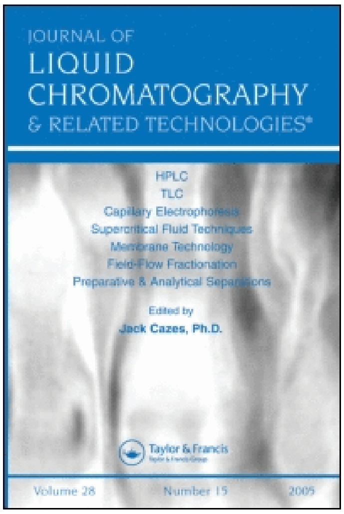

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EXTRA-COLUMN DISPERSION IN LIQUID CHROMATOGRAPHY SYSTEMS

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ABSTRACT

The various sources of extra-column dispersion are considered in detail and examined, both theoretically and experimentally. It is demonstrated that, if the advantageous high efficiencies obtainable from contemporary liquid chromatography (LC) columns is to be realized, the total mobile phase conduit system, from the sample valve to the detector sensor cell, must be very carefully designed and constructed. In practice, it may be necessary to modify existing equipment to reduce extra-column dispersion, particularly if microbore columns are to be used.

INTRODUCTION

Employing well prepared stationary phases and supports, together with efficient packing techniques, columns with very low plate heights and corresponding high efficiencies can now be easily produced. High column efficiencies, however, are accompanied by very narrow peaks and matching very small peak volumes. As column technology has improved, so the volume of the eluted peaks has become much smaller and, as a consequence, any dispersion that takes place outside the column has become potentially more deleterious. It follows that, for the excellent column performance that can be expected from modern liquid chromatography (LC)

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columns to be realized, the chromatographic apparatus must be designed to provide *minimum extra-column dispersion*. This may also mean that if older chromatographs are employed with modern LC columns (particularly microbore columns), the best results from the column may not be attained.

There are four major sources of extra-column dispersion, all of which are controllable and can be reduced sufficiently by appropriate chromatograph design to allow the optimum performance of modern columns to be realized. The four major sources of extra-column dispersion are as follows:

1. *Dispersion in the Sample Valve*: The sample valve can produce dispersion by two processes. Firstly, dispersion that results from the finite volume of sample itself. Secondly, the dispersion due to the Newtonian flow of sample through the sample cell.
2. *Dispersion from Connecting Tubes*: Dispersion occurs in all connecting tubes due to the parabolic velocity profile of the mobile phase passing through it (Newtonian Flow). Thus, any connecting tube between the sample valve and the column, or the column and the detector, will contribute to the total dispersion of the peak.
3. *Dispersion from Unions and Frits*: Dispersion in unions (e.g., between connecting tube and column or column and detector) and in frits is also due to Newtonian flow.
4. *Dispersion in the Volume of the Detector Sensor*: Dispersion in the detector sensor arises from two sources, i.e., that due to Newtonian flow and that due to sensor having a finite volume (the magnitude of which may be comparable to that of the peak). The latter dispersion may not only broaden the peak, but also tend to *distort* the peak, particularly if two solutes are eluted close together.

None of these sources of extra-column dispersion can be completely eliminated but, by careful design, they can be significantly reduced so that they no longer impair the performance of the column. Before the extra-column dispersion can be considered in detail, however, it is necessary to determine the volume of the peaks produced by contemporary high efficiency columns so that a limit can be placed on the acceptable level of extra-column dispersion.

Dispersion in Contemporary LC Columns

The standard deviation volume of an eluted peak (σ_v) from the Plate Theory[1] is given by,

$$\sigma_v = \frac{V_r}{\sqrt{n}}$$

where (V_r) is the retention volume of the solute and (n) is the column efficiency.

Now,

$$V_r = V_0(1 + k') = \varepsilon l \pi r^2 (1 + k')$$

where (V_0) is the thermodynamic dead volume of the column,

$$V_0 = \varepsilon l \pi r^2$$

where (r) is the radius of the column, (l) is the length of the column, and (ε) is the fraction of the mobile phase in the column that is available to the solute. Consequently,

$$\sigma_v = \frac{\varepsilon l \pi r^2 (1 + k')}{\sqrt{n}}$$

Now,

$$n = \frac{l}{H}$$

where (H) is the Height of the Theoretical Plate.

For a well packed column, $H = 1.6\, d_p$, where (d_p) is the particle diameter of the packing.[2]

Thus,

$$n = \frac{l}{1.6 d_p}$$

and

$$\sigma_v = \frac{\varepsilon l \pi r^2}{\sqrt{l/1.6 d_p}}(1 + k') = \sqrt{1.6 d_p l}\, \varepsilon \pi r^2 (1 + k') \tag{1}$$

Consequently, the Volume variance of the peak (${\sigma_v}^2$) is given by

$${\sigma_v}^2 = 1.6 d_p l \left[\varepsilon \pi r^2 (1 + k')\right]^2 \tag{2}$$

Employing Eq. (1), it is now possible to calculate the volume standard deviation for some popular, contemporary LC columns eluting solutes of different (k') values. The type of packing is not defined and may be silica, bonded silica, or polymeric in character, but all will be assumed to be spherical in form. In general, it will only be the particle diameter of the stationary phase that determines column dispersion, not its chemical character. A generally accepted value of 0.6 is taken for (ε).[3] The properties of the three columns are shown in Table 1. The *peak volume*, taken as ($4\sigma_v$), which is included as this value, has more significance to the practicing chromatographer.

