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LOW-DISPERSION CONNECTING TUBES FOR LIQUID CHROMATOGRAPHY SYSTEMS

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SUMMARY

The dispersion that takes place in coiled and serpentine tubes of different dimensions is reported. The advantage of using serpentine tubes suitably encased as connecting tubes in liquid chromatography is demonstrated. Such connecting tubes have been shown to have a variance contribution of less than 0.05 μl^2/cm of linear length and a pressure drop of less than 0.05 MPa/cm at a flow-rate of 3 ml/min.

INTRODUCTION

Extra-column dispersion occurring in injection systems, connecting tubes and detector cells has become of primary importance since the introduction of small diameter columns and packing materials having particle diameters of a few microns.

Laminar flow in a cylindrical tube is accompanied by a parabolic velocity profile, the flow velocity in the center of the tube being at a maximum, whereas the velocity at the tube wall is zero. This range of solvent velocity from the wall of the tube to the center causes significantly increased dispersion of any solute band passing through it.

Over a decade ago, Scott and Kucera[1] pointed out that connecting tubes could contribute significantly to the total observed band dispersion. Low-dispersion connecting tubes are highly desirable in a chromatographic system and it is conventionally achieved by using tubes of a short length and small diameter which results in unnecessary inconvenience in the operation of the chromatographic system. If, however, the parabolic flow profile is disturbed in an appropriate manner, band dispersion in a tube can be significantly reduced. This is easily achieved by the introduction of radial convection which can greatly improve the radial mass transfer and, consequently, reduce the longitudinal dispersion characteristics of the tube. Radial convection, often called secondary flow, can be produced using various techniques that have been described in the literature. Radial mass transfer phenomenon in geometrically deformed (squeezed, twisted, and coiled) tubes has been examined by Halász and co-workers[2–4]. The effect of secondary flow introduced by tightly coiling a tube on the resulting dispersion has been extensively studied by Tijssen[5] who showed that under certain conditions, dispersion in a tube can be drastically reduced by up to two

orders of magnitude. In this paper the work of Tijssen has been extended to include the study of the dispersion that takes place in various forms of coiled and serpentine capillary tubes.

THEORETICAL

The onset of radial mixing or radial flow in coiled tubes was examined both theoretically and experimentally by Tijssen[5]. Tijssen approached the problem from an engineering point of view, utilizing hydrodynamic principals, and he concluded that the function that described the longitudinal dispersion in a cylindrical tube changed as the flow-rate increased. At low linear mobile phase velocities a function similar to that derived by Golay[6] related H (the variance per unit length or the plate height) with u (the linear mobile phase velocity) *viz.*

$$H = \frac{Ar^2u}{D_m} \tag{1}$$

where r is the radius of the tube, D_m is the diffusivity of the solute in the mobile phase and A is a constant over a given velocity range. At higher linear velocities Tijssen suggested that a function of the following form described the relationship between H and u:

$$H = \frac{B}{\lambda u} \tag{2}$$

where B is a constant over a given velocity range, λ is the coil aspect ratio and $\lambda = r/r_{coil}$ (r_{coil} is the radius of the coil).

It is seen that at low linear velocities the value of H increases linearly with u whereas at high linear velocities, H decreases as the reciprocal of u. Such a relationship between H and u also predicts that there will be a maximum value for H at a particular value of u. Tijssen, in fact, developed an equation to predict a value for H_{max} and the value of $\bar{u}$ at which H_{max} occurred for coiled capillaries. If in practice a tube could be designed where $\bar{u}$ could be made sufficiently small such that the dispersion at normal operating flow-rates in liquid chromatography was minimal, then such a tube would be ideal for use as a connecting tube in a liquid chromatographic system.

EXPERIMENTAL

A low-volume injection valve, together with a detector having a small-volume cell and a fast response time was employed in conjunction with a Bascom–Turner recorder. In order to reduce instrument dispersion to a minimum, the injection valve was the UHP series Valco valve with a 0.2-μl sample volume and the detector the Perkin-Elmer LC-85B. Benzyl acetate was used as the test solute and its dispersion was studied in ethyl acetate–*n*-hexane (5:95) at room temperature.

