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Band dispersion in chromatography – a new view of *A*-term dispersion

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Abstract

Flow dispersion in chromatography arises from several independent kinetic processes. We show that in liquid chromatography by far the most important contribution to dispersion under practical operating conditions comes from the flowing part of the mobile phase. Contrary to widely held belief, slow mass transfer within the porous particles normally used in HPLC is unimportant. This is demonstrated by data from both gas and liquid chromatography. The consequences of this change of emphasis are important. Better packing of HPLC columns can certainly produce much more efficient columns. Previous interpretations of the rates of mass transfer of partially excluded polymers in size-exclusion chromatography should be reassessed. Further work should be carried out with monodisperse particles in both HPLC and CEC. New structures made by microfabrication methods hold the promise of much higher efficiencies. Turbulent flow chromatography may yet provide good efficiency by using pellicular particles.

Keywords: Band dispersion; Dispersion

1. Band dispersion up to 1960

The first realistic theory of band dispersion in chromatography was the plate theory of Martin and Synge [1] who in 1941 defined the height equivalent to a theoretical plate (HETP) as ''the thickness of the layer such that the solution issuing from it is in equilibrium with the mean concentration of solute in the non-mobile phase throughout the layer.'' This was the first recognition that imperfect equilibrium between the mobile and stationary phases was a major cause of band broadening in chromatography. Giddings [2,3] (1963) was later to develop his masterly non-equilibrium theory to give mathematically rigorous expressions for dispersion arising from this phenomenon. This theory was fully presented in his classic book ''Dynamics of Chromatography – Part 1'' published in 1965 [4].

Following Martin and Synge, little progress was made with the theory of dispersion until 1956 when van Deemter et al. [5] proposed their famous equation for the then new technique of gas chromatography (GC). They recognised that in addition to non-equilibrium as conceived by Martin and Synge there were two further contributions to band broadening, the first arose from the complex flow pattern in a packed bed, and the second from axial molecular diffusion. Their final equation [6], published at an Informal Gas Chromatography Symposium in 1957 took the form:

$$H = 2\lambda d_p + 2\gamma D_m/u + (1/100)\{k'/(1+k')\}2d_p^2 u/D_m + (2/3)\{k'/(1+k')^2\}d_f^2 u/D_s \quad (1a)$$

$$= \boldsymbol{A} + \boldsymbol{B}/u + \boldsymbol{C}_m u + \boldsymbol{C}_s u \quad (1b)$$

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The *A* term represented the dispersive contribution from the flow profile or Eddy diffusion as van Deemter et al. called it, and it was considered to be independent of the flow velocity, *u*, the *B* term arose from axial molecular diffusion and was inversely proportional to velocity, and the *C* terms or mass transfer terms were proportional to velocity. The C_m term arose from slowness of equilibration or mass transfer in the mobile (gas) phase, and the C_s term from slowness of equilibration or mass transfer in the stationary phase.

The following year, 1958, saw Golay [7] make his classic contribution to the theory of chromatography at the Amsterdam Symposium by working out a mathematically precise solution for the dispersion in the open tube. This equation contained no *A* term in the van Deemter sense, but only the *B*, C_m and C_s terms. The Golay treatment provided a mathematically rigorous expression for the plate height arising from the elution of a solute through an open tube whose wall was lined with a uniform layer of stationary phase. It was an extension of earlier expressions due to Taylor [8] (for an unretained solute in an uncoated open tube) and to Aris [9] (for a retained solute in a tube where the eluent showed a plug flow profile). The Aris equation has recently turned out to be informative in capillary electrophoresis (CE) where plug flow occurs.

At this time the main conclusion drawn from these equations, particularly for the packed tube, was that *H* had a minimum value, and that one should try to use chromatographic conditions which enabled one to work close to this minimum *H*. There was little attempt to match experimental data against theory in a quantitative way, other than to show that the general form of the equations was correct.

2. Reduced parameters

It was during the decade that followed Golay's contribution, that Calvin Giddings made his seminal contributions to the theory of chromatography which he brought together in ''Dynamics of Chromatography'' [4]. Giddings saw that there had to be a general unity which embraced all forms of chromatography and that this unity could be encapsulated by defining dimensionless parameters, namely the reduced plate height and reduced velocity:

$$\text{Reduced plate} \quad h = H/d_p \tag{2}$$

$$\text{Reduced velocity} \quad \nu = ud_p/D_m \tag{3}$$

The rationale behind these definitions was that the plate height was in general scaled to the particle size, the degree of granulation of the column, or the tube diameter for an open tubular system, while the kinetic processes, which were responsible for band spreading, were largely governed by a balance between the ''convective'' velocity of the eluent along the column, and the ''diffusive'' velocity of the analyte across a characteristic length such as the particle diameter. Expressing plate height equations in reduced or dimensionless terms generally simplified them considerably. For example Eqs. (1a) and (1b) becomes:

$$h = 2\lambda + 2\gamma/\nu + (1/100)\{k'/(1+k')\}^2\nu + (2/3)\{k'/(1+k')^2\}(d_f/d_p)^2(D_m/D_s)\nu \tag{4a}$$

$$= \boldsymbol{A} + \boldsymbol{B}/\nu + \boldsymbol{C}_m\nu + \boldsymbol{C}_s\nu \tag{4b}$$

This type of equation enabled widely differing chromatographic systems to be compared directly. For example Knox and Saleem [10] in 1969 showed that, with the same column, essentially identical (h, ν) plots were obtained in gas and liquid chromatography as seen in Fig. 1.

The same general form of the (h, ν) plot that is familiar in GC was demonstrated by Laird [11] for high-performance liquid chromatography (HPLC) as seen in Fig. 2.

Reduced plate height–velocity plots were widely exploited by Knox and co-workers for monitoring the performance of HPLC columns in general.

