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Extra-column band broadening in high-temperature open-tubular liquid chromatography

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ABSTRACT

In the development of high-efficiency split high-temperature open-tubular liquid chromatographic systems, the study of the extra-column band broadening and the "cold-point" effect is of great importance. For a rectangular sample plug of volume V_{inj}, $\sigma^2_{inj} = 0.181\ V^2_{inj}$ if the peak width at half-height is used in the column efficiency calculations. In a split system, the contribution of the connection tube between the split tee-piece and the sample valve to the observed peak variance can be calculated with the Taylor equation and the known splitting ratio. As this tube also performes as a preheating tube, a Perkin-Elmer serpentine tube is recommended from considerations of the heating efficiency and the resistance to mass transfer. In order to obtain a higher sensitivity, an external small volume Z-shaped cell was used in series with the on-column cell. The results show that a large decrease in efficiency occurs at the low-temperature column outlet outside the oven. If the Z-shaped cell is connected to the column with a very narrow-bore tube (half of the column I.D.) and the connection is made inside the column oven, higher selectivity could be obtained with little efficiency loss.

INTRODUCTION

In the development of high-efficiency open-tubular liquid chromatographic (OT-LC) systems, the study of the extra-column band broadening is of great importance. Although many studies have been published on the different extra-column effects and equations have been proposed for calculating individual extra-column variances [1–9], few, if any, have discussed the extra column band broadening in a split OT-LC system. In addition, the validity of the theoretical calculation for use under high-temperature conditions (above the normal boiling points of solvents) has not so far been proved.

In addition to the commonly discussed extra-column factors, such as the sample size, the connection tube and the detector cell volume, the "cold-point" effect is another source of band broadening in high-temperature (HT) OT-LC [10]. This not only reduces the column efficiency itself, also causes much greater band broadening in the connection tubes.

This paper discusses the contributions of the sample volume and the connection tube to the overall band broadening in an HT-OT-LC system. The influence of the "cold-point" effect is also discussed.

EXPERIMENTAL

The same experimental set-up as in previous work [10] was used except that a 10 mm × 25 μm I.D. Z-shaped cell (LC Packings, Amsterdam, Netherlands) was inserted between the column outlet and the flow restriction tube (Fig. 1). The column outlet (outside the oven) was 4 cm long and the cell inlet tube was 40 cm long. The cell and its hold-

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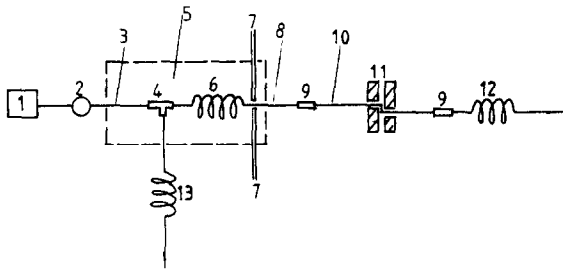

Fig. 1. Experimental set-up. 1 = Solvent pump; 2 = sample valve; 3 = preheating tube; 4 = split tee-piece; 5 = column oven; 6 = chromatographic column; 7 = optical fibres and on-column cell, 8 = column outlet piece; 9 = zero-dead volume unions; 10 = inlet tube of the Z-shaped cell; 11 = Z-shaped cell and holder; 12 = restriction tube; 13 = split tube.

er were placed in a Kontron (Watford, UK) Model 430 UV detector. For the study of the influences of the sample size and the connection tube, signals from the on-column cell were used. For the study of the "cold-point" effect, signals from both the on-column cell and the Z-shaped cell were used.

SB-Methyl-100 and SB-Octyl-50 open-tubular capillary columns were obtained from Lee Scientific (Salt Lake City, UT, USA), fused-silica capillaries from Polymicro Technologies (Phoenix, AZ, USA) and a stainless-steel serpentine tube from Perkin-Elmer (Norwalk, CT, USA). Acetonitrile and methanol were HPLC grade solvents from Rathburn (Walkerburn, UK) and chlorobenzenes of with different Cl substitutions were HPLC test substances from Aldrich-Chemie (Steinheim, Germany). Other chemicals were of analytical-reagent grade from Fluka (Buchs, Switzerland). All chemicals were used as received.

