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FILM THICKNESS REQUIRED OF THE STATIONARY PHASE IN THE SEPARATION COLUMN WHEN USING LARGE RETENTION GAPS IN CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The reconcentration of bands broadened in space by the use of retention gaps depends on the ratio of the retention powers in the retention gap and the separation column. The reconcentration is more efficient than necessary if uncoated column inlets with lengths of a few metres and diameters not far exceeding that of the separation column are used. However, if large retention gaps are applied for on-column injections of large sample volumes or for direct coupling of high-performance liquid chromatography with capillary gas chromatography, the retention power, *i.e.*, the film thickness of the stationary phase in the separation column, may need to be increased in order to provide a sufficient reconcentration effect. An equation is presented that allows the calculation of the required film thickness for given geometries of the retention gap and the separation column, the retention power in the retention gap and a given tolerance to peak broadening.

INTRODUCTION

A retention gap is a deactivated but uncoated column inlet[1] with a length ranging between 30 cm and 50 m. It is used for two purposes: first, to reduce the effects of non-volatile sample by-products on the column performance[2] and second, to reconcentrate solute bands that are broadened in space[3]. The solute bands that are spread in the column inlet due to the flow of the sample liquid are focused on the beginning of the coated column owing to the faster movement of the solute material in the uncoated inlet than in the coated part.

Retention gaps are effective at column temperatures at least about 50°C above the injection temperature. Peaks eluted at lower temperatures are not affected by band broadening in space. However, at low temperatures solvent effects (solvent trapping and phase soaking[4]) are important.

The reconcentration power provided by the use of a retention gap is more than sufficient if retention gaps a few metres long are used for the injection of sample volumes up to about 10 μl and if the inner diameters of the retention gap and the separation column do not differ by more than a factor of 2. However, for a number

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of applications it is attractive to exploit the full potential of the technique, and to use uncoated pre-columns that are as large as can be tolerated —large either in terms of length to allow injections of large sample volumes or in terms of internal width to accept thick syringe needles.

The reconcentration effect when using a retention gap is finite because even an uncoated capillary tube exhibits some retention power. Therefore, there are limits to the size of the retention gap and the length of the flooded zone beyond which the initial bands are no longer satisfactorily focused. This paper contributes to the exploration of these limits.

There is a need for a simple way of calculating how efficiently solute bands are reconcentrated by a given combination of a retention gap and a separation column in order to avoid at least part of the experimental "trial and error" procedure. There are a number of parameters to be considered, but most of them cannot be freely chosen. The length of the retention gap is determined by the sample volume to be injected, its inner diameter by the injection method, and the treatment of the internal wall of the uncoated inlet by the requirement to obtain good wettability with the sample liquid, and there is little scope for achieving a further substantial reduction in the retention power in the retention gap. Therefore, from a practical point of view we are concerned with the film thickness of the coating in the separation column, an increase in which increases the reconcentration of the bands broadened in space. What minimal film thickness is required in order to restrict the peak broadening to, say, 10%?

An equation is derived for answering the above questions mathematically. This relatively simple approach produced guidelines that proved to be reasonable in practice, although some simplifications are involved that are discussed in the concluding section.

THEORY

The standard deviation of a peak, *i.e.*, of the solute concentration profile at the exit of the separation column, σ_p, is related to the contributions due to the chromatographic band broadening in the separation column, σ_{ch}, and the initial band width, σ_i, by a square relationship[5]:

$$\sigma_p^2 = \sigma_{ch}^2 + \sigma_i^2 \quad (1)$$

A convenient unit for the quantitation of the initial bands broadened in space is column length. Chromatographic band broadening is usually expressed in terms of time (or millimetres on the chart paper). However, Saxton[6] recently suggested that column efficiency be expressed by the terminal band length of the solutes, b, *i.e.*, the length of the dispersed solute zone as it reaches the column end; b is assumed to be equivalent to four times the standard deviation, σ, of the solute band concentration profile, and hence to the width of the peak near its base. This terminal band length is convenient for our purposes as it is compatible with the initial band lengths.

The initial band due to band broadening in space has a rectangular (plug) shape rather than fitting a Gaussian distribution. It causes the final peak to be deformed towards a rectangular shape and results in greater broadening at the top than

at the bottom of the peak. This complicates our further considerations because the results depend on whether the peak broadening is critical at the bottom or at the top of the peak. With a nearly complete separation, only the broadening at the bottom of the peak is of interst. With fused peak the broadening further up the peak is decisive, depending on how far the peaks are separated.