3. The *B* term

At the First Houston Symposium in 1962, Cal Giddings and I had a bright idea during a taxi ride. This was that, since dispersion giving rise to the *B* term was due exclusively to axial molecular diffusion partly obstructed by the packing, the apparent

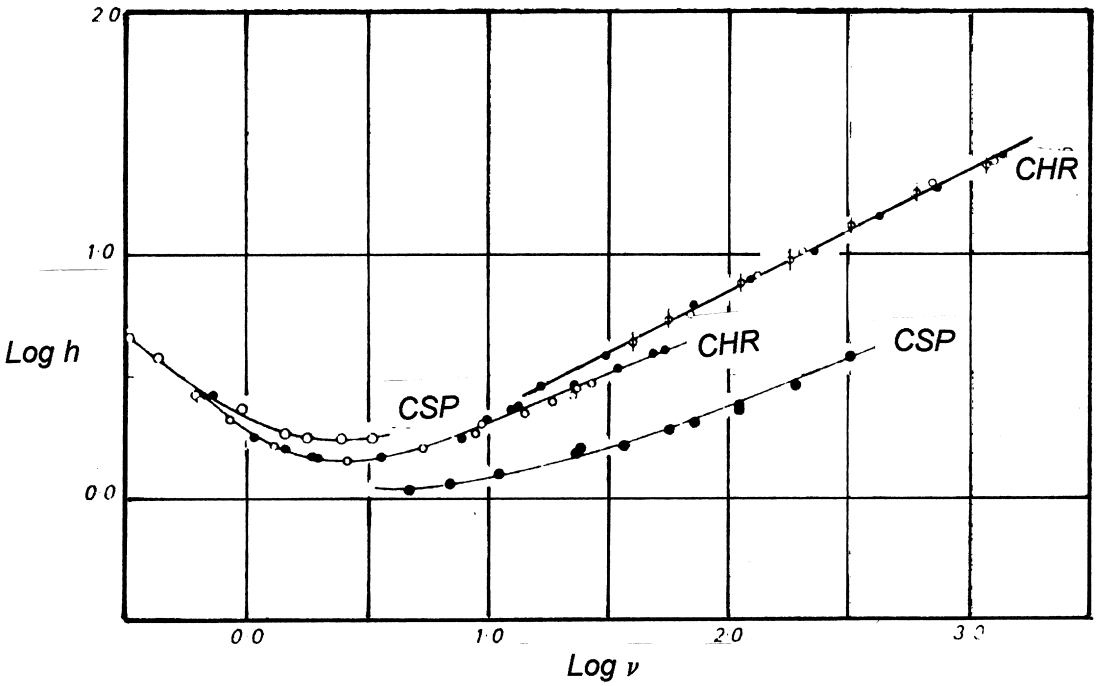

Fig. 1. Demonstration of continuity of gas and liquid chromatography from Knox and Saleem [10]. Solutes unretained; curves for GC on left, curves for LC on right. Packings: CSP = 113 μm controlled surface porosity beads, CHR = 235 μm Chromosorb G particles. Note gradients of plots are in the range 0.3 to 0.5 at high reduced velocities.

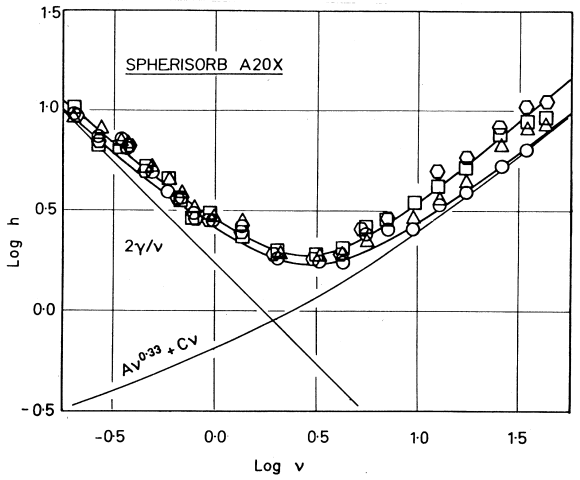

Fig. 2. (Log h, log ν) plot for LC using 20 μm particles of Spherisorb alumina. Data from Laird [11]. Solutes: aromatic hydrocarbons; k' from 0.5 to 3.2.

diffusion rate could be measured by observing the spreading of a peak while it was arrested in the column. Thus the band of analyte would be injected and eluted half way along the column, allowed to broaden for a measured time, and finally eluted at a known rate through the detector. The variance of the band (in length units) when plotted against the time of arrest would give the apparent diffusion coefficient as the gradient. The ratio of this to the diffusion coefficient in an open tube would be the obstructive factor, γ. This was put into effect by Knox and McLaren [12] using ethylene as unretained solute and nitrogen as the carrier gas. We determined γ for a variety of GC columns popular at the time. For columns packed with glass beads γ was close to 0.6. For the porous packing then used in GC, γ was between 0.5 and 0.7.

In GC diffusion in the stationary phase is approximately 10 000-times slower than in the mobile (gas) phase and so it can be ignored as far as the B term is concerned. This is no longer true in liquid chromatography (LC) where the diffusion coefficients in the

two phases are likely to be comparable. The equation for the *B* term is then more complex. If the diffusion paths in the two phases can be thought of as parallel, which is reasonable for a chromatographic packing in which both phases are more or less continuous, the equation for the *B* term takes the form of Eq. (5a):

$$H_B = \sigma_z^2/z = (2\gamma_m D_m + 2\gamma_s D_s k')/u \quad (5a)$$

$$= 2D_{eff}(1 + k')/u = \boldsymbol{B}/u \quad (5b)$$

The alternative form, Eq. (5b) involved an ''effective'' diffusion coefficient, D_{eff}. This is the apparent diffusion coefficient which would be measured by observing how a narrow band kept stationary in the column would broaden by molecular diffusion. Although the diffusion occurs partially in the mobile phase and partly in the stationary phase, the observer is not necessarily aware of this, and can in principle imagine the spreading to occur in a homogeneous body. D_{eff} is exactly what is measured in an arrested elution experiment. Subsequently it can be interpreted in terms of the two diffusion coefficients in the two phases. D_{eff} will, of course be dependent upon k' as a comparison of Eqs. (5a) and (5b) shows. In this way, Knox and Scott [13] found that for ODS silica gel, using methanol–water as eluent, diffusion in the stationary phase occurred at about half the rate that it did in the mobile phase. The concept of an effective diffusion coefficient is also useful when considering slow mass transfer within a single particle of packing, for once again we have regions within the particle of different composition and retentivity but now reticulated on a very small scale (a few Angstrom).

