Band dispersion in chromatography—a universal expression for the contribution from the mobile zone

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Abstract

It is generally assumed that the dispersion which is covered by the C term of Van Deemter type equations arises from processes occurring in the static zone, while the dispersion covered by the A term arises from processes occurring in the mobile zone. It is also now widely accepted that the contribution to h, the reduced plate height, from mobile zone processes increases with a modest power of ν, the reduced flow velocity. A reassessment of data acquired since the 1960s suggests that this power falls with increasing velocity, but may be relatively high at reduced velocities, ν, in the range 1–30. Data for a wide variety of materials over a wide range of ν have been re-examined and are well fitted by an equation of the form: $h = B/\nu + \{1/A + 1/(D\nu^n)\}^{-1} + C\nu$. With $C \leq 0.02$ in accordance with the theoretical value for slow equilibration in the static zone, n is found to be in the range 0.5–1.0 with the lower values applying to glass bead packings, and the higher values applying to porous spherical packing materials. The equation provides a decreasing power of velocity in the A term in agreement with experimental data. It is now clear that nearly all of the dispersion previously assigned to processes in the static zone actually occurs in the mobile zone. Accordingly, substantial improvements in column performance in LC may well be achieved by better packing of columns, or by designing structures such as monolithic beds and two dimensional designs on chips, which can provide more uniform structures than the beds of spherical particles widely used in current HPLC.   © 2002 Published by Elsevier Science B.V.

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1. Introduction

Since the early days of gas chromatography the mechanism of band dispersion had been keenly discussed and argued about. It is now universally accepted that three major processes contribute to band dispersion: axial molecular diffusion, flow and diffusional processes in the mobile zone which comprises the fluid outside the particles, and diffusion plus mass transfer processes in the static zone which comprises the particles themselves. These three different contributions each produce random or Gaussian dispersion and so the band-variances pro-

duced by them can be added. Hence the three term plate height equation of Van Deemter et al. [1]. Slightly broadening the scope of their original equation, this dispersion can be expressed in terms of the plate height in the form:

$$H = B/u + Af(u) + Cu \qquad (1)$$

where A, B and C are constants, u is the linear velocity of the eluent, and $f(u)$ is a constant or a function of u which rises gradually with velocity. In the original formulation, van Deemter et al. had the A term independent of velocity, but they later added a further term for mobile zone mass transfer which depended linearly upon velocity [2]. This contribution to the A term was similar to that in the Golay

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equation [3], and showed a linear dependence upon flow velocity. Work since then has shown that for packed columns the *A* term rises with a fractional power of *u*. But the question remains, just what that power of *u* actually is.

Josef Huber [4], along with Jack Kirkland [5] are generally regarded as the fathers of high-performance liquid chromatography (HPLC). Both employed small particles and used high pressure pumps to deliver eluent. Kirkland introduced the first column packing materials, Zipax, which was specifically designed for HPLC. Josef Huber, with a particular interest in band dispersion in LC, advanced a modified Van-Deemter-type equation, which can be cast in the form of Eq. (2), where the symbols are defined in Section 4:

$$H/d_p = BD_m/(ud_p) + A_1/\{1 + 1/D(ud_p/D_m)^{1/2}\}$$
$$+ A_2\{k'/(1 + k')\}^2\{ud_p/D_m\}^{1/2}$$
$$+ Ck'/(1 + k')^2\{ud_p/D_s\} \qquad (2)$$

Following the introduction of reduced or dimensionless parameters by Giddings [6], Eq. (2) can be written in terms of the reduced plate height, *h*, and the reduced velocity or Peclet number, ν, according to Eq. (3):

$$h = B/\nu + 1/\{1/A_1 + 1/D\nu^{1/2}\}$$
$$+ A_2\{k'/(1 + k')\}^2\nu^{1/2}$$
$$+ C\{k'/(1 + k')^2\}\{D_m/D_s\}\nu \qquad (3)$$

where the reduced parameters are defined in Eqs. (4a) and (4b):

Reduced plate height $\quad h = H/d_p \qquad (4a)$

Reduced velocity $\quad \nu = ud_p/D_m \qquad (4b)$

The *B* and *C* terms are the same as in the original van Deemter equation, but the original constant *A* term is now replaced by two terms: the A_1 term has some similarity to what was proposed Giddings in his ''coupling'' theory of 1962 [7]. The A_2 term arises from empirical chemical engineering studies. Thus the original velocity independent *A* term, now shows a rising trend with velocity, with the steepest

dependence being to the power of 0.5 at low values of ν.

