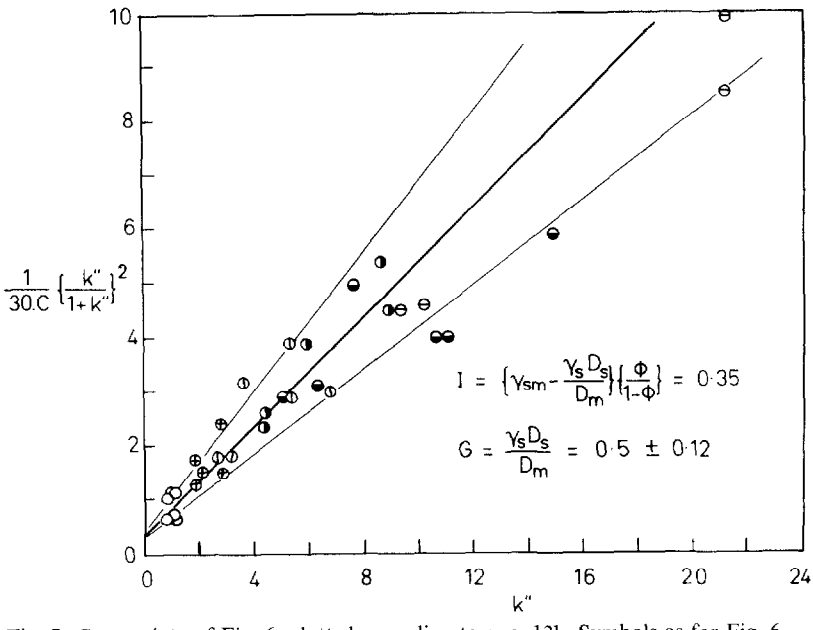

Fig. 7. *C*-term data of Fig. 6, plotted according to eqn. 13b. Symbols as for Fig. 6.

fer processes other than diffusion been important, for example interfacial mass transfer between the stationary and mobile phases, then the *C* values from the larger particles should have been well below those for the 50-μm particles. The data from the 540-μm material confirm that diffusion processes across distances scaled to the particle size predominate in achieving equilibration between the different regions in the column.

OVERALL CONCLUSIONS

The main conclusions to be drawn from the work reported above are:

(1) New theoretical equations have been derived for the *B* and C terms in the equation for the reduced plate, which recognise contributions from the different rates of diffusion in different regions of the packed column.

(2) The basic theory of dispersion by axial molecular diffusion, flow inhomogeneity in the mobile zone, and slow mass transfer within the stationary zone holds qualitatively and quantitatively in liquid chromatography.

(3) The most appropriate formulation of the velocity-dependence of the contribution to the reduced plate height from flow in the mobile zone is $Av_0^{1/3}$ although, over a limited velocity range (say up to a factor of 10), the form $A_0 + A_1 v_0$ is adequate. However, for reduced velocities in the region of 1000, A_0 has a large value of about 20. Since both A_0 and A_1 depend on the velocity range being used, unlike *A*, they do not have any simple geometrical significance.

(4) Measurements of D_{eff} by the arrested elution method and *C* by elution at high reduced velocities give values of $\gamma_s D_s/D_m$ which are self-consistent and around 0.5 when the stationary phase is a unimolecular bonded layer of octadecyl groups on

silica gel and the eluent or mobile phase is methanol–water (60:40). Diffusion thus occurs at comparable rates in the two phases. The same experiments provide more or less self-consistent values of γ_m and γ_{sm} close to unity. For sulphanilic acid under conditions of total or partial exclusion $\gamma_m = 0.65$ is in excellent agreement with previously measured values of the obstructive factor in glass bead columns using gases.

The experiments described indicate the following directions for future work.

(a) It is crucial to further tests of the plate height model to achieve a higher degree of precision in the measurement of both D_{eff} and C. Equipment of the highest quality will be required, coupled with computer handling of the output of detector and ancillary equipment.

(b) The form of $f(v_0)$, the flow contribution to the plate height, should be established for retained solutes: very little is known of the dependence of $f(v_0)$ on k''. To some extent, this can be achieved simply by improving the precision of measurement of h at high reduced velocities, using fully porous packing materials. However, it would preferable to obtain $f(v_0)$ by an independent experiment. This should be possible by using pellicular materials with a measured but extremely thin pellicular layer of, say, 1-μm thickness on a 50-μm diameter bead.

(c) Given data of high precision, it is of importance to establish whether D_s/D_m is a function of the solute type, especially in adsorption chromatography where there is discussion about the mobility of solutes on the adsorbent surface[16].

(d) It is important to establish how D_s/D_m for any solute depends upon the eluent. This will give an indication of the true configuration of the stationary phase, whether it has to be regarded as a material of fixed geometry (in which case D_s would be expected to be constant) or as analogous to a swollen polymer (in which case D_s would tend to follow D_m).

The present work provides a self-consistent framework for studying dispersion processes in liquid chromatography enabling quantitative data to be obtained on diffusion rates in different regions of a packed column.

SYMBOLS

A, A_0, A_1	Constants in equation for reduced plate height relating to dispersion by flow
B	As for A, but relating to dispersion by axial molecular diffusion
C, C_1, C_m	As for A, but relating to slow mass transfer: in the stationary zone; overall; in the mobile zone
D_{eff}, D_i, D_m, D_s, D_{sz}	Diffusion coefficients: effective in the column as a whole; in region i; in the mobile phase (unobstructed); in the stationary phase (unobstructed); in the stationary zone
d_c, d_{coil}, d_p	Diameters of: open tube; helical coil; particle
De	Dean number
H, h	Plate height; reduced plate height ($h = H/d_p$)
K	Exclusion coefficient = fraction of pore volume accessible to a solute
k', k''	Phase capacity ratio; zone capacity ratio $k' = (t_R - t_m)/t_m$; $k'' = (t_R - t_0)/t_0$

L	Column or tube length
m, $\mathrm{d}m/\mathrm{d}t$	mass of eluent; flow-rate of eluent in mass per unit time
N	Number of theoretical plates
Sc	Schmidt number
T	Absolute temperature
t_i, t_m, t_0, t_R, t_{sm}	Time of residence: in region i; in mobile phase; in mobile zone; in column (retention time); in stagnant mobile phase
u, u_0	Linear velocity of: mobile phase; mobile zone
γ_m, γ_s, γ_{sm}	Obstructive factors for diffusion: in mobile phase; in stationary phase; in stagnant mobile phase
η	Eluent viscosity
φ	Fraction of mobile phase which is stagnant
ρ	Density of eluent
σ_m^2, σ_t^2, σ_z^2, $\sigma_{z,0}^2$	Variance of peak: in mass units; in time units; as distance within column; variance arising from processes other than axial molecular diffusion as distance within column
v, v_0	Reduced velocity of mobile phase; reduced velocity of mobile zone. $v = L\,d_p/D_m t_m$; $v_0 = L d_p/D_m t_0$

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