Broadly the axial-diffusion contribution to the plate height is now well understood.

4. The *A* term

When van Deemter et al. proposed their original equation they did not include any C_m term for slow equilibration in the mobile phase and they considered that the dispersion caused by the flow pattern was a purely geometrical effect independent of flow velocity and which could be scaled to the particle size. When they later recognised that slow mass transfer or equilibration in the mobile phase could contribute to dispersion they simply added a further term similar to the C_s term for the stationary phase. The work of Taylor [8] followed by that of Golay [7] clarified the situation by showing that, in an open tube, without transverse diffusion there would be near infinite dispersion due to the wide range of velocities of the stream lines.

Immediately after the Golay contribution it was therefore recognised that there were two processes which took place in the mobile phase which were relevant to band dispersion in a packed column. The first was the tortuous and obstructed flow of eluent through and around the particles of packing; the second was the flow profile in the mobile phase whose dispersive effect was controlled by transverse diffusion. The question was how these two contributions to *H* should be combined. van Deemter et al. [6] simply added them together, and some chromatographers continue to argue that this is the best way to combine them. However Giddings [2,3] gave a different and more logical interpretation. He recognised that the two kinetic processes combined to reduce and control dispersion not to increase it. He argued that, in an open tube, a solute unable to diffuse would be more or less infinitely dispersed since any molecule of the analyte would remain in streamline of constant velocity from injection to elution. To avoid dispersion the individual analyte molecule had to be able to sample the complete range of linear flow velocities in a random way as it moved down the column; it did this by transverse diffusion. However, in a packed column, a molecule of analyte could randomly sample the complete range of flow velocities without moving from any streamline. In a packed column there were therefore two processes which combined to reduce dispersion, transverse diffusion and tortuous flow. Accordingly, Giddings proposed that they be combined harmonically, that is:

$$H_A = \{(1/\boldsymbol{A}) + (1/\boldsymbol{C}_m u)\}^{-1} \quad (6)$$

Eq. (6) implies that the mobile phase contribution at low velocity will be proportional to flow velocity, *u*, but will become constant at high velocity. It also implies that the change will occur over about two-

orders of magnitude in velocity. It was soon recognised that this was an oversimplification and that the transition was likely to be much more gradual. Giddings [4] then proposed that five contributions should be added together covering inhomogeneities of flow and packing ranging from trans-particle to trans-column.

When I was privileged to spend eight months in Gidding's laboratory in Salt Lake City in 1964, I decided to investigate the problem of ''coupling'' and, because of the wide range of reduced velocity which would need to be covered, I decided to work with a liquid eluent and columns packed with glass beads. This also meant that the range of Reynolds number for the experiments would be well below that for turbulence to occur. The experiments showed that flow dispersion rose with a low power of the velocity – about 0.3 – over the reduced velocity range from 10 to 1000. At still higher reduced velocities h flattened off and then fell gradually. Here the Reynolds number was becoming high, and the decline was attributed to the onset of turbulence. For my work in Utah [14], I used glass beads of different sizes in columns of the same diameter. I found that although the (h, ν) plots were parallel there was a substantial dependence of h upon particle size with the smallest particles giving the highest reduced plate heights. Later Parcher [15], in Edinburgh, refined these experiments: he used the same size of particles in columns of different diameter so as to cover the same range of column to particle diameter, ρ. There was now much less dependence upon ρ. Comparative data are shown in Fig. 3.

Comparison of these two sets of results suggests that the ''goodness of packing'' has a strong influence on the size of the A contribution but does not influence the gradient of the (h, ν) plot. This is a surprising but important conclusion which was not recognised at the time. Why were the original (h, ν) curves of Knox parallel when there was a large difference in the ''goodness of packing'' of the columns? We still do not have a good answer to this. A good fit to the (h, ν) dependence with glass bead columns where there is no retention can be obtained either by a weighted integral of the original Giddings formulation, or by a much simpler empirical equation given by Knox and Parcher [15]:

$$h_A = \left(1/A + 1/C\nu^{1/3}\right)^{-1} \quad (7)$$

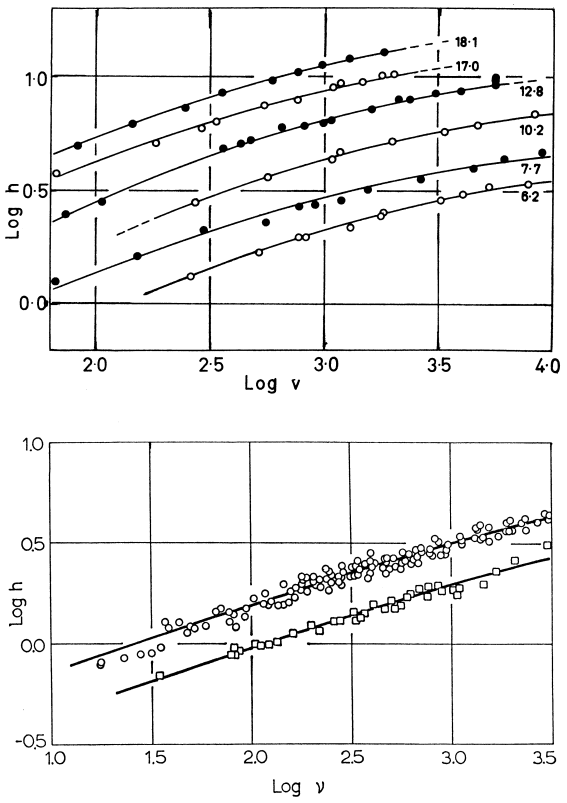

Fig. 3. (Log h, log ν) plots for glass bead columns. Upper: from Knox [14], column diameter 3 mm, particle size varied from 165 μm to 480 μm, column-to-particle diameter ratios given on lines. Lower: from Knox and Parcher [15]; particle diameter 480 μm, column diameter varied from 2.4 mm to 11; lower line – column-to-particle diameter ratios, below 6, upper line – above 6.

For columns with $\rho > 8$, and over the narrower range of reduced velocity 30 to 300, the data of Knox and Parcher [15] were well fitted by

$$h_A = 0.33\nu^{0.35} \quad (8)$$

although they did not establish the dependence for columns packed with porous particles. However, Fig. 1 shows that even with porous particles (lines marked CHR) the gradient of the (log h, log ν) plot is well below unity at around 0.5. With such particles one, of course, expects some contribution to h from slow diffusion into and out of the particles themselves, this contribution being linear in ν. It is thus clear that by 1970, it had already been established that A-term dispersion exhibited a dependence upon

velocity for columns packed both with non-porous glass beads, and with porous non-spherical particles of Chromosorb. These data led to the so-called Knox equation [16] for the plate height in LC

$$h = B/\nu + A\nu^{1/3} + C\nu \quad (9)$$

This equation has been widely used to monitor the performance of LC columns. Empirical fitting of this equation to plate height data such as that shown in Fig. 2 gives representative values, $B \approx 2$, $A \approx 1$, $C \approx 0.1$. The minimum value of h is then around 2.5 at a reduced velocity of about 3. Columns giving lower h values can of course be produced, but it is rare to find commercial columns for which h is less than 2. It has often been claimed erroneously that 2 is the theoretical minimum value of h. There is no justification for this. As shown here, we have well established expressions for the B- and C-term dispersion, although the theoretically predicted values of B and C can be somewhat different from those just quoted. There is, by contrast, no good theoretical value for the A term, which seems to depend strongly on what we have termed rather loosely the ''goodness of packing'' of a column. The value $A \approx 1$ appears to hold for well packed columns used with pressure-driven flow, but when electrodrive is used (see below), the empirical value of A in Eq. (9) can be well below 1. Thus it is quite possible to obtain values for h well below 2 and indeed as low as 1. It is indeed conceivable that, with specially designed chromatographic beds, for example by using optimized microfabrication methods, still lower h values may be obtained.

Eq. (7), which is slightly preferable to its approximation (Eq. (8)), resembles an early equation proposed by Huber [17] on the basis of chemical engineering data, except that his exponent was 1/2. It is essentially the same as the equation proposed in 1976 by Horváth and Lin [18] which came from a chemical engineering study of Pfeffer [19]. This work dealt with the dispersion arising from slow mass transfer in the stagnant mobile phase surrounding the particles of packing and lying within the cusp-shaped regions at their points of contact. Still more recently, in 1987, Lenhoff [20] discussed band broadening in general terms using the well known continuity equations for the mobile and stationary phases. His treatment lumps together ''Eddy diffusion'' and axial molecular diffusion (the origin of the B term), and does not discuss the detailed features of this contribution to band dispersion. He does not therefore advance our understanding of dispersion due to flow beyond that given by Horváth and Lin.

There is no doubt that more complex equations than Eq. (9), have been proposed. But measurements of H or h have not generally been sufficiently precise, nor have they cover a sufficient range of ν, for the determination of more than three adjustable parameters (A, B and C in Eq. (9) for example) with any confidence. We therefore feel that efforts to obtain better descriptions of band spreading by adding more terms to the plate height equation are misdirected unless they can be supported by very wide ranging experimental data. Since the forms of the B and C terms are now well established by fairly rigorous theory, it is only the form of the remaining A term contribution for packed columns that is in question. In this paper I have therefore lumped together all processes which lead to dispersion occurring in the ''moving zone'' (defined below) under the umbrella term ''A-term dispersion'', and for ease of calculation I have taken the simple power dependence of h upon velocity for the A term. The question as to whether the various processes which occur in the moving zone of a chromatographic column or packed bed can be further broken down into component parts is not really our concern here. Our object is to establish as clearly as possible the magnitude of A-term dispersion rather than the details of its origin.

5. Separation of A and C terms

The separation of the A and C terms for retained solutes is no longer straightforward when both terms are velocity dependent. Before attempting this it is relevant to go into more detail regarding the column structure. Fig. 4 shows how the column can be divided either according to thermodynamic phases (mobile and stationary phases) or according to kinetic zones (moving and static zones). In GC the distinction between phases and zones is not particularly important since the diffusion coefficient of analyte in the mobile phase is so much greater than

THERMODYNAMIC PHASES	GEOMETRICAL REGIONS	KINETIC ZONES
Mobile Phase	Flowing Mobile Phase (in the interparticle space)	Moving Zone
	Stagnant Mobile Phase (within the pores of the particles of packing)	Static Zone
Stationary Phase	Stationary Phase (on the surface of the support structure)	
Support Structure		

Fig. 4. Packed column structure distinguishing between thermodynamic phases and kinetic zones.

in the stationary phase, but in LC, where the diffusion coefficients are likely to be similar, the distinction is vital.