In expounding his ''coupling'' theory, Giddings [6,7] noted that two contributions from processes in the mobile zone, which had previously been simply added in the van Deemter treatment, should actually be combined so that they collaborated in reducing what would otherwise the infinite dispersion of a band moving along an open tube. The first process was transverse diffusion considered by Golay in his classic paper, and the second was the break up of the parabolic flow profile of the open tube by packing with particles, more usually termed ''eddy diffusion''. Giddings contended that the proper way to combine these processes was to add them harmonically not linearly. This led to the ''coupled'' formulation of the contribution to *h* from processes in the mobile zone:

$$h_A = \{(1/A) + (1/D\nu)\}^{-1} \qquad (5)$$

A plot of log *h* against log ν would then show unit gradient at low values of ν, and zero gradient at high values of ν, with the change over occurring effectively over about two orders of magnitude. Very soon it was recognised that a number of different processes occurring over different ranges within the packed bed should be recognised. This gave a final expression containing five terms, each of the form given in Eq. (5). These terms represented contributions from transchannel, transparticle, short range interchannel, long range interchannel, and transcolumn processes. For each contribution there was a specific transition velocity; that is the velocity at which both flow and diffusion contributed equally in Eq. (5). The transition velocity is $\nu_{trans} = A/D$. For transparticle processes ν_{trans} was so high that the only significant process here was slow diffusion within the particle itself. This contribution is, in fact, the generally accepted *C* term contribution to *h*, namely the contribution from slow equilibration within the particle itself. Accordingly it should no longer be considered as contributing to the *A* term. For the remaining four contributions, the transition velocities ranged from $\nu_{trans} = 0.1$ for long range interchannel processes, to $\nu_{trans} = 100$ for transchannel processes. It would therefore be deduced from the Giddings coupling theory that a more or less linear contribu-

tion to h would arise from transchannel processes at reduced velocities in the range typically used in HPLC (around 10), while the other contributions (with low transition velocities) would be more or less velocity independent. Finally, Giddings considered that the transchannel contribution would be the largest of the five. It would therefore dominate the mobile zone contribution to h, with the result that, real data should after all be represented more or less by the simple coupling Eq. (5).

A few years before Huber published his paper [4], I was fortunate enough to spend a sabbatical period in the laboratory of Cal Giddings where I decided to test the validity of the Giddings proposals for coupling of eddy diffusion and mobile phase mass transfer. I did this by determining (h, ν) curves for columns packed with glass beads [8]. The work was continued in Edinburgh by Jon Parcher [9]. It showed that the gradient of the (log h/log ν) curve for unretained solutes in glass bead columns fell gradually with velocity. The gradient was steeper at low values of ν, less steep as ν rose, finally becoming zero and then negative when turbulence came into effect at ν around 10 000. Contrary to what Giddings had proposed, there was no indication of any rapid transition (over say a ten- or hundred-fold range of ν) suggested by the single term coupling formula, or indeed, even the five-term equation. However the results did generally agree with Gidding's hypothesis that dispersion in the mobile zone had a velocity dependence. In reality the transition was rather gradual, and, if the idea of coupling was to be accepted, there had to be a continuous range of coupling phenomena to produce the results observed experimentally. A weighed integral of coupling terms [8] did produce the experimentally observed dependence for glass bead columns, giving a gradient of around 1/3 in the log h vs. log ν plot at ν of around 100, near zero at around $n = 10\ 000$, and around 0.5 at low values of ν, but the limits of integration did not seem to have any theoretical significance. A much simpler equation reproduced the data equally well [9], namely Eq. (6):

$$h_A = \{(1/A) + (1/D\nu^n)\}^{-1} \qquad (6)$$

Knox and Parcher [9] found that $n = 1/3$ gave the best fit to their data, but n is essentially a variable

which can be chosen to obtain the best fit. The use of a power of ν lower than unity in the denominator restricts the maximum gradient of the (log h/log ν) plot and at the same time extends the range over which the gradient of the plot changes from n (at low ν) to zero (at high ν). This contribution to h is similar to Huber's A_1 term, in which n was fixed at 0.5. Eq. (6) is, of course, essentially empirical and has no simple theoretical justification. It conforms broadly to the Giddings proposal of coupling, but, as with the equation involving an integral, A, D and n again have no simple theoretical interpretation. Over a 30-fold range of ν, a simple power law is adequate:

$$h_A = A\nu^n \qquad (7)$$

The LC results with glass beads were also borne out by early studies in gas chromatography with porous particles [10], recently reviewed [11]. These early studies were carried out with relatively large particles ranging from 50 to 1000 μm in diameter, which were dry packed into columns typically 3–6 mm in bore.

Subsequent to these experiments in the 1960s, HPLC was developed. In 1969 Kirkland [5] presented the first specifically designed HPLC packing material tradenamed Zipax. This was a pellicular 30–40 μm material. It was followed in 1972 by the spherical Zorbax [12] whose particles were fully porous, and were much smaller, in the 5–10 μm range. Since then, many new packing materials have been developed. The conventional HPLC column is now a stainless steel tube with a bore of 4.6 mm, a lengths between 50 and 250 mm, and packed with 3–5 μm porous spherical particles. Columns are now universally packed by the slurry method. Recently much narrower columns have appeared, firstly the so-called 1 mm "microbore columns" and most recently true microbore quartz tubes 30–300 μm in bore and packed with particles as small as 1 μm.