Six tubes were examined, four of which were coiled tubes and two of which

TABLE I

COILED TUBES EXAMINED

Tube	r (O.r.) (cm)	r (I.r.) (cm)	l (cm)	r_{coil} (cm)	λ (r/r_{coil})	l_{coil} (cm)
1	0.80	0.019	365.8	0.50	0.038	18.5
2	0.035	0.20	365.0	0.085	0.235	65.8
3	0.025	0.0127	998.0	0.0765	0.166	128.0
4	0.025	0.0127	337.5	0.0498	0.255	73.3

were zig-zag or serpentine tubes. The physical dimensions of these tubes are shown in Tables I and II. It is seen from Table I that the coiled tubes 1 and 2 have similar radii but coil 1 was coiled into a relatively wide helix whereas coil 2 was coiled into a very tight helix. Coils 3 and 4 had smaller internal radii but both were coiled into very tight helices, coil 4 being coiled as tightly as was practically possible. The zig-zag tubes 5 and 6, described in Table II, differ in their internal radii but the amplitude of the serpentine form of each tube being similar and equal to *ca.* 0.05 cm. These serpentine tubes were lamina in shape and were formed by passing the straight stainless-steel tubes through a pair of toothed gear wheels, the relative positions of which had been carefully adjusted such that the tube was molded into the serpentine shape. Care was taken, however, to ensure that the gear wheels were not so close together that the central aperture of the tube was deformed. The serpentine tube was then threaded through a 1/6 in. O.D. × 1.25 mm I.D. tube and the ends of the serpentine tube silver soldered into two small disks, 1.25 mm O.D., at either end. In this way the serpentine connecting tubes could still be connected to different parts of the chromatographic system employing standard 1/16 in. unions. A section of a serpentine connecting tube is shown in Fig. 1. Part of the outer 1/16 in. O.D. tube has been cut away to expose the serpentine tube contained inside.

    The dispersion in each tube was determined in a similar manner. Initially, the dispersion of the instrument system, which comprised of an injection valve and detector connected by a 5-cm length of 0.007 in. I.D. tubing, was measured at several flow-rates up to 3 ml/min for samples of benzyl acetate; the average of three replicate measurements was taken as the dispersion for each flow-rate. The band dispersion was measured in two ways, either as the equivalent plate height or in terms of the actual peak variance per centimeter of tube length in μl^2/cm. The plate height, H, was taken as the ratio of the tube length to the tube efficiency in theoretical plates and the efficiency was calculated as four times the square of the ratio of the retention distance

TABLE II

SERPENTINE TUBES EXAMINED

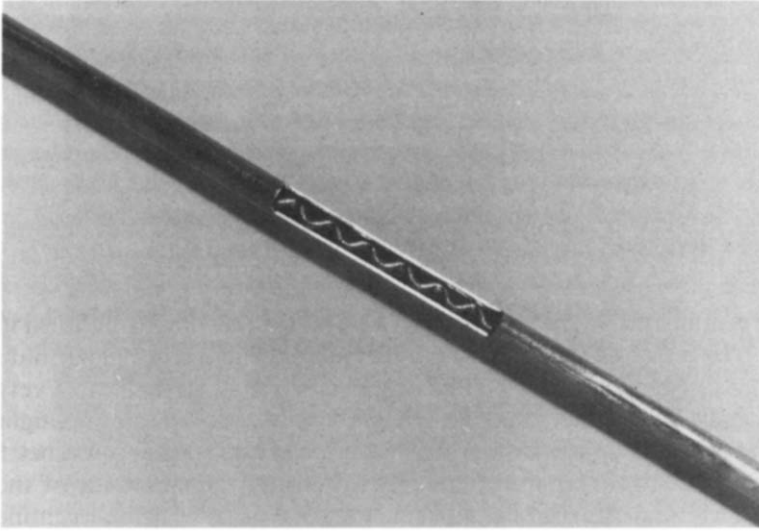| Tube	r (O.r.) (cm)	r (I.r.) (cm)	l (cm)	ζ^* (cm)	$l_{zig-zag}$ (cm)
5	0.025	0.0089	38.2	0.05	35.0
6	0.025	0.0127	42.46	0.05	38.5

    * ζ = Serpentine amplitude.