According to the Giddings analysis [4], the dispersion leading to the *A* term arises in the moving zone, while that leading to the *C* term arises in the static zone. The static zone contains stagnant mobile phase and stationary phase. While this at first sight is a complication, the situation is much simplified conceptually if we regard the inside of a particle of packing as homogeneous with its own effective diffusion coefficient, rather than as multiphase. The effective diffusion coefficient can then be expressed as a weighted combination of the diffusion coefficients in the various phases comprising the static zone.

The distinction between phases and zones then requires the definition of two types of capacity ratio. The capacity ratio in terms of quantities of solute in the mobile and stationary phases is denoted by k', while the capacity ratio in terms of the quantities of solute in the moving and static zones is denoted by k''. Likewise, u and ν will be used for the linear velocities of the mobile phase; u_o and ν_o will be used for the linear velocities of the moving zone.

There are two ways of separating the *A* and *C* contributions to the plate height. The first applies in GC where it is possible to vary D_m without changing other column characteristics such as k' for any solute. This can be done either by changing the carrier gas (e.g., helium to nitrogen decreases D_m about five-fold), or by changing the operating pressure (D_m is proportional to $1/p$). The second, applicable to LC, is to work at very high reduced velocities when the *A* term will become less important due to its lower dependence upon velocity, and to cover a wide range of velocity so as to establish a reliable dependence of h upon ν.

6. Experiments in gas chromatography

The separation of mobile and stationary phase contributions to h was first carried out by Perrett and Purnell [21] and later by Knox and Saleem [22]. D_m was altered by changing either pressure or carrier gas while keeping k' constant. This method separates the contributions to H which arise in the mobile phase from those which arise in the stationary phase. It does not allow the moving and static zone contributions to be determined independently. Knox and Saleem [22] (1969) found that with squalane coated Chromosorb the stationary phase contribution to H was 20 to 40% of the total. They also showed that mobile phase contribution varied with a fractional power of the velocity as shown in Fig. 5 when *B*-term dispersion was allowed for. Under these conditions, h increased with ν with a power dependence between 0.4 and 0.6. This confirmed the earlier findings of Knox and Saleem illustrated in Fig. 1 for unretained solutes.

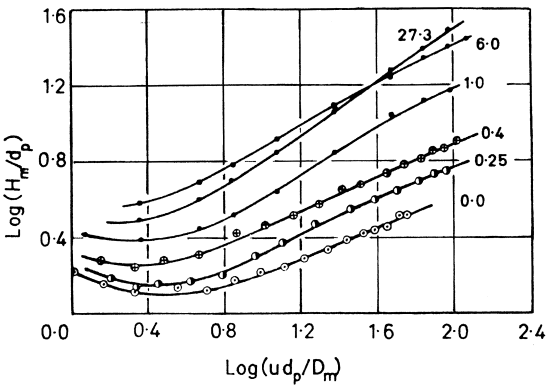

Fig. 5. (Log h, log ν) plots from Knox and Saleem [22] showing mobile phase contribution to h in packed column GC. Particle size 235 μm. Solutes: hydrocarbons; k' values given on lines. Lower three lines data for uncoated Chromosorb G; upper three lines data for Chromosorb G coated with 1% and 6% squalane. Note gradients of plots are around 0.5.

This mobile phase contribution includes the contribution from slow mass transfer within the stagnant mobile phase in the pores of the particles. It may be estimated from the theoretical equation for spherical particles namely:

$$h_C = (1/30)\{k''/(1+k'')\}^2\{(1-\phi)/\phi\}\nu_o/\gamma_{sm} \qquad (10)$$

where ϕ is the fraction of mobile phase which is stagnant. Taking ϕ as 0.5 and γ_{sm} as 0.6, Eq. (10) is simplified to $h_C = (1/20)\{k''/(1+k'')\}^2\nu_o$. Typically for the uppermost curves in Fig. 5 at the highest reduced velocity of 100, $h_C = 5$ compared with the mobile phase contribution of 25, i.e., about 20%, while at a reduced velocity of 10, $h_C = 0.5$ compared with the mobile phase contribution of 6, i.e., less than 10%. For the curve with $k' = 1$, at reduced velocity of 200, $h_C = 1.25$ compared with the mobile phase contribution of 15, i.e., about 8%. Thus, even in GC, the largest contribution to the plate height at moderate reduced velocities arises from the flowing part of the mobile phase and not from either the stationary phase or the stagnant part of the mobile phase. This is further supported by the dependence of h_A upon velocity which follows a fractional power of around 0.5. This is not very different from that obtained with glass bead columns. This conclusion differs markedly from the conventional view that the major contribution to band dispersion in GC arises from stationary phase dis-equilibrium.

7. Experiments in liquid chromatography

In LC as already noted there is much less distinction between the mobile and stationary phases in terms of diffusion rates. Indeed with bonded phases it is even difficult to say where the boundary between the mobile and stationary phases resides. As a result it is most convenient in the first instance to regard the particles of packing as homogeneous from the point of view of diffusion and to assign an effective diffusion coefficient which gives the overall diffusion rate within the particle. The contribution to h from slow equilibration within spherical particles, as shown by Giddings, then becomes:

$$h_C = (1/30)\{k''/(1+k'')^2\}\nu_o D_m/D_{eff} = C_{sz}\nu_o \qquad (11)$$

D_{eff} can be expressed in terms of the diffusion coefficients in the two phases. This was done by Knox and Scott [13]. Their overall equation for h_C is rather complex, but results in two limiting cases are fairly simple:

Case (a). When there is negligible diffusion in the stationary phase (for example as in GC), we can set $D_s = 0$. This gives Eq. (12) (already quoted as Eq. (10)):

$$h_C = (1/30)\{k''/(1+k'')\}^2\{(1-\phi)/\phi\}\nu_o/\gamma_{sm} \qquad (12)$$

Case (b). When the diffusion rates in the two phases are the same, that is if $\gamma_{sm}D_m = \gamma_s D_s$, the result is Eq. (13). This is identical to Eq. (11) except that the effective diffusion coefficient is replaced by $\gamma_{sm}D_m$ which is the same as $\gamma_s D_s$.