Table 1. Properties of Three Common Types of Contemporary LC Columns

Column	1	2	3
I.D. (mm)	4.6	3	1
Length (cm)	3	10	20
Part. diam. (μm)	3	5	10
Flow rate (mL/min)	1	0.5	0.020
Efficiency (*n*)	6,250	12,500	6,250
Ther. dead vol (mL)	0.299	0.424	0.094
$\sigma_{v(k'=0)}$ (μL)	3.78	3.79	1.19
$\sigma_{v(k'=0)}{}^2$ (μL^2)	1.43×10^{-5}	1.44×10^{-5}	1.41×10^{-6}
$\sigma_{v(k'=5)}$ (μL)	22.68	22.74	7.14
$\sigma_{v(k'=5)}{}^2$ (μL^2)	5.15×10^{-4}	5.18×10^{-4}	5.08×10^{-5}
$\sigma_{e(k'=0)}$ (μL)	1.19	1.20	0.38
	$4\sigma = 4.76$	$4\sigma = 4.8$	$4\sigma = 1.52$
$\sigma_{e(k'=0)}{}^2$ (μL^2)	1.43×10^{-6}	1.44×10^{-6}	1.42×10^{-7}
$V_{i(k'=0)}$	5.0	5.0	1.57
$V_{i(k'=5)}$	30.0	30.0	9.42
Practical $V_{i(k'=0)}$	0.87	0.88	0.27
Practical $V_{i(k'=5)}$	5.24	5.25	1.65

It is seen that the dead volume peak widths are very narrow (assessed as ($4\sigma_{v(k'=0)}$)) and range from about 5 μL for the peak from the microbore column to about 15 μL for the 3 mm I.D. and 4.6 mm I.D. columns. From these data, the maximum permissible extra-column dispersion can be calculated.

Maximum Permissible Extra-Column Dispersion

The total variance of an eluted peak ($\sigma_r{}^2$) will be the sum of the variance due to column dispersion ($\sigma_v{}^2$) and the variance from extra-column dispersion ($\sigma_e{}^2$), i.e.,

$$\sigma_r{}^2 = \sigma_v{}^2 + \sigma_e{}^2$$

Klinkenberg[4] suggested that the maximum increase in peak variance from extra-column dispersion that could be tolerated, while not significantly affecting the resolution, is 10%. Thus,

$$\sigma_r{}^2 = 1.1\sigma_v{}^2 = \sigma_v{}^2 + \sigma_{e(\mathrm{Max})}{}^2$$

or,

$$\sigma_{e(\mathrm{Max})}{}^2 = 0.1\sigma_v{}^2$$

Substituting for ($\sigma_v{}^2$) from Eq. (2),

$$\sigma_{e(\mathrm{Max})}{}^2 = 0.16 d_p l \left[\varepsilon \pi r^2 (1 + k')\right]^2 \tag{3}$$

and,

$$\sigma_{e(\mathrm{Max})} = \sqrt{0.16 d_p l}\, \varepsilon \pi r^2 (1 + k') \tag{4}$$

Values for the standard deviation and variance arising from permissible extra-column dispersion are included in Table 1. It is seen that the permissible peak widths and variances are very small [assessed as ($4\sigma_{v(k'=0)}$)] and range from about 1.5 μL for the peak from the microbore column to about 4.8 μL for the 3 mm I.D. and 4.6 mm I.D. columns. These limits are for peaks eluted close to the dead volume where the conditions are most stringent.

Now, the extra-column dispersion must be shared between all the possible dispersion sources, one of which, the dispersion due to a finite sample volume, may be critical for a satisfactory analysis. This is because the sample volume controls the maximum amount of the solute mixture that can be placed on the column.

Now, treating the effect of sample volume in the same manner as general extra-column dispersion (i.e., by summing the variances),

$$\sigma_r{}^2 = \sigma_i{}^2 + \sigma_c{}^2$$

where

$\sigma_r{}^2$ is the overall variance of the eluted peak,
$\sigma_i{}^2$ is the variance of the sample volume, and
$\sigma_e{}^2$ is the variance due to column dispersion.

It has been established that the variance of a rectangular distribution of sample volume (V_i) will be ($V_i{}^2/12$). Thus, it can again be assumed that the peak variance can be increased by 10%, as a result of extra-column dispersion, without seriously denigrating column performance. However, it can *not* be assumed that *all* the permitted extra-column dispersion can be allotted to the effect of a finite sample volume, as some must be allowed for the other dispersion sources. Therefore, at least half of the permissible extension of peak width must be allotted to the effect of the sample volume (i.e., the peak variance can be increased by 5%). Thus,

$$\frac{V_i{}^2}{12} + \left(\frac{V_r}{\sqrt{n}}\right)^2 = 1.05\frac{V_r{}^2}{n}$$

where dispersion due to the column alone ($\sigma_v{}^2$) is $\left(V_r/\sqrt{n}\right)^2$

Thus,

$$\frac{V_i^2}{12} = 0.05\frac{V_r^{\,2}}{n} \quad \text{and} \quad V_i = 0.77\frac{V_r}{\sqrt{n}} \tag{5}$$

Now, from Eq. (2),

$$\sigma_v^{\,2} = \frac{V_r^{\,2}}{n} = 1.6d_p l\left[\varepsilon\pi r^2(1+k')\right]^2$$

Thus, substituting for $(\sigma_v^{\,2})$ in Eq. (5),

$$V_i^2 = 0.6\sigma_v^{\,2} = 0.96d_p l\left(\varepsilon\pi r^2(1+k')\right)^2 \tag{6}$$

and

$$V_i = 0.77\sigma_v = 0.98\sqrt{d_\mathrm{p} l}\,\varepsilon\pi r^2(1+k') \tag{7}$$

Equation (6) also gives a value for permissible extra-column dispersion variance from all the remaining dispersion processes not associated with sample volume dispersion. The maximum sample volume and the maximum extra-column variance for each column are included in Table 1. It is seen that, if the solutes of interest are eluted close to the dead volume, the maximum sample volume is very small, about 1.57 μL for the microbore column and about 5.0 μL for the 4.6 mm I.D. and 3 mm I.D. columns. Such small sample volumes may be difficult to work with in practice, particularly if the solutes of interest are present at trace levels. If the phase system is adjusted so that the solutes of interest are eluted at a capacity ratio of 5, then much larger sample volumes are permissible e.g., 9.4 μL for the microbore column and 30 μL for the large bore columns.