Fig. 1. View of a serpentine tube placed inside of a straight tube (1/16 in. O.D.). Dimensions: serpentine tube: r (l.r.) = 0.0127 cm, r (O.r.) = 0.025 cm, ζ (amplitude) = 0.05 cm; outer straight tube: r (I.r.) = 0.0635 cm, r (O.r.) = 0.0794 cm.

to the peak width measured at 0.6065 of the peak height. The actual peak variance was taken as the ratio of the square of the tube volume to the tube efficiency. Tube lengths were chosen such that all the tubes had more than 65 theoretical plates and all eluted peaks had asymmetry values of less than 1.1 and 1.35 taken as the ratio of the rear portion of the peak to the front portion of the peak, measured at 0.6065 and 0.10 of the peak height, respectively. The tube under examination was then interposed
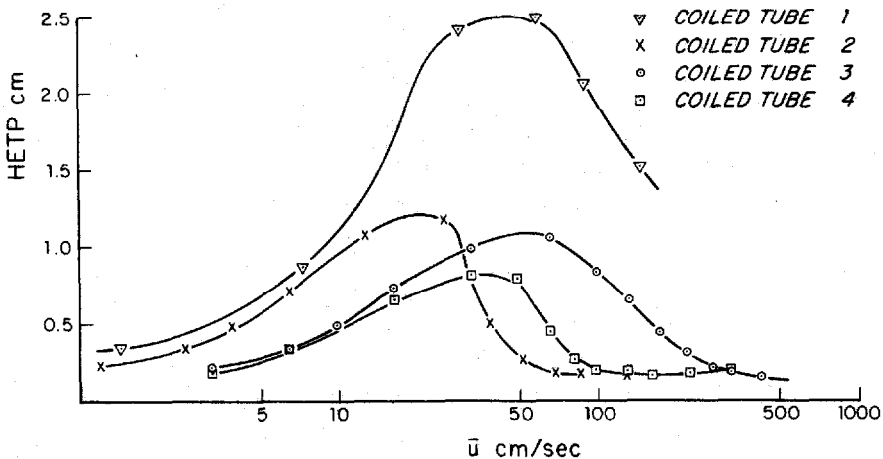

Fig. 2. HETP curves for different type coiled tubes. Mobile phase, ethyl acetate–n-hexane (5:95); solute, benzyl acetate.

between the sample valve and detector and the dispersion measurements repeated; all dispersion values were also taken as the average of three replicates. The specific dispersion for each tube was taken as the the variance of each solute band as the instrumental contribution to the peak dispersion was shown to be negligible over the range of flow-rates examined.

RESULTS AND DISCUSSION

The results obtained for the coiled tubes 1, 2, 3 and 4 (Table I) are shown in Fig. 2. It is seen that the greatest dispersion, *i.e.* the highest value of the height equivalent to a theoretical plate (HETP) for the tubes, was observed in the tube coiled to the largest radius and, consequently, the coil of the lowest aspect ratio. Curves depicted in Fig. 2 exhibited the two different types of relationship between HETP and linear velocity. At low velocities the HETP increases rapidly as the linear velocity increases. However, taking tube 1 as an example, radial mixing appears to commence somewhere around 20–30 cm/sec, and consequently reduces the rate of increase in the dispersion. At *ca.* 50 cm/sec, the dispersion reaches a maximum and subsequent to that, strong radial mixing occurs, fully disrupting the parabolic velocity profile, causing the band dispersion to decrease rapidly.

Comparing the curves for coils 1 and 2 that had the same internal diameter but different coil aspect ratios, it is seen that the tighter coil exhibited less then half the maximum dispersion and, furthermore, the maximum occurs at a much lower velocity.