$$h_C = (1/30)\{k''/(1+k'')^2\}\nu_o/\gamma_{sm} = C_{sz}\nu_o \qquad (13)$$

On the basis of the work of Knox and McLaren [12] it would be expected that γ_{sm} would be around 0.6. The two special cases (Eqs. (12) and (13)) differ chiefly in their dependence upon k''. When $D_s = 0$ (Case a), the plate height contribution is a maximum when $k'' = \infty$, while, when the two diffusion coefficients are the same (Case b), the maximum occurs at $k'' = 1$. As one expects, when there is no diffusion in the stationary phase, the plate height is larger. For intermediate case we expect that there will be a maximum h_C at k'' above unity but for k'' high, h_C will still be finite. It should be mentioned that this approach does not include any specific contribution from slow mass transfer between the stationary and mobile phases. By taking an effective diffusion coefficient and expressing this in terms of diffusion rates in the two phases it is being assumed that the diffusion in both phases contributes to analyte molecules getting around inside the particles by what amount to parallel paths through the two phases. It is assumed that there is no slow process associated with getting into and out of the stationary phase. This may be justified by observing that, in modern HPLC with bonded phases, the ''thickness'' of the stationary phase is only a few Angstrom units. The situation in GC is very different where the stationary phase can be relatively thick and where D_s is very much less than D_m. Here molecules do not

''get around'' the particle by diffusing in the stationary phase; they get around the particles only by diffusing in the stagnant mobile phase. On the other hand they do have a problem getting across the stationary phase to exit from it into the mobile phase; in other words there is resistance to mass transfer in the stationary phase. The diffusion paths in the two phases in GC are essentially orthogonal not parallel.

In LC, even with the highest possible k'' factor, C will nearly always be below 1/20 (assuming $\gamma_{sm}=0.6$) and in most cases significantly smaller than this. Since the experimental values of C derived by application of the Knox equation are generally in the region of 0.1 to 0.2, there is clearly an anomaly. Is the theory wrong, or has the plate height data been misinterpreted? Is there good evidence for as high a C factor as 0.1?

Knox and Scott [13] examined this by carrying out LC using 50 μm ODS-bonded particles which allowed them to attain reduced velocities up to 5000. With a weak dependence of the A term upon velocity the C term was expected dominate at these high reduced velocities. Fig. 6 shows their (h, ν) plot for a solute (p-cresol) having $k'=1.86$.

The data may be fitted, within experimental error, by either a straight line or a gentle curve. If the simple van Deemter equation is applied (Eqs. (4a) and (4b)) the value of A_{VD} is around 21. This is clearly unacceptable since at low ν values, h is normally around 2 or 3 and cannot possibly have a value of 21. Indeed if the van Deemter equation were to apply A_{VD} could not be more than 2. The simple formulation cannot therefore be correct: the A term must be velocity dependent. If the exponent of velocity is taken as 1/3 as in the Knox equation, then the best fit is obtained with $A=2.5$. This A value could be still somewhat too high; a slightly larger exponent, say about 0.4, would give $A=1.5$. The evidence is clear that the A term has a velocity dependence with an exponent in the range of 0.3 to 0.4. A solute retained on a column packed with porous particles thus behaves similarly to an unretained solute on a glass bead column (Knox and Parcher [15]) The result is also in accord with those of Knox and Saleem [10,22].

The value of C derived from the data is 0.0125 (A term exponent 1/3) or 0.0115 (exponent 0.4). For $k''=1.86$ the values of C derived from Eqs. (11) and (12) would be respectively, 0.012 and 0.020. The agreement is therefore good.

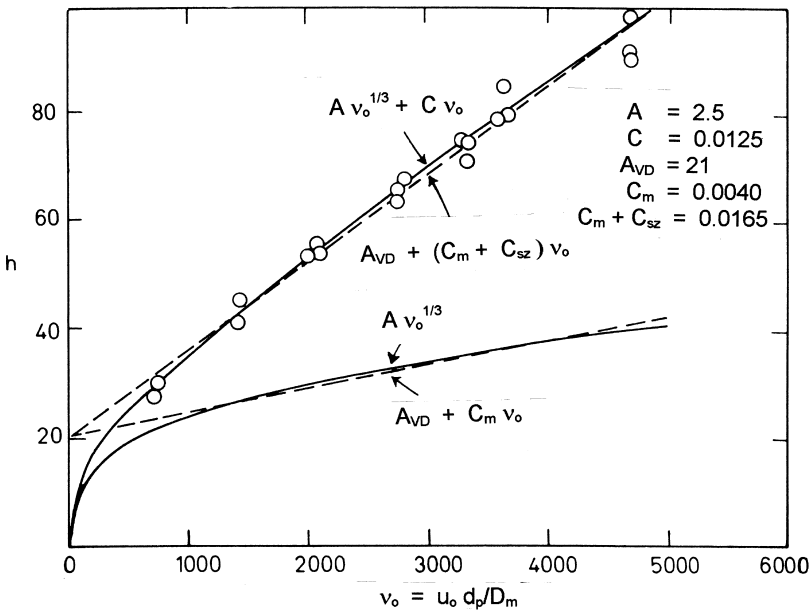

Fig. 6. (h, ν) plots in LC at high values of ν from Knox and Scott [13]. Column packing: 50 μm ODS silica gel. Solute: p-cresol, $k''=1.86$. Best van Deemter plot has form $h=21+0.0165\nu$; best ''Knox'' plot has form $h=2.5\nu^{1/3}+0.0125\nu$.