The Sample Valve

The elution profile of a solute band leaving a sample valve is controlled largely by the geometry of the sample volume itself (i.e., whether it takes the form of an exterior tube or a groove in the spigot of the valve). However, it does also depend, to some extent, on the geometry of the exit ports to the valve–column connecting tube. Contemporary low dead volume unions have largely eliminated dispersion in unions and, thus, they no longer contribute significantly to extra-column dispersion in most column systems. However, they are still not completely dispersion free. If the sample volume consists of an *external tube*, then any dispersion that takes place in it will be the same as that which would occur in a connecting tube of equivalent length; this will be discussed below. Dispersion effects resulting from an internal sample volume that is formed by a groove cut in the valve spigot, together with dispersion arising in the sample valve exit ports, are extremely difficult to treat theoretically and, consequently, need to be determined experimentally. Scott and Simpson[5] measured the dispersion from two different 0.2 μL sample valves and their results are shown in Table 2.

The data was obtained using a specially designed low dispersion chromatograhic system and an electrical conductivity detector with a sensor volume of only 0.08 μL.

It is seen that the internal bore of the valve connection can affect the resulting dispersion very significantly and it could be anticipated that the dispersion would be reduced still more if the diameter of the connection was further reduced. However, there is a limit to reducing the radius of any conduit in the mobile phase system, as apertures less than 0.005 in. I.D. readily become blocked. The absolute minimum internal diameter that is recommended is 0.003 in. (this applies to connecting tubes as well) and, when using such tubes, all samples and the mobile phase should be filtered before use. It should be possible to design exit ports to provide secondary flow and, thus, increase the effective diffusivity of the solute and, consequently, reduce dispersion. The dispersion from an *exterior* sample tube can be reduced very significantly by packing it with appropriately sized glass beads (which would make the sample tube more like a packed column than an open tubular column). Alternatively, crimping the tube as suggested by Halasz,[6] coiling the tube as suggested by Thjisen,[7] or employing low dispersion tubing such as the serpentine tubing designed by Katz et al.[8] can also help reduce dispersion in open tubes.

Dispersion in Connecting Tubes

The dispersion that takes place in sample-valve/column and column/detector cell connecting tubes results from the parabolic velocity profile that always occurs during pressure driven fluid flow through open tubes. The dispersion is described by the Golay equation, for open tubular columns,[9] by

Table 2. Variance of Solute Bands Resulting from Two Different Valco Valves

Internal Bore of Valve Connection	Variance of Peak (μL^2) Mean of Three Measurements
0.030 in.	0.667
0.010 in.	0.338

Flow rate 20 μL/min.

setting the capacity ratio (k') to zero. Thus, the variance due to an open tube ($\sigma_{tu}{}^2$) will be

$$\sigma_T{}^2 = \frac{2\mathrm{D}_m}{u} + \frac{r^2 u}{24 D_m} \tag{8}$$

where (r) is the radius of the tube, (u) is the linear velocity of the mobile phase, and (D_m) is the diffusivity of the solute in the mobile phase.

If $u \gg D_m/r$, then Eq. (7) reduces to

$$\sigma_T{}^2 = \frac{r^2 u}{24 D_m}$$

Now, the peak variance, in volume units ($\sigma_{T(v)}{}^2$), is

$$\sigma_{T(v)}{}^2 = \frac{(\text{Tube volume})^2}{n} = \frac{\left(\pi r^2 l\right)^2}{n} = \frac{\pi^2 r^4 l^2}{n}$$

where (n) is the number of theoretical plates in the tube.

Now,

$$\frac{l}{n} = H = \sigma_T{}^2 = \frac{r^2 u}{24 D_m}$$

Thus, substituting for (l/n),

$$\sigma_{T(v)}{}^2 = \frac{\pi^2 r^6 l u}{24 D_m}$$

Bearing in mind that the flow rate $Q = \pi r^2 u$,

$$\sigma_{T(v)}{}^2 = \frac{Q \pi r^4 l}{24 D_m} \tag{9}$$

As the column should be operated at its optimum mobile phase velocity, the flow rate, (Q), is defined by the characteristics of the column, and cannot be used to control tube dispersion. Similarly, the diffusivity of the solute, (D_m), is determined by the nature of the sample and the mobile phase and is also not a variable available for dispersion control. The remaining factors that can be used to control dispersion are the tube radius and the tube length. The dispersion increases as the fourth power of the tube radius and linearly as the column length. The radius has the major control on dispersion, as a reduction in the tube radius by a factor of two will reduce the dispersion by a factor of sixteen. Unfortunately, there is a limit to the process of reducing (r) as, from Poiseuille's equation, the pressure drop across the tube is given by

$$\Delta P = \frac{8 \eta l u}{\pi r^2}$$

and, as $Q = \pi r^2 u$

$$\Delta P = \frac{8\eta l Q}{\pi r^4}$$

It is seen that the pressure drop across the connecting tube increases inversely as the fourth power of the tube radius. Consequently, as it is inadvisable to dissipate a significant amount of the available pump pressure across a connecting tubing, there will be a lower limit to which (r) can be reduced in order to minimize dispersion.