For any HPLC column, it is very difficult to separate dispersion arising in the mobile zone (comprising the eluent in the interparticle space) from that arising in the static zone (comprising the stagnant eluent within the pores plus any retentive stationary phase) since both have significant velocity dependences. This is particularly so if the A term dispersion has an uncertain velocity dependence. Only one

study [13] has successfully done this and is discussed below. Most chromatographers have therefore chosen a broad-brush approach and have monitored band broadening in HPLC using the simple Knox equation [14] which fits experimental data for conventional columns over a ten- or hundred-fold range of reduced velocity. That is Eq. (8):

$$h = B/\nu + A\nu^n + C\nu \tag{8}$$

The exponent n is generally taken as $1/3$ in line with the original data on glass bead columns [8,9]. For a good column, B will normally be in the range 2–4, A around 1, and C around 0.1. Whether the numerical values of the parameters can be interpreted in terms of the three main band broadening effects is, however, highly questionable. Although the question is rarely addressed, it seems to be widely assumed that the C term indeed represents the contribution to h from slow equilibration within the static zone. This view is almost certainly incorrect, and it is important to address the question, for little can be done about improving mass transfer within the particles of packing, but much can be done to improve the structure of the chromatographic bed. Thus if the dispersion associated with the C term in the Knox equation does indeed arise mainly from mobile zone processes, considerable improvement in column performance should still be possible.

For genuine slow equilibration within spherical porous particles, the theoretical expression for the contribution to h, given by Giddings [6], is

$$h_C = (1/30)\{k''/(1 + k'')^2\}(D_m/D_{sz})\nu \tag{9}$$

D_{sz} is the effective diffusion coefficient of analyte within the static zone. This ''diffusion'' within the static zone encompasses all processes which allow analyte molecules to move around inside the particles of packing material. It thus includes diffusion in the stagnant mobile phase, diffusion in the stationary phase and any rate limiting processes of transition between the stationary and stagnant mobile phases. In HPLC slow phase transition is unlikely, so diffusion within the two phases should be the only important contributions to D_{sz}. Since most stationary phases in HPLC comprise groups such as C_{18} bonded to a silica structure, it is likely that the

diffusion rates in the mobile and stationary phases will be similar.

If the diffusion coefficient in the stationary phase (e.g. the bonded ODS layer) is the same as in the mobile phase then $D_{sz} = \gamma D_m$, where γ is the obstructive factor for diffusion within the particles, and the plate height contribution h_C becomes:

$$h_C = (1/30)\{k''/(1 + k'')^2\}\nu/\gamma \quad (D_m = D_s) \tag{10}$$

If there is no diffusion in the stationary phase, the expression for h_C becomes:

$$h_C = (1/30)\{k''/(1 + k'')\}^2(1 - \varphi)\nu/\gamma\varphi \quad (D_s = \text{zero}) \tag{11}$$

where φ is fraction of the mobile phase which is stagnant within the pores of the packing material. The expression for intermediate cases is given by Knox and Scott [13]. It is important to note that with porous particles k'' will be non-zero even for so-called unretained solutes since these must still permeate the stagnant mobile phase: typically the minimum value for k'' is about 0.4. If the obstructive factor γ for diffusion within the particles is taken as 0.6, and if diffusion within the stationary phase is about half as fast as in the mobile phase (as found by Knox and Scott), a maximum C values in accord with Eqs. (6)–(8) is about 0.02. It is generally expected to be less than this. The values obtained by Knox and Scott for ν between 500 and 5000, rose to a maximum of 0.013 at $k'' = 1$ and then gradually fell to 0.006 as k'' increased to high values.

Thus when empirical C values, are reported in the range from 0.1 up to 0.5 for typical HPLC columns, one has to question how these can possibly be accounted for. Evidently, if the theoretical value for the C term is accepted, then the main part of the band broadening which is conventionally included in the C term of the Knox equation, must arise from processes taking place in the mobile zone. But if this is so then the $1/3$ power law for the A term cannot apply to these columns. It is the purpose of this paper to explore this question, and to determine whether a better expression can be found for the contribution to h from the band spreading processes which occur in the mobile zone.

One possible explanations for high C values is self-heating due to frictional heat dissipation. This has been considered in detail by Kraak and Poppe and coworkers [15,16]. Broadly speaking, self heating arises from the dissipation of the energy required to pump the eluent through the packed bed. This produces a parabolic temperature profile across the column with the highest temperatures along the axis. This temperature profile produces a corresponding profile in the band migration rate due to the temperature variation of viscosity and k'. This in its turn leads to band spreading which will be particularly severe at high flow-rates. Such broadening will produce what could look very like a C term contribution even if it is predicted to have a higher-than-linear dependence upon velocity and to result in the development of non-Gaussian peaks. From the calculations and data acquired by Kraak and Poppe it is, however, clear that band broadening due to thermal effects will be insignificant except at very high velocities with 4.6 mm bore columns packed with 3 μm particles. In other words, thermal effects are not the cause of the high C values in HPLC. Other reasons must be sought. The most likely is that the assumed form of the A term contribution to h is incorrect and that, for the ranges of ν used in HPLC, the dependence is steeper than the 1/3rd power assumed in the Knox Eq. (8). A near linear contribution from processes in the mobile zone could arise from poorly flushed voids within the packing structure which can only be equilibrated by transverse diffusion, or alternatively from transcolumn variations in packing density such as those identified by Knox et al. [17], and recently studied in detail by Shalliker et al. [18]. Such processes according to the Giddings analysis [6] could have relatively high transition velocities so giving rise to near linear dependences of h upon ν.