RESULTS AND DISCUSSION

The total peak variance, σ^2, is the sum of the column variance and all independent extra-column variances:

$$\sigma^2 = \sigma_c^2 + \sigma_d^2 + \sigma_t^2 + \sigma_{inj}^2 + \sigma_s^2 \quad (1)$$

where σ_c^2 is the column variance, σ_d^2 is the variance caused by the detector cell volume, σ_t^2 is the variance caused by the preheating tube, σ_{inj}^2 is the variance caused by the injected sample volume and σ_s^2 is the contribution of all other factors, such as dead volumes between connections, electrical response delay and column end flow patterns.

The influence of the "cold-point" effect on the overall band broadening cannot be expressed as a separate term in eqn. 1. Its contribution is indicated by the increase in the σ_c^2 and σ_t^2 values.

In our system with on-column UV detection, the cell dead volume is in the range 0.2–0.4 nl, depending on the column diameter. The contribution of σ_d^2 to the observed peak dispersion is negligible. Also, σ_s^2 can be made insignificant through a proper choice of electronic devices and through the application of high-quality, zero-dead-volume fittings. Hence, σ_t^2 and σ_{inj}^2 are the only extra-column variances that should be studied carefully.

Influence of sample size

The contribution of the sample size to the total peak variance depends on the sample profile and the method by which the column efficiency is measured. In most instances the sample profile can be considered as a rectangular plug, but there are different ways of measuring column efficiency.

The peak variance σ^2 can be calculated from the eluting peak profile with the computerized second momentum method [5]. For this method, the volume variance of a rectangular sample plug of volume V_{inj} is $V_{inj}^2/12$. As Kirkland *et al.* [5] have pointed out, σ^2 obtained with the second momentum method cannot reflect the real separation quality of seriously skewed peaks. This method is not commonly used.

For a Gaussian peak, the standard deviation σ can be measured from the half-width at 0.607 of the peak height. In this instance, the contribution of a rectangular sample plug of volume V_{inj} is given by $\sigma_{inj}^2 = 0.25\ V_{inj}^2$ or $\sigma_{inj} = 0.5\ V_{inj}$ [6]. This method is rarely used in practice.

As the peak width at half-height, $\Delta V_{1/2}$, is critical in determining resolution, the column efficiency derived from $\Delta V_{1/2}$ is commonly used and σ^2 is calculated from the measured plate number (n) and the known retention volume (V_0). Here, σ_{inj}^2 has to be determined in the following way.

If the influence of the sample size only is considered, *i.e.*, $\sigma^2 = \sigma_{inj}^2$, then

$$n = 5.54\ (V_0/\Delta V_{1/2})^2 = (V_0/\sigma)^2 = (V_0/\sigma_{inj})^2 \quad (2)$$

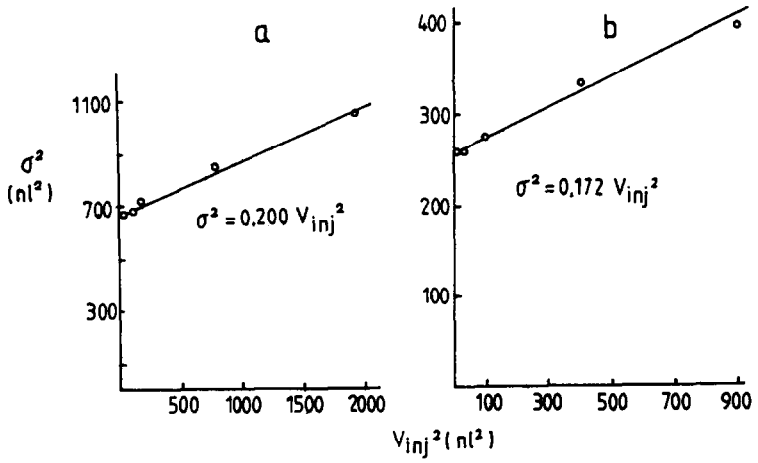

Fig. 2. σ^2 *vs.* V_{inj}^2 plot. (a) column 8.4 m × 0.051 mm I.D. SB-Octyl-50, 100°C, u = 0.27 cm/s; (b) column 1.2 m × 0.051 mm I.D. SB-Methyl-100, 22°C, u = 0.33 cm/s.