Changing the length of the connecting tube has the same effect on both dispersion and pressure drop. Reducing (l) will linearly reduce dispersion and, at the same time, proportionally reduce the pressure drop across the connecting tube. Thus, reducing the length of the connecting tube is, by far, the best method of controlling dispersion and, by making (l) as small as possible, both the dispersion and the pressure drop can be minimized.

However, as already suggested, the diameter of the connecting tube should not be made less than 0.012 cm, (0.005 in. I.D.), not merely because of the pressure drop that will occur across it, but to ensure that the tube *will not become blocked.* Employing Eq. (8), the volume variance and standard deviation contribution from connecting tubes of different lengths was calculated for two different flow rates. The result relating equivalent peak volume ($4\sigma_{T(v)}$) to tube length is shown in Fig. 1. The tube radius was assumed to be 0.005 in., the flow rates 1 mL/min and 20 μL/min, and the diffusivity of the solute in the mobile phase 2.5×10^{-5} cm^2/sec.

Although standard deviations are not additive, from a practical point of view, values of equivalent peak width (4σ) do give a better impression of the dispersion that takes place in the connecting tube. It is seen that a tube 10 cm long and 0.012 cm I.D. can result in a peak having a base width of 10 μL. This means, that if high efficiency columns are to be used, with small plate heights, then connecting tubes should either be eliminated altogether, or reduced to the absolute minimum in length. In practice, it is sometimes difficult to achieve short lengths of a connecting tube, particularly for column detector connections. This is because manufacturers often design detectors such that the sensor cells require significant lengths of tubing to connect them to the exterior union.

Low Dispersion Connecting Tubes

The ideal situation, where the sample valve and the detector sensor cell is coupled directly to the column is usually impossible in practice and, thus, a conduit system that provides little or no dispersion could be very useful. The dispersion (which is due to the parabolic velocity profile of the mobile phase

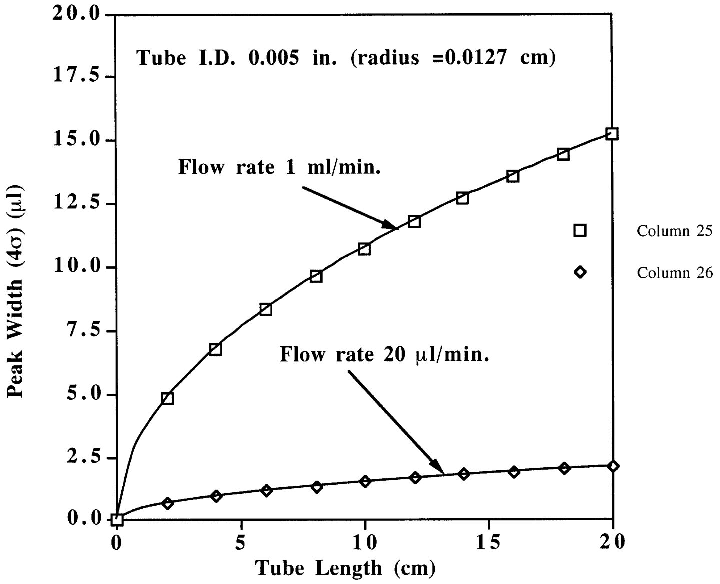


Figure 1. Graph of equivalent peak volume from a connecting tube 0.005 in. I.D., vs. tube length.

flowing through it) can be disturbed, and secondary flow introduced into the tube by deforming its regular geometry.

Dispersion in geometrically deformed tubes (squeezed, twisted, and coiled) has been studied by Halasz et al.[6] and the effect of radial convection introduced in tightly coiled tubes has been examined by Tijssen.[7] The effect of secondary flow introduced in serpentine shaped tubes has been reported by Katz and Scott.[8] The effect of coiling a straight tube on the resulting tube dispersion was examined experimentally by Scott and Simpson.[10] They took a 1 m length of capillary tubing, 0.010 in I.D., and measured the dispersion, first as a straight tube, and then after it had been made into coils of various diameters. The dispersion (variance in μL^2) is shown plotted against the number of turns in the coil (the actual tube length being kept constant at 1 m) in Fig. 2. It is seen that coiling the tube has a profound effect on the consequent dispersion, due to the introduction of radial flow by the flow change of direction as it passes round the coils. It is seen, however, that after about 50 turns have been introduced into the coil, the radial flow effect appears nearly complete and further coiling has only a small effect on dispersion.

Tijssen[7] developed a theory that qualitatively described the relationship between the variance per unit length, (H), of a coiled tube and the mobile phase

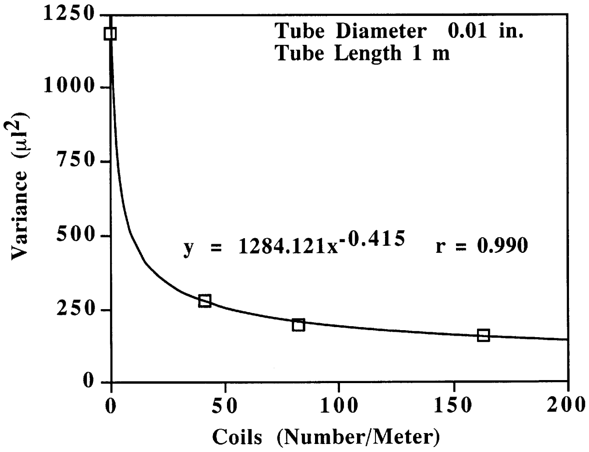


Figure 2. Variance for a 1 m tube vs. number of coils.

velocity. At relatively low linear velocities (but not low relative to the optimum velocity for the tube) Tijssen proposed the equation

$$H = \frac{jr^2u}{D_m} \tag{10}$$

where (j) is a constant over a given velocity range, and the other symbols have the meanings previously ascribed to them. It is seen that the band variance is directly proportional to the square of the tube radius and the relationship is very similar to that derived by Golay[9] for a straight tube.