If the peak broadening at the bottom of the peak is considered, the full terminal band length ($4\sigma_{ch}$) is introduced into eqn. 1, resulting in the following approximation:

$$b_p^2 = b_{ch}^2 + b_i^2 \tag{2}$$

However, in the interest of effecting a compromise with regard to the different peak broadenings at the bottom and the top of the peak, in the following calculations we concentrate on the peak broadening at 60% of the peak height, where the Gaussian distribution has a width of 2σ, half of b_{ch}. Then

$$w_{p,60}^2 = w_{ch,60}^2 + b_i^2 \tag{3}$$

The peak broadening due to the initial band length, b_i, is of interest as a proportion x of the chromatographic contribution to the terminal band length:

$$w_{p,60} = (1 + x)\, w_{ch,60} \tag{4}$$

The tolerable length of the initial band in the separation column, b_i, if a peak broadening x is accepted, is

$$b_i = w_{ch,60} \sqrt{x^2 + 2x} \tag{5}$$

According to Saxton[6], the height equivalent to a theoretical plate, h, of a column of length L is related to the terminal band length b by

$$b_{ch} = 4 \sqrt{L\, h} \tag{6}$$

and, as $w_{ch,60}$ is $b_{ch}/2$,

$$w_{ch,60} = 2 \sqrt{L\, h} \tag{7}$$

The value of h is assumed to be similar to the inner diameter of the separation column ($2r_{sc}$), which is a reasonable approximation for highly efficient columns used under optimal separation conditions.

The initial band length in the separation column, b_i, is equal to the length of the flooded zone, l, in the retention gap, divided by the reconcentration factor achieved during the passage from the low retention power in the retention gap to the high retention power in the separation column. This reconcentration factor is assumed to be equal to the ratio of the retention powers in the two parts of the column[7,8], where d_{rg} is the apparent film thickness in the retention gap (retention power

calculated as if it were due to a film of an apolar stationary phase[8]) and d_{sc} is the real film thickness in the separation column (also assumed to be apolar). If the retention gap has a bore different to that of the separation column, the difference in column diameters must be considered according to ref. 8, taking into account the change in length of the solute vapour cloud and the dependence of the phase ratio β on the column diameter for a given apparent film thickness:

$$b_i = l \cdot \frac{d_{rg} r_{rg}}{d_{sc} r_{sc}} \tag{8}$$

Using eqns. 5 and 7 and specifying h as $2r_{sc}$:

$$l \cdot \frac{d_{rg} r_{rg}}{d_{sc} r_{sc}} = 2\sqrt{2 r_{sc} L (x^2 + 2x)} \tag{9}$$

If the peak broadening is relatively small, x^2 is negligible and then peak broadening x is

$$x = \frac{l^2 d_{rg}^2 r_{rg}}{16 d_{sc}^2 r_{sc}^3 L} \tag{10}$$

As mentioned in the Introduction, the most important parameter to be adjusted in practice is the film thickness in the separation column. An increased film thickness enhances the reconcentration effect and allows the use of larger retention gaps, longer flooded zones and larger sample volumes. The film thickness for a given length of the flooded zone and a given tolerated peak broadening is (starting from eqn. 9):

$$d_{sc} = \frac{l d_{rg}}{\sqrt{8 L r_{sc} 2x}} \cdot \frac{r_{rg}}{r_{sc}} \tag{11}$$

DISCUSSION OF SOME CALCULATED EXAMPLES

Long retention gaps

In Fig. 1, the required film thickness of the stationary phase in the separation column (assumed to be 15 m × 0.30 mm I.D.) is plotted against the length of the retention gap. It is assumed that the retention power in the retention gap corresponds to a coating 3 nm thick[9] and that the inner diameters of the retention gap and the separation column are equal. The length of the retention gap is used synonymously with the length of the flooded zone, assuming the exploitation of the full length of the pre-column. The three curves give the results if 5, 10 and 20% peak broadening is tolerated. If a (considerable) peak broadening of 20% is tolerated, a 50-m retention gap requires the use of a separation column with a 1.25 μm film thickness and a 100-m retention gap one of 2.5 μm thickness. A peak broadening of 10% may be tolerated for most applications. In this instance a separation column of standard film thickness (0.25 μm) tolerates a retention gap of about 7 m, whereas a 50-m retention gap presupposes a thick-filmed column (1.7 μm).