For a rectangular sample plug,

$$\Delta V_{1/2} = V_{inj} \quad (3)$$

From eqns. 2 and 3, we obtain

$$\sigma_{inj}^2 = V_{inj}^2/5.54 = 0.181 V_{inj}^2 \quad (4)$$

To prove this relationship, different volumes of acetophenone in methanol were injected into the methanol mobile phase under the same chromatographic conditions. For each injection, the plate number (n) was measured and the peak variance (σ^2) was calculated. Calculated σ^2 values were then plotted against V_{inj}^2 (Fig. 2). The slope of this plot equals the proportionality constant of eqn. 4. The experiments were repeated at 22 and 100°C, and the slope values obtained were 0.171 and 0.200, respectively. As the experimental values approximate the theoretical value of 0.181, eqn. 4 must give a correct description of the influence of the sample size in HT-OT-LC.

If a 20% efficiency loss is the acceptable limit, it can be calculated that a 9.6-nl injection volume is allowed for a 1.2 m × 0.05 mm I.D. column and a 35-nl volume can be used for a 16-m column.

Influence of the connection tube

In our HT-OT-LC system, a connection tube (preheating tube) was inserted between the sample valve and the split tee-piece (Fig. 3). Unlike room-temperature OT-LC, where the connection tube is always cut as short as possible, the connection tube in HT-OT-LC must have a certain size so as to provide enough residence time for the cold liquid (mobile phase and sample) to be heated up. In this instance, the contribution of the connection tube to the overall band broadening must be taken into consideration.

The volume variance in the connection tube, σ_{tube}^2, can be calculated with the Taylor equation:

$$\sigma_{tube}^2 = \pi d_t^4 F l_t / 384 D_m \quad (5)$$

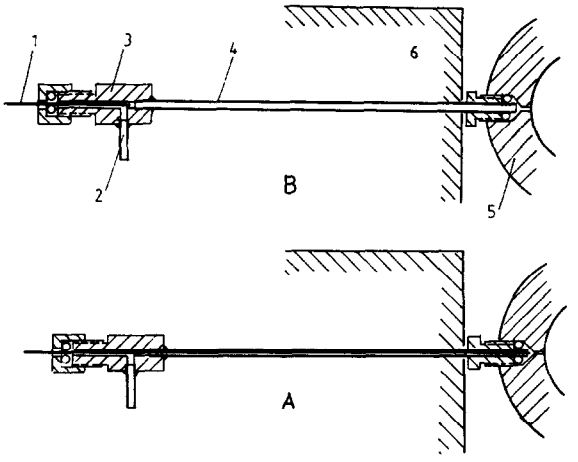

Fig. 3. Column inlet position. 1 = Capillary column; 2 = split outlet; 3 = split tee-piece; 4 = connection tube; 5 = sample valve; 6 = column oven.

where d_t is the inner diameter of the connection tube (cm), l_t is its length (cm), F is the mobile phase flow-rate (ml/s) and D_m is the diffusivity of the solute in the mobile phase. In a split OT-LC system, however, we are interested only in the contribution of the connection tube to the observed peak dispersion (σ_t^2). This can be derived as follows.

As the time variance at the end of the connection tube and the time variance at the inlet of the capillary column should be the same, we have

$$\sigma_t^2/F_c^2 = \sigma_{tube}^2/F^2$$

$$\sigma_t^2 = \sigma_{tube}^2 (F_c/F)^2 = \sigma_{tube}^2 / (SR)^2 \quad (6)$$

where F_c is the flow-rate through the column and $SR = F/F_c$ is the splitting ratio.

To prove the correctness of eqns. 5 and 6 under HT-OT-LC conditions, a piece of 12 cm × 0.52 mm I.D. stainless-steel tubing was used as the connection tube and the following experiments were carried out. First, the column inlet was pushed directly to the sample valve outlet (Fig. 3A) and the peak variance (σ_A^2) was measured. As there was no connection tube, the measured value was the sum of all other variances except σ_t^2. The column inlet was then withdrawn to the position shown in Fig. 3B and the peak variance was measured again (σ_B^2). The contribution of the connection tube, σ_t^2, can be obtained as the difference between σ_B^2 and σ_A^2,

$$\sigma_t^2 = \sigma_B^2 - \sigma_A^2 \quad (7)$$

The experiments were repeated at different column temperatures and different mobile phase flow-rates. The results are given in Table I, where the experimentally obtained σ_t^2 values are compared with the theoretical values calculated from eqns. 5 and 6, using known values for d_t, l_t, D_m, F and SR.