Comparing coils 2 and 4 which had relatively the same coil aspect ratio, but with coil 4 having a significantly lower internal diameter than coil 2, it is seen that
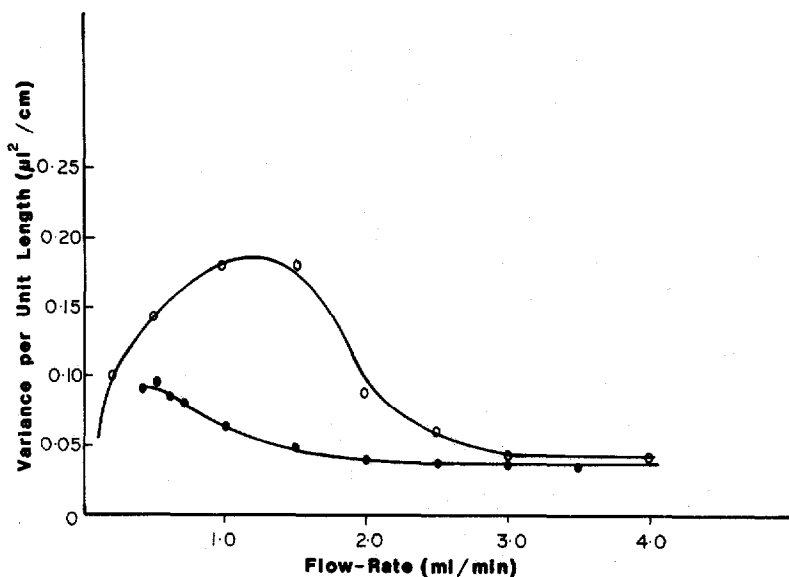

Fig. 3. Graphs of peak variance against flow-rate for coiled and zig-zag tubes. ○, Coiled tube ($r = 0.013$ cm, $l = 337.5$ cm, $d_{coil} = 0.996$ mm); ●, zig-zag tube ($r = 0.013$ cm, $l = 42.46$ cm).

reducing the diameter has reduced the maximum HETP but at the same time caused the maximum to occur at a significantly higher mobile phase velocity. On examining the results for coils 3 and 4 the same effect as that for coils 1 and 2 was observed. The higher aspect ratio reduces both the maximum HETP and also reduces the velocity at which the maximum HETP was realized.

Examining the serpentine tubes in a similar way it was found that tube 5 had a much lower HETP of *ca.* 0.05 μl^2 per unit length of serpentine tube. Furthermore, this value was nearly constant over the entire range of flow-rates examined. However, tube 5 had a very high flow impedance and required an inlet pressure of 23 MPa to provide a flow-rate of 3 ml/min.

Tube 6, which was constructed from a wider-diameter tube and thus presented much less flow impedance, only required an inlet pressure of 1.3 MPa at a flow-rate of 3 ml/min (actual pressure drop was 0.034 MPa/cm). It was thought that the smaller internal diameter used in tube 5 was further restricted when the tube was molded into the serpentine form. The results obtained from tube 6 are shown plotted as variance per unit length in μl^2/cm against flow-rate in ml/min in Fig. 3. The abscissa was changed to flow-rate as this had more chromatographic significance. The linear velocity in the connecting tube is not the pertinent variable as the chromatographic system is adjusted to a particular *column* flow-rate. In Fig. 3, the results obtained for coiled tube 4 are also included for comparison purposes. It is seen that the serpentine tube performs far better and has a much lower dispersion than the coiled tube. This coiled tube in fact, was about the smallest coil diameter that could be made in practice. It is seen that for serpentine tube 6, not only was the magnitude of the maximum much lower then that for the coiled tube but it also occured at a much lower flow-rate. A variance of 0.05 μl^2/cm of connecting tube would permit a 10-cm
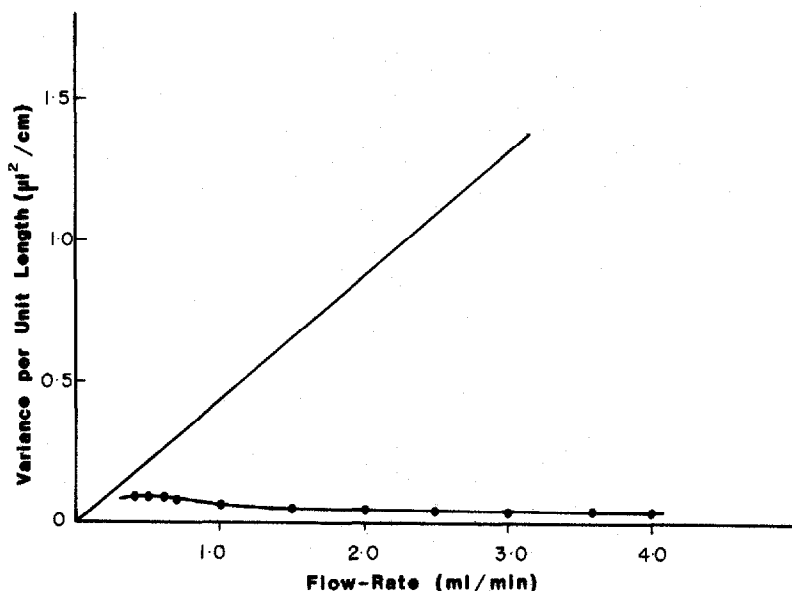