2. Some examples

Since the 1960s a vast amount of data on band dispersion in HPLC columns has been reported. A selection of this body of data is now examined in detail to establish whether there is a single plate height equation which can accommodate it.

2.1. Knox, and Knox and Parcher

Knox and Parcher [9] studied 3–11 mm bore columns dry-packed with 480 μm glass beads. They found that, over a range of ν from about 30 to 3000, their data were well fitted by Eq. (6) with $n = 1/3$. This is a special example of the more general Eq. (12):

$$h = B/\nu + \{1/A + 1/(D\nu^{n})\}^{-1} + C\nu \tag{12}$$

Knox and Parcher did not include the B term in their equation, and the effect of this was to overestimate the contribution from mobile zone dispersion at low ν. Fig. 1 shows the best fit of Eq. (12) to their data replotted from the original paper. The fitting was carried out manually by a process of successive approximation using an EXCEL spreadsheet. The sensitivity and possible correlation of values of parameters, A, B, D, n and C was not specifically tested, but changes of around 10% in any parameter resulted in obvious misfitting. For impermeable non-retaining glass beads, C is of course zero, but inclusion of the B term now leads to a much higher value of $n = 0.5$. Best fit values of A, B, D, n and C are listed in Table 1.

2.2. Done and Knox—Zipax

Zipax [5] is a pellicular material in which glass beads are coated with five to ten monolayers of colloidal silica, the total layer thickness being of the order of 100 nm. Solute equilibration in this layer is expected to be extremely fast and therefore the true
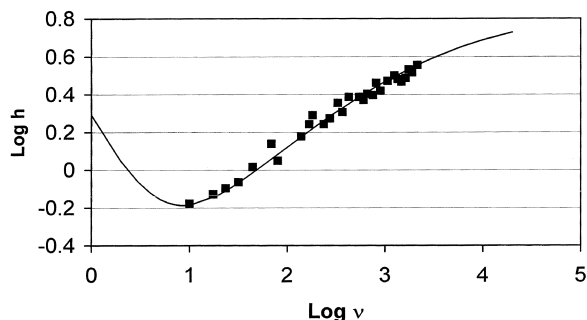

Fig. 1. Replot of data of Knox and Parcher [9] for 480-μm glass beads, with best fit line according to Eq. (12).

Table 1
Parameters of Eq. (1)2 for various LC columns

Material	Particle diameter (μm)	Reference (figure)	k', k'', designation	B	A	D	n	C
Glass beads	480	Knox and Parcher [9] (1)	0	1.8	7	0.16	0.50	0.00[a]
Zipax	29	Done and Knox [19] (2)	0 1.7 7.8	2.5	19	0.50	0.48	0.00[a]
				2.5	21	0.62	0.50	
				2.5	22	0.65	0.55	
Porasil	44–53	Kennedy and Knox [20] (3)	Unretained	3.5	20	0.40	0.78	0.025
			Retained	3.5	22	0.50	0.85	0.035
Spherisorb alumina	20	Knox et al. [17] (4)	All data	3.6	22	0.23	0.90	0.016
Porous ODS silica	50	Knox and Scott [13] (4)	1.83					
ODS silica	1	MacNair et al. [21] (5)	0.07–0.11	1.45	15	0.48	0.75	0.01[a]
			0.29–0.35	1.82	17	0.41	0.85	
			0.81–1.05	2.1	20	0.34	1.05	
Merck particulates	3.5 and	Merck [23]						
	5	Sinz and Cabrera [24] (6)	Z3.5, Z5	4.5	20	0.42	1.00	0.01[a]
Merck particulates	5	Merck [23]	S5, X5	3.5	14	0.42	0.82	
Merck Chromolith	9[b]	Sinz and Cabrera [24] (6)	Chromolith	3.6	20	0.105	0.72	0.00[a]

[a] Values of C are assumed to be in line with Eq. (9).
[b] Effective particle diameter to give same flow permeability.

value of C should be negligible. Done and Knox [19] determined (log h/log ν) curves for a number of particle sizes from 29 to 106 μm and established that they gave superimposable plots. For the 29 μm particles they determined (h/ν) curves for k'' values of 0, 1.7 and 7.8. Their data are replotted in Fig. 2. Assuming that C = zero, the simple Knox equation (n variable) fits the data well with B = 2.5 and the following values of A and n: k'' = 0, A = 0.57, n = 0.40; k'' = 1.7, A = 0.64, n = 0.44; k'' = 7.8, A = 0.68,
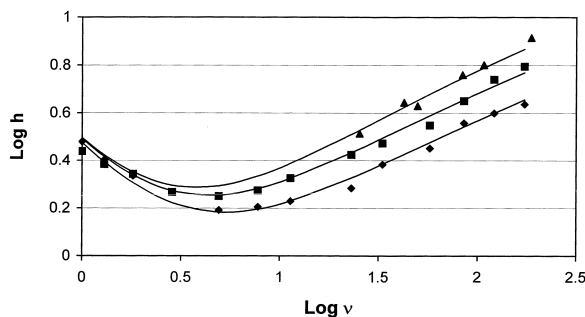