At room temperature, the agreement between the experimental and the theoretical values was acceptable, but at 100°C the experimental values were always higher than the theoretical values. This must be due to the insufficient heating of the sample in the connection tube. The real D_m value of the solute was much lower than that expected for 100°C, resulting in a much higher band broadening in the tube. Considering the heating efficiency and the resistance to mass transfer, a Perkin-Elmer 40 cm × 0.2 mm I.D. serpentine tube was used as the connection tube.

"Cold-point" effect

Above we have seen that insufficient heating of the sample causes a decrease in the overall efficiency. This is the so-called "cold-point" effect. As was

TABLE I

PEAK VARIANCE CAUSED BY THE CONNECTION TUBE

Column, 85 cm × 0.075 mm I.D. fused silica; mobile phase, methanol; test sample, acetophenone in methanol.

Column temperature (°C)	Flow-rate (ml/min)	σ_A^2 (nl^2)	σ_B^2 (nl^2)	σ_t^2 (nl^2)	σ_t (nl)	σ_t[a] (nl)
100	1.00	873.0	1137.3	264.3	16.26	15.31
	0.60	564.1	748.7	184.6	13.59	11.63
	0.40	468.7	635.5	166.8	12.91	9.84
	0.30	436.3	565.5	129.2	11.37	8.57
	0.20	427.8	500.4	72.6	8.52	7.18
	0.10	569.5	622.2	52.7	7.26	5.34
22	0.30	709.7	903.7	194.0	13.93	15.75
	0.20	544.3	729.1	184.8	13.59	12.97
	0.10	426.8	491.2	64.4	8.03	9.22
	0.07	417.3	490.0	72.7	8.55	7.87
	0.05	456.6	488.7	32.1	5.66	6.50

[a] Theoretical values.

shown previously [10], the efficiency loss caused by the "cold-point" effect in the inlet region can be made insignificant if a serpentine tube is used as the connection tube and is separately heated to a few degrees higher than the column oven temperature.

Sometimes, a Z-shaped UV cell outside the column oven was used in order to obtain a higher sensitivity. The sensitivity of the LC Packings cell was 10–20 times higher than that of our on-column cell (a window on the 50 μm I.D. capillary column). However, in this instance, a large efficiency loss resulted owing to the "cold-point" effect (Table II).

The volume of the Z-shaped cell is about 5 nl and its contribution to the overall band broadening is negligible. The efficiency loss mainly arises from the extra band broadening in the column outlet and/or in the Z-shaped cell inlet tube. Several conclusions could be drawn from the data in Table II. First, k' values measured with the Z-shaped cell are smaller than those with the on-column cell. This is due to the 40-cm long inlet tube of the Z-shaped cell. There is no stationary phase in this tube, so the average k' values must decrease. The second fact is that the efficiency loss of the slightly retained solute, 1,4-dichlorobenzene, did not change with the column temperature. This means that the inlet tube of the Z-shaped cell itself does not give rise to a noticeable "cold-point" effect. As this tube is very narrow (25 μm I.D.), extra band broadening in this tube, even at a much lower temperature, is very small (see eqn. 5). The 15–18% efficiency loss must come from the connection between the column outlet piece and the Z-shaped cell inlet tube.

The most important finding is that the efficiency loss of the more retained pentachlorobenzene increases rapidly with increasing column temperature. This is explained as the result of the additional retention of the solute on the stationary phase in the low-temperature column outlet. Solute diffusivity in the stationary phase decreases rapidly with decreasing temperature, resulting in very large band broadening. This additional retention became noticeable when the column temperature was raised to 200°C. Here, the k' value of pentachlorobenzene on the Z-shaped cell became larger than that on the on-column cell. We noticed that column bleeding often leads to very thick films at the column outlet. This is why the 4-cm long column outlet created such a large "cold-point" effect. Fig. 4 shows the chromatograms of a chlorobenzene mixture, obtained with the Z-shaped cell and the on-column cell. Broadening of the pentachlorobenzene band is clearly seen with the Z-shaped cell. As the column was operated at a much higher flow-rate than optimum, the column efficiency was relatively low.