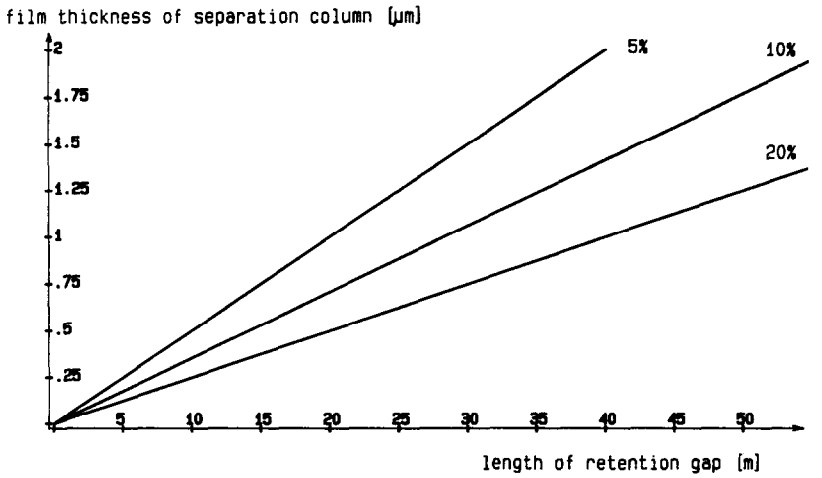

Fig. 1. Required film thickness in the separation column (15 m × 0.30 mm I.D.) *versus* length of the retention gap (0.30 mm I.D.), assuming a retention power in the retention gap corresponding to a 3-nm film of apolar stationary phase and flooding by the sample liquid for the full length of the retention gap. The three curves indicate calculated results for cases where 5, 10 or 20% peak broadening at 60% peak height is tolerated.

According to eqn. 11, there is a direct proportionality between the required film thickness and the length of the retention gap. The required film thickness decreases with increasing length of the separation column, but only as a function of the square root of the latter. The separation column must be elongated to 60 m to reduce the required film thickness in Fig. 1 by half. The inner diameter of the column parts (which are still assumed to be equal) influences the required film thickness in the same manner as the length of the separation column. However, strong deviations from the standard 0.3 mm I.D. are not likely to become important in the near future.

Wide-bore retention gaps

Retention gaps with an inner diameter exceeding that of the separation column require an additional reconcentration power because the plug of sample vapour is elongated during the transfer from the wide-bore retention gap to the more narrow-bore separation column. On the other hand, wide-bore retentions gaps (*e.g.*, if used for automatic on-column injection) are seldom used for injections of large sample volumes, so they do not need to be wide and long at the same time. A 2 m × 0.5 mm I.D. pre-column allows injections of volumes up to about 10 µl.

Table I lists the calculated film thicknesses in the separation column if a (fully flooded) 2 m × 0.5 mm I.D. retention gap is combined with separation columns of length 15 m and various inner diameters, assuming a retention power in the retention gap corresponding to a 3-nm film and a peak broadening of 10%. The results clearly show that the reconcentration of the band broadening in space using such pre-columns is more efficient than required. The calculated data even suggest that a 0.2 mm I.D. separation column with hardly more than a standard film thickness would be applicable. However, if very narrow-bore separation columns are used, other problems become predominant, such as the longitudinal diffusion in the retention gap[10] and the large dead time of the pre-column[11].

TABLE I

REQUIRED FILM THICKNESS IN THE SEPARATION COLUMN FOR THE COMBINATION OF A 2 m × 0.5 mm I.D. RETENTION GAP WITH SEPARATION COLUMNS OF LENGTH 15 m AND VARIOUS I.D.s, ASSUMING 10% PEAK BROADENING

Separation column I.D. (mm)	*Required film thickness (μm)*
0.5	0.055
0.3	0.118
0.2	0.217
0.1	0.612

EXPERIMENTAL RESULTS

Fig. 2 shows results obtained using a 9 m × 0.32 mm I.D. separation column coated with a 0.2-μm film of SE-54 and a retention gap of 15 m × 0.32 mm I.D., leached and deactivated with hexamethyldisilazane and exhibiting a retention power for alkanes corresponding toa 0.8-nm film of an apolar stationary phase.