Fig. 2. Replot of data of Done and Knox [19] for 29-μm Zipax, with best fit lines according to Eq. (12). Lower line k'' = 0; middle line k'' = 1.7; upper line k'' = 7.8. For parameter values see Table 1.

n = 0.48. The coupled Eq. (12) also provides an excellent fit with n = 0.48–0.55 as shown in Fig. 2. The other parameters for Eq. (12) are listed in Table 1. The values of n are very similar to those for the glass beads of Knox and Parcher. The major difference between the two sets of data are the much lower h values for the 480-μm glass beads than for the 29-μm Zipax. This is shown up by the much lower values of D for the former. It is the value of D which largely determines the A term plate height contribution at low values of ν for any given value of n.

2.3. Kennedy and Knox

In a paper predating that of Done and Knox [19], Kennedy and Knox [20] determined (log h/log ν) curves for a number of porous supports including the spherical silicas marketed under the name Porasil. The particle size was 44–53 μm. The data have been replotted in Fig. 3. The data can be well fitted by Eq. (8). If C is taken taken as 0.02, then with B = 2, the best fit is obtained with A = 0.9 and n = 0.45 for the unretained solutes, and n = 0.55 for the retained solutes. Formula (12) gives a slightly better fit;
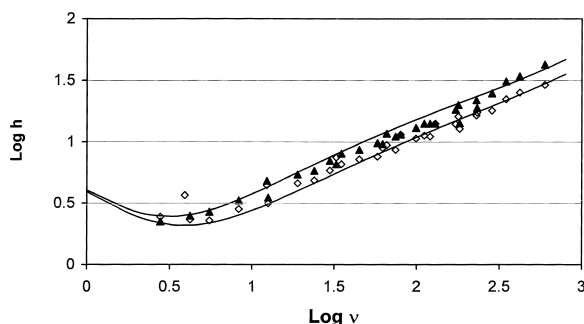
Fig. 3. Replot of data of Kennedy and Knox [20] for 44–53-μm Porasil, with best fit line according to Eq. (12). Upper line for retained solutes; lower line for unretained solutes. For parameter values see Table 1.

Fig. 4. Replot of data of Knox et al. [17] for 20-μm Spherisorb alumina (data at low ν), and of Knox and Scott [13] for 50-μm porous ODS silica gel (data at high ν) with best fit line according to Eq. (12). For parameter values see Table 1.

parameter values listed in Table 1. Values of n range from 0.78 to 0.85 depending upon the degree of retention. Significant values of C are required to obtain a good fit; these are still somewhat high when compared with the theoretical values but this may be due to slower mass transfer within this early silica gel than in more modern materials.

2.4. Knox et al., and Knox and Scott

Knox et al. [17] made extensive plate height measurements with 20-μm Spherisorb alumina. Their data have been used as the basis for Eq. (8). They covered a range of log ν from -0.5 to 1.8. The best fit of Knox Eq. (8) to their data required C in the range 0.09–0.16. Such values are too large for them to be ascribed entirely due to static zone equilibration. Subsequently Knox and Scott [13] noted that to separate the A term from the C term in LC it would be necessary to work at very high values of ν in order that the A term contribution with its lower dependence on velocity could be made relatively small compared to the C term contribution. By working with ν in the range 500–5000, they indeed showed that the C values for retained solutes on a 50-μm laboratory-made ODS silica were in the range predicted by theory with maximum values of about 0.013. By covering a range of k'' values, it was also shown that diffusion in the stationary ODS phase occurred at about half the rate observed in the mobile phase.

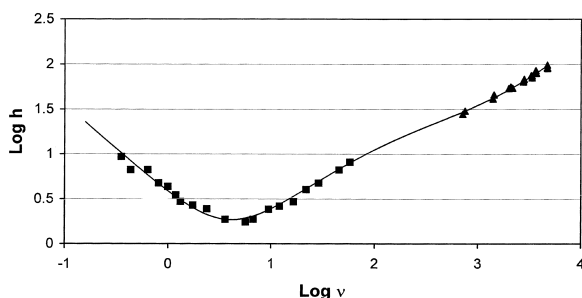Fig. 4 shows the replotted data of Knox and Scott

(K&S) for a solute with $k'' = 1.83$ alongside the replotted data of Knox et al. (KL&R). While it is possible to get a good fit of the K&S data to the Knox Eq. (8) with $n = 1/3$ and $A = 2.5$, this gives a very poor fit to the data of KL&R: the calculated curve being much too high at low ν. The coupled Eq. (12) does however provide an excellent fit to both sets of data with $n = 0.9$ and $C = 0.016$. The other parameters for Eq. (12) are listed in Table 1. The plate heights are generally lower than those of Kennedy and Knox [20] indicating that the columns were better packed, but the values of n are very similar.

The reconciliation of the data of Knox et al. [17] with those of Knox and Scott [13] is evidence that an equation such as (12) gives the best representation of the contribution to the plate height from processes occurring in the mobile zone. The essential requirement for fitting any equation to the combined data is that the gradient of the log h_A vs. log ν plot decreases as ν increases.