At high linear velocities, Tijssen deduced that

$$H = \frac{bD_m{}^{0.14}}{\psi} \tag{11}$$

where (b), is a constant for a given mobile phase and (ψ) is the ratio of the tube radius to the coil radius, and was given the term the *coil aspect ratio*.

Consequently,

$$j = \frac{r_{\text{tube}}}{r_{\text{coil}}}$$

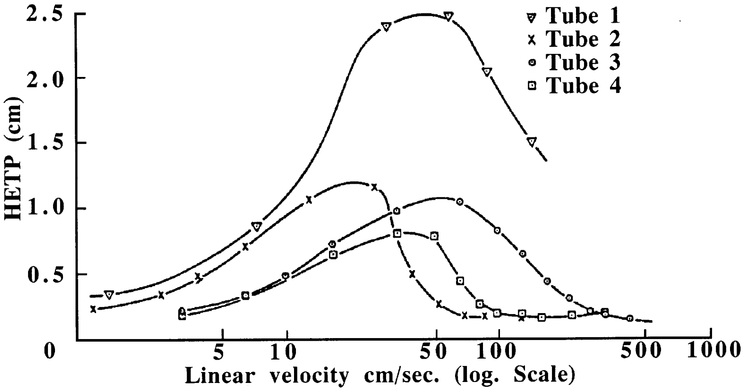
At the higher linear mobile phase velocities, it is seen that the value of (H) depends on (D_m) taken to the power of 0.14 and inversely dependent on the coil aspect ratio and the linear velocity. Thus, at low velocities, the band dispersion *increases* with (u) whereas, at high velocities, the band dispersion *decreases* with (u). Consequently, a plot of (H) against (u) will show a maximum at a certain value of (*H*). By combining Eqs. (9) and (10), an equation can be obtained that predicts the value of (*u*) at which (*H*) is a maximum, viz.,

$$u = \frac{c}{r\sqrt{j}}$$

where (*c*) is a constant for a given solute and given mobile phase.

The above equations were employed to investigate the effect of tube radius and coil aspect ratio on the onset of radial mixing in coiled tubes. The curves relating (*H*) and (*u*) are shown in Fig. 3.

It can be seen that, at low linear velocities, where radial mixing is still poor, the values of (*H*) increase as (*u*) increases. Furthermore, the dispersion in coiled tubes (1) and (2) of larger radii is greater than that in tubes (3) and (4) which had smaller radii. At high linear velocities, where radial mixing commences, the values of (*H*) decrease as (*u*) increases. As the range of linear velocities is approached where radial mixing dominates, the solute dispersion becomes independent of the linear velocity (*u*). It is also seen the maximum value of (*H*) for any particular coil occurs at different values of (*u*), depending on the combined values of *r* and (*j*). In general, it would seem that a high coil aspect ratio reduces both the maximum value of (*H*) and the value of (*u*) at which it occurs.



Mobile Phase 5 Ethyl Acetate in n-Hexane Solute Benzyl Acetate

Tube	(r)cm	(L) cm	r(coil) cm	(j)	L(coil)cm
1	0.019	366	0.5	0.038	18.5
2	0.020	365	0.085	0.235	65.8
3	0.0127	998	0.077	0.166	128
4	0.0127	338	0.050	0.026	73.7

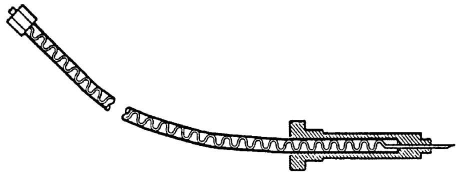
Figure 3. HETP of various coiled tubes vs. Log (linear velocity).

However, at high velocities, the effect of secondary flow causes the diffusivity of the solute to dramatically increase, eventually reducing the resistance to mass transfer function to virtually zero. This results in a corresponding reduction in the value of (*H*). Finally, at very high velocities, the greatly reduced longitudinal diffusion effect is again the only dispersion process that dominates. At this point, the value of (*H*) is very small indeed and, in fact, decreases even further as the mobile phase velocity is increased more.

Serpentine Tubes

The low dispersion serpentine tube developed by Katz et al.[8] was an alternative approach to the coiled tube and was designed to increase secondary flow by actually *reversing* the direction of flow at each serpentine bend. A diagram of a serpentine tube is shown in Fig. 4. In fact, the serpentine tubing shown in Fig. 4 was designed as an interface between a liquid chromatograph and an atomic absorption spectrometer. The serpentine tube is encased in an outer sheath to protect the tube and provide some rigidity. A graph relating the variance per unit length of the tube (*H*) against flow rate is shown in Fig. 5, for a serpentine tube with the dimensions given in Fig. 4.

The flow rate is employed as the independent variable, an alternative to the more usual linear velocity, because the flow rate is defined by the column with which the low dispersion tubing is to be used. It is seen that a similar curve (but much reduced in amplitude) is obtained for the serpentine tube, as that which was observed for the coiled tube, but the maximum value of (*H*) is reached at a much lower flow rate than that with the coiled tube. Furthermore, the variance remains more or less constant over a wide range of flow rates that encompass those usually employed in normal LC separations.