Fig. 4. Graphs of peak variance against flow-rate for straight and zig-zag tubes. ——, Straight tube, 0.007 in. I.D.; ●—●, zig-zag tube, 0.010 in. I.D.

linear length to be employed with a total contribution of only 0.5 μl^2 to the peak variance. The performance of the serpentine tube relative to a straight tube is shown in Fig. 4. The variance per unit length of the straight tube is calculated from the Golay equation[6] which has been shown to hold precisely for a straight tube. Employing the Poiseuille equation and a value for the solvent viscosity of 0.30 cp (measurement by the Ostwald Viscometer[7], the radius of a tube 1-cm long that would provide an equivalent pressure drop to that of the serpentine tube (0.034MPa/cm) was calculated and found to be 0.0058 cm (I.D. 0.0046 in.). Employing the Golay equation the variance per unit length of this tube can be shown to be 0.24 μl^2/cm [the value of $3.1 \cdot 10^{-5}$ cm^2/sec was taken as the diffusivity of benzyl acetate in ethyl acetate–n-hexane $(5:95)^7$]. It is seen that the dispersion per centimeter length of the serpentine tube is one fifth of that obtained by a straight cylindrical tube having the same pressure drop. In fact, the equivalent cylindrical tube having an I.D. of 0.004 in would be extremely difficult to use in practice due to its tendency to become blocked. The dispersion from one centimeter of cylindrical tubing having the same I.D. as the serpentine tube (0.010 in.) would be 5.5 μl^2 and consequently the use of the serpentine tube would, in this instance reduce the peak dispersion by two orders of magnitude. It is also seen that the serpentine tube of 0.025 cm I.D. can operate over a very wide flow-rate range and this range is that commonly employed in liquid chromatography today. It should be noted that the results obtained for the coiled and serpentine tubes are in qualitative agreement with the theoretical predictions of Tijssen.

CONCLUSION

The serious band dispersion that takes place in a cylindrical tube due to the parabolic velocity profile in the tube can be significantly reduced by introducing radial flow. In practice, this can be satisfactorily achieved by molding tubing of appropriate size into a serpentine form making the aspect ratio of the serpentine coil as large as possible.

Serpentine tubes of 0.025 cm I.D. can be inserted into standard tubes of 1/16 in. O.D. and 1.25 mm I.D. to form useful connecting tubes. Such tubing can be connected into the chromatographic system employing standard 1/16 in. unions. The contribution to the normal peak variance by serpentine connecting tubes can be as little as 0.05 μl^2/cm of linear length and provide a pressure drop of less than 0.05 MPa/cm at a flow-rate of 3 ml/min. Such connecting tubes could also be employed as the sample loop in injection valves and for post-column reactors to reduce band dispersion.

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