2.5. MacNair et al.—miniaturised HPLC at very high pressure

Very recently, MacNair et al. [21] have obtained impressive performance under extremely high pressures in an HPLC system using a 30-μm bore quartz tube packed with 1-μm non-porous ODS silica particles. The replotted plate height data are shown in Fig. 5.

A typical best fit of the Knox Eq. (8) with $n = 0.33$, has $A = 0.3$, $B = 1.7$ and $C = 0.25$. The C value
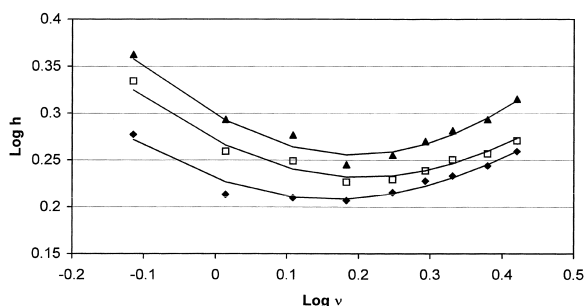
Fig. 5. Replot of data of MacNair et al. [21] for 1-μm porous ODS silica gel, with best fit lines. Lower line, hydroquinone, $k' = 0.11$; middle line, catechol, $k' = 0.35$, upper line, 4-methylcatechol, $k' = 1.05$ with best fit lines according to Eq. (12). For parameter values see Table 1.

is disappointingly high if it is assumed that it arises entirely from static zone processes. The authors, who applied the simple van Deemter equation, (A term constant), also noted ''The C terms are larger than expected for the non-porous particles used''. When Eq. (12) is applied, the data for the three solutes can be well fitted, as shown in Fig. 5, using the constants given in Table 1. The value of n is in the range 0.75–1.05. Of course, five parameters cannot be reliably determined when the range of ν is less than a factor of 10. The high value of n for this column packed with non-porous particles is similar to the values for porous particles, and contrasts with the much lower values of n (around 0.5) for 480-μm glass beads and 29-μm Zipax. This suggests that the value of n is related more to the regularity of packing than to whether the particles of packing are porous or non-porous.

2.6. Monolithic columns

Monolithic column structures for use in LC have been described by Minakuchi et al. [22]. They are produced by a unique process whereby silica gel is precipitated as a porous monolith from tetramethoxy-silane in an aqueous solution of containing poly-ethylene oxide. The mixture, acidified with acetic acid, is reacted at 40 °C in a polycarbonate mould to form porous monolithic silica rods. The monolithic structures have pores around 2 μm across. The fragile porous rods are removed from their poly-carbonate moulds, and coated with polyether ether

ketone (PEEK) tubing so that they can be used for pressure-driven flow. The process has been adopted and refined by the Merck company [23]. The mono-liths exhibit a superior combination of porosity and plate height. This is demonstrated by Sinz and Cabrera [24] who have plotted the separation impe-dance $E = H^2/K$ as defined by Bristow and Knox [25]. The Merck Chromoliths show a ten-fold im-provement in E over conventional columns packed with particulate materials.

Another way to compare the data on monoliths with particulates is to calculate what the particle diameter would, in a conventional column, give equivalent linear flow-rates. This is equivalent to normalising the data to channel size rather than to particle size. Since we are primarily concerned with what happens within the mobile zone, the channel dimension is the most appropriate dimension to use for any comparison. From the permeability data given by Sinz and Cabrera it is readily calculated that the channels in Chromolith are equivalent to those in a bed packed with 9-μm diameter porous spheres. However the ''thickness'' of the structural elements of the monolithic bed are much less than this particle diameter, and from the electron mi-crographs appears to be around 1 μm. With such ''thin'' retentive structures the contribution to h from the static zone equilibration is likely to be negligible, and C can be taken as zero.

Fig. 6 shows the data of Sinz and Cabrera [24]

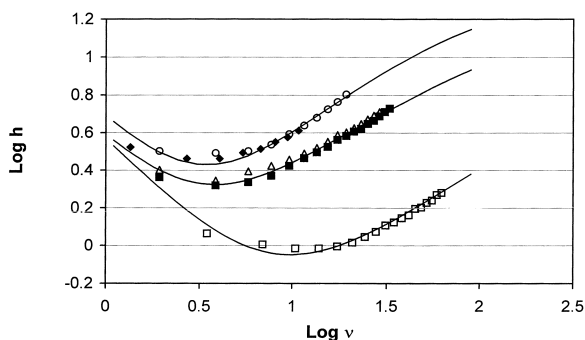Fig. 6. Replot of data from Sinz and Cabrera [24] for Merck particulates and Chromolith [23]. Upper line Z3.5, 3.5 μm, X5, 5 μm; middle line, Z5 and S5, 5 μm; lower line, Chromolith 2 μm channel diameter equivalent to those in bed packed with 9 μm spheres, with best fit lines according to Eq. (12). For parameter values see Table 1.

replotted on a reduced basis with h for the Chromolith column calculated as if it were for particles of 9-μm diameter. This makes the assumption that the channels in the Chromolith column are of the same size as those in a bed of 9-μm slurry-packed spheres. The parameters for the fits of Eq. (12) to the data for the different materials are listed in Table 1.