In future work, connection between the column outlet and the Z-shaped cell inlet will be made inside the column oven. In this way, the high sensitiv-

TABLE II

INFLUENCE OF THE "COLD-POINT" EFFECT

Column, 19.5 m × 51 μm I.D. SB-Methyl-100; mobile phase, acetonitrile–water (50:50).

Column temperature (°C)	Detector	1,4-Dichlorobenzene		Pentachlorobenzene	
		k'	n	k'	n
22	On-column	0.1627	37270	0.5722	11330
	Z-shaped	0.1622	30780	0.5691	8610
	%[a]	−0.31	−17.4	−0.54	−24.0
100	On-column	0.08625	143000	0.2791	62270
	Z-shaped	0.08588	120500	0.2785	28580
	%[a]	−0.43	−15.7	−0.21	−54.1
200	On-column	0.03468	815400	0.07933	616300
	Z-shaped	0.03465	683100	0.08077	176000
	%[a]	−0.12	−16.2	+0.93	−71.4

[a] % = 100 $(k'_Z - k'_{oc})$ or 100 $(n_Z - n_{oc})/n_{oc}$, where the subscript oc refers to the on-column cell and Z to the Z-shaped cell.

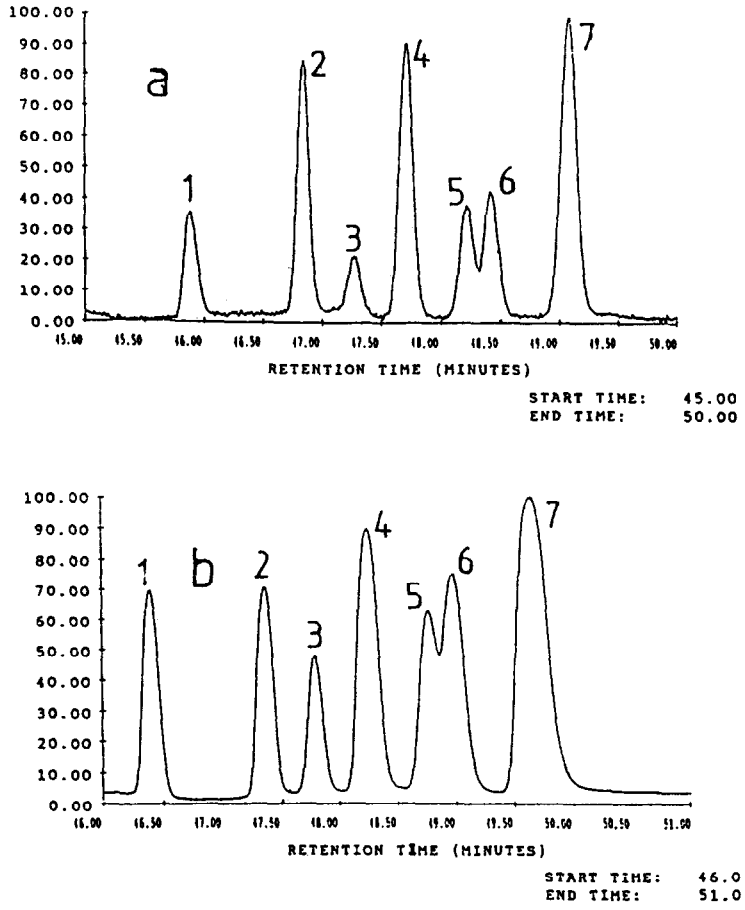

Fig. 4. Chromatograms of a chlorobenzene mixture. Column, 19.5 × 51 μm I.D. SB-Methyl-100, 200°C, mobile phase, acetonitrile–water (50:50), u = 0.584 cm/s; UV detection at 210 nm. (a) On-column cell; (b) Z-shaped cell. Peaks: 1 = tropolone; 2 = benzene; 3 = 1,4-dichlorobenzene; 4 = 1,2,4-trichlorobenzene; 5 = 1,3,5-trichlorobenzene; 6 = 1,2,4,5-tetrachlorobenzene; 7 = pentachlorobenzene.

ity of the Z-shaped cell could be utilized with little efficiency loss.

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