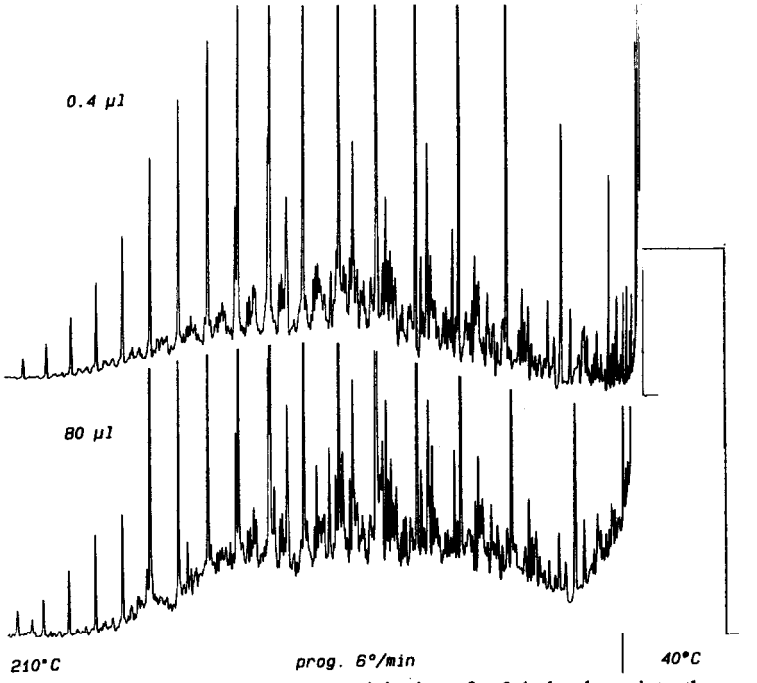

Fig. 2. Heating oil in *n*-pentane. Top: injection of a 0.4-μl volume into the separation column alone. Bottom: 80 μl of a 200-fold diluted solution injected into a 15 m × 0.32 mm I.D. retention gap ahead of the separation column. The band broadening in space causes the solute bands to be spread through about 60% of the column. Nevertheless, no significant decrease in separation efficiency is observed, indicating virtually complete reconcentration of the solute bands at the beginning of the separation column. The film thickness in the separation column is 0.2 μm. The film thickness calculated to cause a 5% peak broadening is 0.26 μm, indicating that there is little peak broadening beyond the calculated one.

A 0.4-μl volume of heating oil dissolved in *n*-pentane was injected directly into the separation column in order to obtain a reference chromatogram. Then 80 μl of a 200-fold diluted solution were injected, using the above-mentioned retention gap attached to the separation column by means of shrinkable PTFE tubing. The same flow-rate and temperature-programme were applied. The initial isothermal temperature for the solvent evaporation (40°C) was maintained up to the completion of the solvent peak.

Comparison of the two chromatograms (Fig. 2) shows no significant decrease in separation efficiency, excluding a noticeable peak broadening. The required film thickness in the separation column calculated to obtain a 5% peak broadening is 0.26 μm and the calculated peak broadening expected from the separation column actually used is 8.4%.

DISCUSSION

The use of large (primarily long) uncoated pre-columns presupposes the selection of suitable separation columns to ensure sufficient reconcentration effects. The equation presented here facilitates this selection.

The most difficult quantity in eqns. 9 and 10 is the retention power in the retention gap. This must be determined experimentally by a suitable test[9]. The calculated results assume an apparent film thickness of 3 nm, which is high, being appropriate for phenyldimethylsilylated pre-columns but several times too high for trimethylsilylated retention gaps. The accepted peak broadening of 5–20% is small. In every-day gas chromatography much stronger peak broadening is accepted or even not recognized.

On the other hand, the basic assumptions in eqn. 8 are optimistic in other respects: they neglect the dead time of the flooded section in the retention gap[11]; this dead time causes peak broadening if the column is heated too rapidly to give the solute material the necessary time to pass through the pre-column at sufficiently low temperatures. However, if suitable chromatographic conditions are used, peak broadening due to this dead time is small.

The experimental results shown in Fig. 2 and considerable practical experience have shown that the discrepancy between the calculated and the experimental results is small.

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