Dimensions : (r), (internal), 0.0127 cm (0.010 in. I. D.)
(r), (external), 0.025 cm (0.020 in. O. D.)
(L), length (linear) 42.5 cm.
(l), length (coil) 38.5 cm.
(s), (serpentine amplitude) 0.05 cm.

Figure 4. Serpentine tube design.

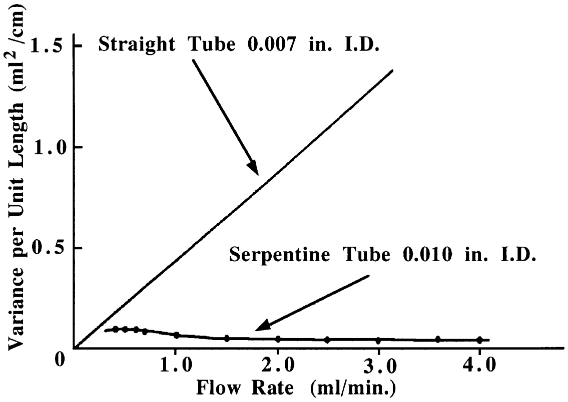


Figure 5. Peak variance vs. flow rate for straight and serpentine tubes.

It is seen that the numerical value of the peak variance per unit length for the serpentine tube (0.010 in I.D.) is 0.05 $\mu L^2/cm$ and, consequently, a tube *10 cm long* would contribute a variance of 0.5 μL^2. In contrast, the dispersion of a straight tube of the same internal diameter and *only 1 cm in linear length* would be 5.5 μL^2, which is an order of magnitude larger.

Low dispersion connecting tubes are still not in common use in LC equipment today although, at least one manufacturer provides serpentine tubing as a standard column/detector connection in a combined sample valve injector/column/detector system. Low dispersion tubing has another feature that, in fact, could be anticipated from its principle of operation. The secondary flow, which results from its serpentine form, also greatly improves its thermal conducting properties and, thus, serpentine tubes can be used as highly efficient heat exchangers. Consequently, another instrument manufacturer utilizes serpentine tubing as a heat exchanger between a thermostatting medium and the inlet tube carrying mobile phase to the column. It was found that only a few centimeters of serpentine tubing were necessary to achieve complete thermal equilibrium between the thermostatting medium and the mobile phase.

Frits and Unions

By their very design, which is basically a packed bed fused into a single entity, the stainless steel frit is unlikely to provide significant dispersion. This was confirmed by Scott and Simpson[10] who measured the dispersion of a series of stainless steel frits placed one upon the other. The results they obtained are shown in Fig. 6 as a graph relating total peak variance to the number of frits in the pile. It is

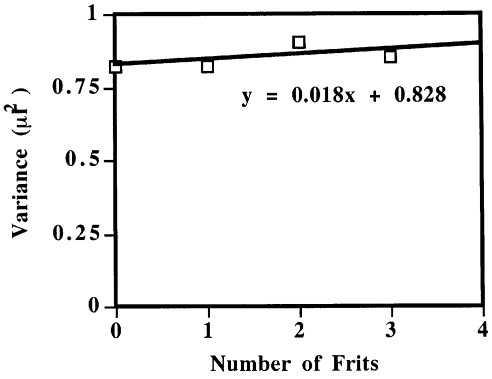


Figure 6. Total peak variance vs. number of frits.

seen that the contribution of each frit to the overall variance (0.018 μL^2) is, indeed, minimal and, for most applications, can be ignored. Simpson and Scott also measured the dispersion resulting from *normal* and *drilled-out* unions (low dispersion unions). Low dispersion unions can take two forms. In the first, the connecting aperture between the ends of the connecting tubes is made very small and short to reduce dispersion. This is the more common type of low dispersion union.

The other allows the connecting tube to butt up to the sintered disk of a column or the face of another connecting tube so that the union itself is not part of the mobile phase conduit. The latter type of low dispersion union is the type examined by Scott and Simpson. The results they obtained are shown in Table 3.

It is seen that, by drilling out the union so that direct contact can be made between the connecting conduits, reduces the dispersion contributed by the union from 1.464 (μL^2) to 0.113 (μL^2). This reduction is very significant and emphasizes the need for using low dead volume unions (or preferentially drilled

Table 3. Dispersion in Unions

Union Type	Variance $(\mu L)^2$
Normal	1.983
Drilled out	0.632

Variance from sample valve = 0.054 $(\mu L)^2$.
Variance from 5 cm of tubing = 0.465 $(\mu L)^2$.
Total variance other than that from unions = 0.519 $(\mu L)^2$.
Variance due to drilled-out union = 0.632 − 0.519 = 0.113 $(\mu L)^2$.
Variance due to normal union = 1.983 − 0.519 = 1.464 $(\mu L)^2$.

out unions) when employing columns of high efficiency and low intrinsic volume (e.g. microbore columns).

Dispersion in the Volume of the Detector Sensor

Dispersion in detector sensor volumes relate to sources as already discussed, that caused by Newtonian being more easy to reduce, or even eliminate, than that due to the volume dilution effect. Scott and Simpson[10] also measured the overall effect of dispersion in sensor cells by simulating them from simple short cylindrical tubes and measuring the net dispersion of a peak passing through them. The results they obtained are shown in Table 4.

It is seen that both the length and the I.D. of the cell are critical factors that affect the dispersion. In addition, the magnitude of the dispersion can be comparable to that produced by the column alone and, consequently, will seriously affect the performance of microbore columns. The major source of the dispersion, however, could not be identified by these experiments. Scott and Kucera[11] examined the effect of sensor volume on peak dispersion in their work on microbore columns. The results they obtained are shown in Fig. 7.