The comparison corroborates the claims that the monolithic column provides superior performance to columns packed with particulates. The parameters for the particulate materials are similar to those for other particulates. But the value of n for the Chromolith column is significantly lower and the value of D is much lower.

3. Discussion

The main conclusion from the above analysis of LC data obtained over the last four decades is that most of the dispersion which we see under normal HPLC operating conditions arises from processes in the mobile zone, not from processes in the static zone. A similar conclusion was reached previously [11]. An equation which satisfactorily unites the data from a wide range of studies is Eq. (12).

The fact that Eq. (12) unites the data, does not, of course, prove that it is unique. Indeed an alternative which fits the data almost as well is Eq. (13):

$$h = B/\nu + A\nu^n + C\nu \quad n = n_o(1 - 0.125 \log \nu) \quad (13)$$

where n_o is chosen for each set of data. This equation, like Eq. (12), ensures that the gradient of the $\log h/\log \nu$ plot decreases steadily as ν increases, with the gradient becoming zero at high values of ν. Eq. (12) is preferable since it does represent a version of the Giddings' coupling equation. Nevertheless, while the main empirical features of the dependence of h_A upon ν have, I believe, been established, a proper theoretical interpretation has yet to be provided. Computer modelling should be able to provide a more precise dependence of the dispersion upon velocity, but there will always be a major difficulty of devising an adequate representation of a typical packed bed such as that found in an HPLC column. In particular, the variations of packing

density and bed structure across the chromatographic bed along with the effects of walls will eventually have to be built into the model, although initially modelling of the dispersion characteristics of an infinitely wide, randomly packed bed would be the place to start, and would provide fascinating information.

Returning now to the parameters derived using Eq. (12), that is, the values of B, A, D, n and C for the materials given in Table 1. These comprise particulates with diameters from 1 to 480 μm and, materials from solid glass beads through surface layered beads (Zipax) to porous particles and a monolith (Chromolith).

Chromatographers are very familiar with the U-shaped curves obtained when h is plotted against ν or $\log h$ against $\log \nu$, which have long been interpreted by van Deemter-type equations. The left hand side of the U is determined by the B term representing axial molecular diffusion. The right hand side of the U, particularly at high values of ν, is determined by the C term representing slow equilibration within the stationary zone. Around the minimum all three terms are, or may be, important. Various formulations have been proposed for the A term representing the dispersion arising from processes in the mobile zone. Eq. (12) gives the best representation so far. Eq. (12) has a somewhat peculiar form. At low values of ν, i.e. below the transition value of $\nu = (D/A)^{(1/n)}$, the term is dominated by and approximates to $D\nu^n$. At high values of ν, the term is dominated by and approximates to A, and tends to velocity independence. There is therefore a transition from quite a steep dependence of h upon ν, to independence of h upon ν. The number of orders of magnitude of ν over which the gradient of the $(\log h/\log \nu)$ plot changes from, say 90% of n to 10% of n is around $2/n$. Thus, the larger n, the faster the transition: with values of n close to unity, for example, the transition will occur over about two orders of magnitude in ν. A high value of n would, according to the Gidding's analysis, be associated with a closely similar group of coupled processes, having roughly the same geometrical range within the column structure, while a low value of n would be associated a more diverse range of coupled processes. If one particular type of process dominated the mobile zone dispersion, such as trans-

channel or transcolumn processes, then n would be close to unity, and the transition from a steep dependence to a flat dependence (on a logarithmic scale) would be quite sharp.

3.1. Values of n

The most striking differences between the parameters, listed in Table 1, are to be found in the values of n. For glass beads, whether bare or coated, n is around 0.5, and this provides quite a slow flattening off in the log h vs. log ν plot covering some 4 orders of magnitude in ν, while for the porous materials, n is closer to unity, and the flattening off occurs in 2–3 orders of magnitude. For the monolith n has an intermediate value of around 0.7. An exception to this general trend is found with Jorgenson's 1-μm particles packed into a 30-μm tube, where n is around 0.9, the same as for columns packed with porous materials.

If, as Giddings proposes, the main source of dispersion in the mobile zone is transchannel processes, then we deduce, from the values of n, that these have the greatest effect in columns packed with porous spheres, while they have the least effect in columns packed with glass beads. This is not unreasonable, since the transchannel effects are probably dominated by what happens in relatively large voids (on a particle scale) formed by particle bridging during the column packing process. Glass beads being very smooth should pack with minimal bridging, while the less dense and rougher porous particles will show greater friction and form bridges more readily. Monoliths, of course, are formed by a physicochemical process in situ, so bridging cannot occur. The cavities or channels will undoubtedly have a random variation in size but this will be relatively small: there is no reason to expect that there will be any especially large cavities such as those formed by bridging in particle beds. But in addition to transchannel processes, transcolumn processes are likely to be important, especially in the narrow quartz capillaries now becoming increasingly popular. The fact that the 1-μm porous spheres in a 30-μm bore column give a high value of n suggests that they like the porous particulates are difficult to pack uniformly. The overall conclusion therefore has to be that the regularity of the packing rather than the

configuration of the particles is the main factor governing n. Accordingly improving transcolumn equilibration via better packing is likely to be the key to reducing n and generally improving column performance.