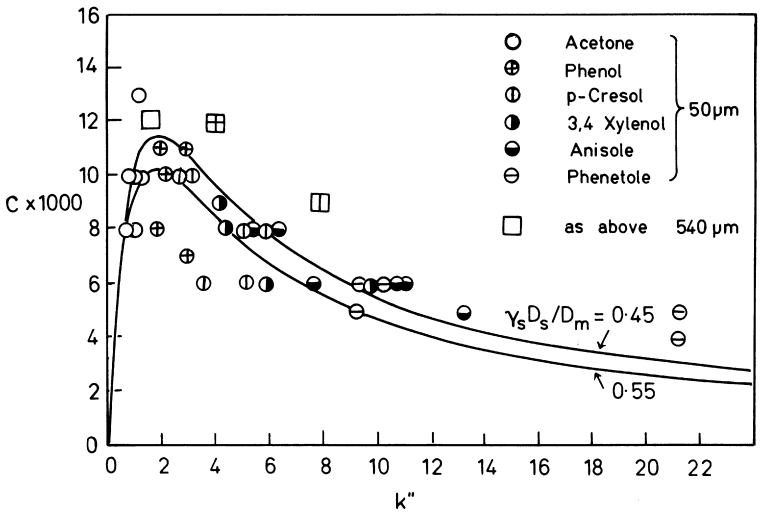

Fig. 7. Dependence of C upon k'' from Knox and Scott [13], assuming $A=2.5$ in Knox equation. Column packing: 50 and 540 μm ODS silica gels. Note the range of C from about 0.0004 to 0.013.

The results of further experiments carried out to determine how C varied with k'' are shown in Fig. 7.

The points are experimental and the lines calculated according to the full equation for h_C assuming two values for $\gamma_s D_s/D_m$. h_A has been taken throughout as $2.5\nu^{1/3}$. While there is considerable scatter of the data it is clear that the magnitude of the C values is correct. It falls in the range 0.004 to 0.014. There does not appear to be any significant dependence of the A upon k'', since, if there were, we would have obtained irreconcilable values of C. Further experiments to check this would nevertheless be highly desirable.

What now becomes apparent particularly from Fig. 6, is that, over the range of reduced velocities normally used in HPLC (say 3 to 10), the contribution from the C term to the total plate height is going to be essentially negligible, at less than 5%. Virtually all dispersion in current LC arises in the flowing part of the mobile phase.

We conclude that: (1) the apparent high C values obtained from data on conventional HPLC columns are erroneous, and probably arise because of the range of velocity is too small and/or because instrumental broadening is emphasised at high flow-rates. Or to put another way: the coefficients of the various terms in the Knox equation when fitted to the experimental data obtained from the normal type of experiments carried out over a small range of reduced velocity (say a factor of 10), cannot adequately separate the dispersion processes which occur in the mobile (the A term) and the stationary zones (the C term). (2) At flow-rates around the minimum in the (h, ν) curve the contribution from the C term in HPLC is below 5% and virtually all the broadening arises from the A term, that is from processes occurring within the flowing part of the mobile phase outside the particles.

These conclusions are at variance with conventional wisdom which would claim that a major sources of band broadening in LC comes from slow mass transfer within the particles themselves. A number of interesting conclusions or predictions now emerge, and are discussed in the following sections.

8. Exclusion chromatography: data of Knox and McLennan, and of Dawkins and Yeadon

Both Knox and McLennan (K&M) [23] and Dawkins and Yeadon (D&Y) [24,25] made measurements of plate height dependence on velocity for polystyrenes, partially excluded from porous silica

gels. Their conclusions were that diffusion within the silica gel particles became increasingly restricted, as the polystyrenes were more highly excluded. Previously van Krefeld and van den Hoed (VK&VDH) [26] had reached similar conclusions. However they had worked with much higher reduced velocities, and their calculated diffusion rates were significantly higher than those of K&M and D&Y.

These results should now be reconsidered in the light of the conclusions that slow mass transfer within the particles of packing is normally an unimportant in LC. An important observation made by K&M was that, for polystyrenes with M_r ranging from 2000 to 33 000, the (h, ν) curves were essentially superimposable. The slope of these curves was interpreted in terms of slow mass transfer within the particles of packing, and gave $C=0.07$ independent of M_r. Because of the k'' factor in the equations for h, (see Eq. (13)), γ_{sm} would have to change to compensate to give the value found for C. Thus K&M, and D&Y concluded that diffusion within the particles became more restricted as the polymer became more excluded, i.e., γ_{sm} declined. If the dispersion was in fact due mainly to A-term dispersion then the commonality of the (h, ν) curves, would be readily explained, especially if, as suggested above A was independent of k'. It seems the results of K&M and D&Y did not in fact indicate that diffusion within the particles became more restricted, as exclusion increased, since the true value of C was probably never actually measured. To establish the true value of C we would need to work with much higher reduced velocities in the range 1000 to 10 000, rather within the range up to 200. VK&VDH indeed worked at very much higher reduced velocities, in fact so high that flow through the particles occurred. When this was allowed for, their C values were about half those of K&M and D&Y. The simplest explanation of this is that the relative contribution of A-term dispersion in their experiments was less and that of C-term dispersion more. The results are thus in line with the findings of Knox and Scott.

Further experiments in this area are now desirable to establish once and for all the relative contributions of A- and C-term dispersion in exclusion chromatography. Exclusion chromatography in fact provides the perfect test-bed since there is no involvement of a stationary phase.

9. Electrochromatography

The new field of capillary electrochromatography (CEC) [27,28] which is developing rapidly, uses an electric field of up to 100 kV/m to drive the eluent through an LC column. The column diameter is kept below 150 μm to avoid self heating. One of the striking features of CEC is that the plate height is lower than with pressure drive using the same column. h values in the region of 1 can be obtained in CEC [28], whereas the minimum for pressure driven HPLC is about 2. This reduction has been ascribed to the better flow profile generated by electrodrive, and there seems little doubt that this is correct. However it is still widely assumed that much of the remaining dependence of h upon ν arises from C-term dispersion. This is now questionable. It seems that the remaining velocity-dependent dispersion is still largely due to the uneven flow profile outside the particles. It is highly probable that dispersion can be further reduced by improving the uniformity of the packing. The use of narrow bore columns is an advantage since more sophisticated and expensive packing materials can be tested, for example monodisperse porous and non-porous beads.