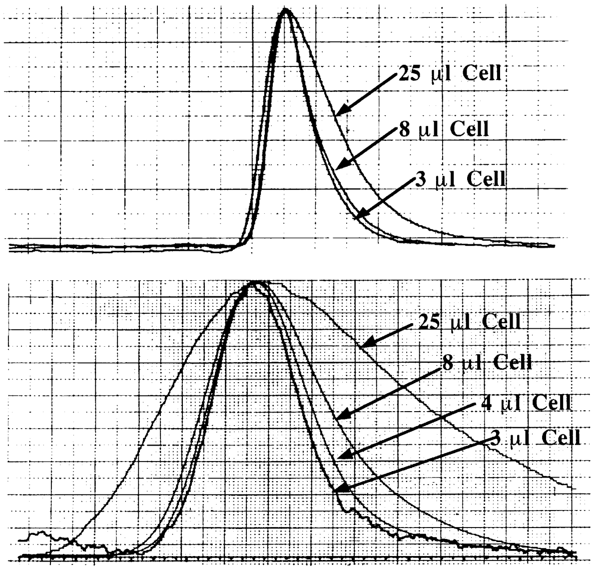
The column used in the upper chromatogram was 24 cm long, 4.6 mm I.D., and operated at a flow rate of 1 mL/min. The mobile phase was tetrahydrofuran and the solute benzene. The column used in the lower chromatogram was 1 m long, 1 mm I.D., and operated at a flow rate of 40 μL/min. It is seen that the reduction in cell volume has a dramatic effect on both peak width and peak shape. The large 25 μL cell causes significant peak asymmetry, as well as excessive peak dispersion, which is predicted by the work of Atwood and Golay[12] which is discussed below. Clearly, even cell volumes of 3 μL are too large for use with 1 mm I.D. columns; relatively few contemporary detectors have cell volumes less than 3 μL.

Most sensor volumes are cylindrical in shape, relatively short in length, and have a small length-to-diameter ratio. The small length-to-diameter ratio is in

Table 4. Dispersion in Sensor Cells

Cell Volume	Cell I.D.	Cell Length	Volume Variance	Equilibrium Peak Volume
0.59 (μL)	0.5 mm	3.0 mm	0.45 (μL^2)	2.68 (4σ) (μL)
1.96 (μL)	0.5 mm	10.0 mm	1.19 (μL^2)	4.36 (4σ) (μL)
2.75 (μL)	1.0 mm	3.5 mm	1.19 (μL^2)	4.36 (4σ) (μL)
7.85 (μL)	1.0 mm	10.0 mm	4.92 (μL^2)	8.88 (4σ) (μL)

The measurements were made on a linear reconstruction of the cell.



Courtesy of Elsevier Publications (Ref. [11])

Figure 7. Peak profiles from detectors having various cell volumes.

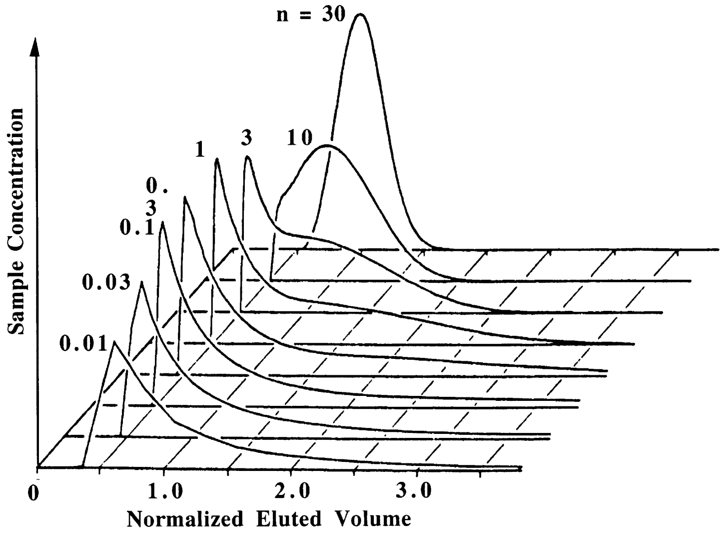


Figure 8. Elution curves presented as a function of the normalized tube length.

conflict with the premises adopted in the development of the Golay equation for dispersion in an open tube and, consequently, its conclusions are not pertinent to detector sensors. Atwood and Golay[12] extended the theory of dispersion in open tubes to tubes of small length-to-diameter ratio. The theory developed is not pertinent here, as it will be seen that, with correctly designed cells, dispersion from viscous sources can be made negligible. Nevertheless, the effect of the cell on solute profiles is shown in Fig. 7.

It is seen that, depending on the length of the cell (i.e., the number of theoretical plates it contains), the peak can exhibit various types of dispersion and distortion. However, this results only when true Newtonian flow is present and if the parabolic velocity profile can be disrupted, the dispersion and distortion arising from such conditions can be virtually eliminated.

Fortunately, this can readily be achieved in practice by modifying the manner of entrance and exit of the mobile phase to the cell. The conduit connections to the cell are designed to produce secondary flow and, thus, break up the parabolic velocity profile, which causes the peak distortion in the manner shown in Fig. 9. Mobile phase enters the cell at an angle that is directed at the cell window. As a consequence, the flow has to virtually reverse its direction to pass through the cell, producing a strong radial flow and disrupting the Newtonian flow.