3.2. Values of A

The values of A mostly fall in the range 15–20 with the exception of the low value of 7 for the 480-μm glass beads. This low value could be explained by the low aspect ratio (column to particle diameter ratio) of the columns used by Knox and Parcher. The high values of A compare with the typical van Deemter values of 1 or 2. They suggest that in the absence of transverse diffusion the spreading due to tortuous and irregular flow in a packed bed is very large. Transverse diffusion in a packed bed is therefore much more important than has often been thought in moderating the dispersion arising from the packing.

3.3. Values of D

The values of D for a particular type of packing give a measure of how far on average molecules have to diffuse to iron out the irregularities of flow. They, like the values of A, indicate how well a column is packed. Thus the 480-μm glass beads of Knox and Parcher ($D = 0.16$) were better packed than the 29-μm Zipax beads of Done and Knox ($D = 0.50$–0.65). Likewise the 20- and 50-μm particles of K&S and of KL&R ($D = 0.23$) were better packed than the 50-μm Porasil beads used in the early study of Kennedy and Knox (0.40–0.50). The very low value of D along with an n value of 0.72 for the Chromolith, suggests that here the channels are much more uniform in their size than in packed beds but that there is a range of geometries for the coupling of flow and diffusion processes. The overall effect of a very homogeneous bed is greatly to enhance chromatographic efficiency.

3.4. Values of B and C

The values of B listed in Table 1 are within the expected range. The C values have mostly been assigned on the basis of theory, but some of the

values have been determined by the best fit procedure, namely the values for the combined data from Spherisorb alumina and porous ODS silica ($C = 0.016$) and the values for Porasil ($C = 0.025$ and 0.030). The last are somewhat high, but may result from restricted diffusion in this early silica gel.

The major practical conclusion from the above assessment is that the great part of the dispersion in present HPLC columns arises in the mobile zone. It is caused by the irregularity of the flow pattern in the packed bed, and not by slow equilibration within the particles of packing material. This slow equilibration only begins to contribute to the plate height when the reduced velocities is far above that for the minimum h. In line with Giddings' original analysis, the largest contributors to h in the mobile zone most probably arises from "transchannel" and "transcolumn" processes which have a relatively high transition velocity. The monolith structure with its much more regular cavities or channels produces much less dispersion than would be expected from a packed bed with the same mean channel size. The formation of yet more uniform structures should lead to much improved performance in chromatography.

It may now be possible to devise specific structures for chromatography such as specialised regular 2-dimensional structures etched on silicon or other chips [26]. The object of such a structure would be to provide regular streamlined flow around retentive pillars which could be either porous or surface coated. The sort of structure which might provide more efficient chromatography is shown in Fig. 7. The struc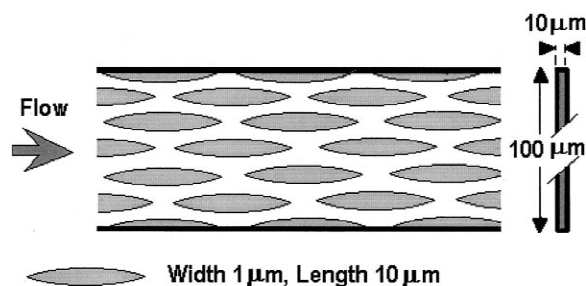ture envisaged consists of a series of pillars of high aspect ratio, formed by etching to give a 2-dimensional array. The pillars would typically be $1–10$ μm in height, 10 μm long and 1-μm wide with channels between them of about 1-μm width. This structure would be closed by a close fitting sealed plate. Retention could be introduced by bonding suitable groups to the walls of the pillars. Alternatively the pillars could possibly be made porous so that they could be accessed by diffusion through the walls. The internal parts of the pillars would then be bonded in the same way as current HPLC materials using suitable silanes.

4. Nomenclature

A, A_1, A_2	Constants in plate equations for mobile zone processes
B	Constant in plate height equations for axial diffusion
C	Constant in plate height equations for static zone processes
D	Constant in "coupled" plate height equations
D_m, D_s, D_{sz}	Diffusion coefficient in mobile phase, in stationary phase and in static zone
d_p, d_c	Particle diameter, column diameter
H	Plate height
h, h_A, h_C	Reduced plate height in general, for mobile zone processes, for static zone processes
K	Column permeability coefficient
k', k''	Phase capacity ratio, zone capacity ratio
n, n_o	Power of reduced velocity in plate height equations
u	Linear flow velocity
γ	Obstructive factor
φ	Fraction of mobile phase which is stagnant
ν	Reduced velocity or Peclet number

Fig. 7. Possible configuration of two dimensional column-on-a-chip, consisting of short pillars (shaded) some $1–10$ μm high, 10 μm long and 1-μm wide etched from a flat plate. The pillars could be edge coated, or porous with a bonded monolayer to provide retention, the whole to be capped with a sealing lid.

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