10. Future design of LC column systems

The improvement of LC packings and columns has largely concentrated on trying to reduce the contribution to dispersion arising from slow mass transfer in the static zone. This is now seen to have been misguided. At the flow-rates used in modern HPLC, which are near those giving minimum h, the contribution to dispersion from slow mass transfer in the static zone (C-term dispersion) is a very small proportion of the total. By far the most important contribution comes from the moving zone (A-term dispersion). This has important consequences for the design of improved packings. Firstly column packing is now seen to be all-important and substantial improvements can undoubtedly be made. For example the data on glass beads already referred to above (Fig. 3) shows that, with large particles, reduced plate heights well below unity can be achieved even with pressure driven flow. The difficulty is to pack smaller particles as well as larger ones. There is no

justification for the widely held view that h has a ''theoretical minimum'' value of 2, although it does seem to represent the practical minimum with 5 mm bore columns packed with 3 to 5 μm particles. Recent work in electrochromatography, just cited, shows that the improved flow profile coming from electrodrive can give h values of 1. These low values are not entirely due to the use of very narrow tubes since the same columns with pressure drive still produce h values of close to 2. It may be that still better fractionation of particles or the use of the recently available monodisperse particles will offer the way toward substantially more efficient columns – especially narrow bore capillaries.

As an alternative to improving methods of packing existing spherical particles, it should be possible to devise micromachined structures which are much more regular than conventional packed beds, and which are optimised to provide ideal flow patterns giving minimal dispersion.

11. Turbulent flow chromatography

Another area which is currently being explored is ''turbulent flow'' chromatography [29]. Knox [14] showed in 1966 that with glass beads, when ν was increased to above about 5000, the (h, ν) plot flattened off and then declined. This decline occurred at ν values of around 5000, and was put down to the onset of turbulence. A much more dramatic effect occurred for an unretained solute in an open tube, as shown by Pretorius and Smuts, also in 1966 [30]. The onset of turbulence is characterised by the attainment of a specific Reynolds number which depends upon the structure of the flow channel. In a packed bed turbulence starts at R_e of about 5 whereas in an open tube it starts at R_e around 2000. The Reynolds number is defined by Eq. (14)

$$R_e = ud\rho/\eta \tag{14}$$

and the ratio of Reynolds number to reduced velocity is $R_e/\nu = D_m\rho/\eta$. For a gas R_e/ν is around unity, while for a liquid it is about 1000. Thus turbulent flow will occur in GC at typical operating velocities, whereas in LC it will occur only at ν values of the order of several thousand. Turbulence thus occurs at such high ν values in LC that a substantial contribution from C-term dispersion must be expected when porous packings are used (k'' can never be less than about 0.6 even for so-called unretained solutes). The data of Fig. 6, for example, shows that C-term dispersion contributes around 60 to h at $\nu = 5000$, when the total h value is about 100. Although A-term dispersion will fall when turbulence sets in, there can be no corresponding fall in the C-term dispersion. One cannot therefore expect high efficiencies with columns packed with porous particles when they are used in this velocity range. Nevertheless the use of large particles and very high linear velocities [31] (e.g., $d_p = 50$ μm, $u = 0.5$ m/s, giving $\nu \approx 10\,000$) can give separations of simple mixtures if gradient elution is used. Similar results are demonstrated for proteins by Kopaciewicz et al. [32]. But under these conditions one is effectively carrying out programmed desorption rather than true chromatography. For the efficient separation of retained analytes by isocratic turbulent flow chromatography, C-term dispersion will have to be dramatically reduced. The future may lie with the long neglected pellicular packings originally devised for the then new technique of HPLC by Kirkland [33] in 1969. With their much thinner static zone they could provide the very fast mass transfer required for retentive chromatography under turbulent flow conditions.

12. Glossary of symbols

$\boldsymbol{A}$, $\boldsymbol{B}$, $\boldsymbol{C}_m$, $\boldsymbol{C}_s$	Constants in the Van Deemter equation, subscripts m and s refer to mobile and stationary phases
A, B, C_m, C_s, C_{sz}	Constants in the reduced form of the van Deemter equation or Knox equation, subscripts m, s and sz refer to mobile, stationary and stagnant phases or zones
D_m, D_s, D_{sz}, D_{eff}	Diffusion coefficients in mobile phase, stationary phase, static zone and effective diffusion coefficient in packed bed or particle
d	Characteristic dimension in relation to turbulence
d_p, d_f	Particle diameter, film thickness of stationary phase (in GC)

H	Plate height
h	Reduced plate height, $h=H/d_p$
H_A, H_B, H_C	Plate height contributions from different dispersion mechanisms
h_A, h_B, h_C	Reduced plate height contributions from different dispersion mechanisms
k', k''	Phase capacity ratio, zone capacity ratio
p	Pressure
R_e	Reynolds number $=ud\rho/\eta$
u, u_o	Linear velocities of mobile phase and mobile zone
z	Distance moved along column by band
ϕ	Fraction of mobile phase which is stagnant
γ, γ_m, γ_s, γ_{sm}	Tortuosity factor in B term of van Deemter equation, for mobile phase, for stationary phase and for static zone
η	Viscosity
λ	Geometric factor in A term of van Deemter equation
ν, ν_o	Reduced velocity, reduced velocity of mobile zone: $\nu=ud_p/D_m$
ρ	Density
σ_z	Standard deviation of band profile resulting from diffusion

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