The same arrangement is employed at the exit end of the cell. The flow along the axis of the cell is made to reverse its direction to pass out of the port that

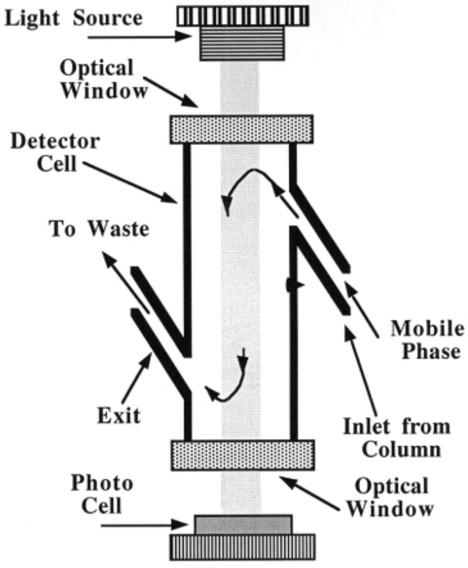


Figure 9. Design of a modern absorption cell.

is also set at an angle, as shown in the diagram. Employing this type of cell geometry, dispersion resulting from Newtonian flow can be practically eliminated. There remains, however, the dispersion that arises from the effect of the finite volume of the sensor relative to the volume of the eluted peak.

Apparent Dispersion from Detector Sensor Volume

In practice, the detector does not respond to a specific concentration in the column eluent, but to an *average value of the total amount of solute in the sensor cell.* This situation is depicted in Fig. 10. It is clear that the average concentration of solute contained in the slice representing the cell volume will differ considerably from the true concentration of solute in the column eluent. For a single peak, if the sensor volume is significant compared with the peak volume, the mean concentration will always be *less* than the true concentration; in addition, the peak width as measured by the sensor will always be *significantly wider* than the actual peak width. If the sensor volume is *very* large it could contain two closely eluted peaks and, thus, give a response (although very distorted in shape) that would appear as a single peak. However, whatever the volume, the output of the sensor will always represent the *average concentration* in the sensor cell.

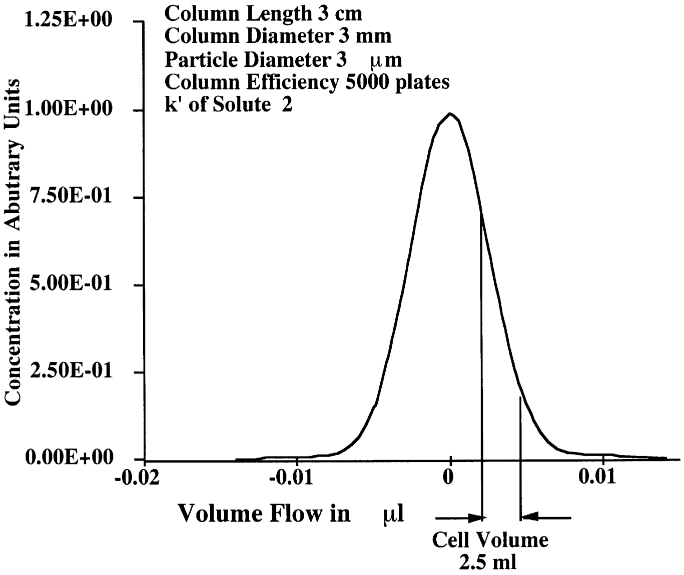


Figure 10. Effect of sensor volume on detector output.

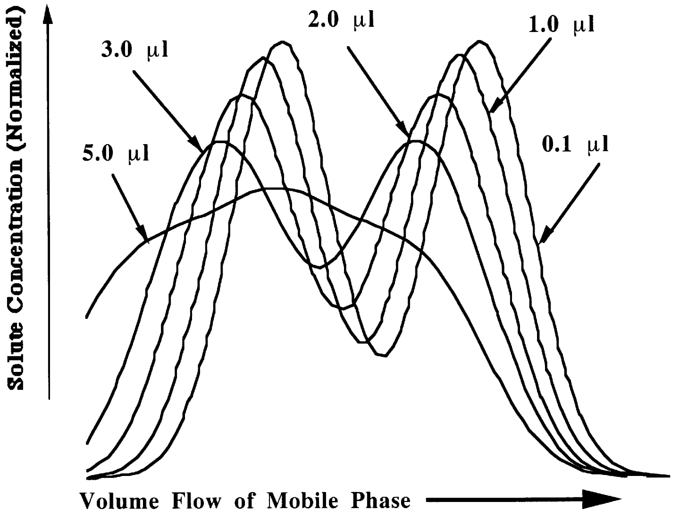


Figure 11. Effect of the detector sensor volume on the resolution of two solutes 4σ apart on a microbore column. Col length, 15 cm; column diameter, 1 mm; particle diameter, 5 µ; k′ of first eluted peak, 1.

The effect of a finite sensor volume on a peak profile can be easily simulated with a relatively simple computer program; the output from such a program is shown in Fig. 11. The example given, although not the worst case scenario, shows a condition where the sensing volume of the detector has a very serious effect on the peak profile and, consequently, the resolution. The column that is simulated is small bore and, thus, the eluted peaks have a relatively small peak volume. The peak volumes are, in fact, commensurate with that of the sensing cell. It is seen that even a sensor volume of 1 µL has a significant effect on the peak width and it is clear that, if the maximum resolution is to be obtained from the column, then the sensor cell volume should be certainly no greater than 2 µL.

It is clear that the results from the use of a 5 µL sensor cell are virtually useless; unfortunately, many commercially available detectors do have sensor volumes as great as, if not greater than 5 µL. Consequently, if small bore columns are to be employed, such sensor volumes must be studiously avoided. It should also be pointed out that small volume cells must be very carefully designed. In general, reducing the diameter of the cell reduces the volume and increases the noise, thus reducing the usable sensitivity. Reducing the length of the cell maintains the same noise, but reduces the response and, thus, also the sensitivity. It follows that the design of the sensor cell requires some considerable skill and care and a good understanding of cell dispersion.

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