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ZONE COMPRESSION EFFECTS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A mathematical description of the post-column compression and acceleration of peaks eluted from a liquid chromatographic column is presented. By using specialized on-column concentration instrumentation for the sequential entrapment, compression and acceleration of individual solute zones, automatic enrichment of long retained solutes is possible. The technique is useful for preparative separations, where gradient elution cannot always be utilized.

INTRODUCTION

The apparent width of a chromatographic peak in an elution method is a function of two independent factors; the physical width of the solute zone as it leaves the column, and the speed at which it exits. It is important that the mass of solute be concentrated into the minimum possible volume, in order to increase detection sensitivity in analytical separations, to minimize zone dispersion in multidimensional separations[1–3], or to maximize collection efficiency in preparative chromatography[4]. Although on-column concentration and similar techniques are widely used in practice, there have been only a few quantitative treatments of zone compression phenomena in the literature[5]. During research within our laboratory regarding preparative separations[4], investigations arose concerning the principles of how solute zones can be concentrated without resorting to gradient elution. It became interesting to consider how such concentration can be achieved, and the limits to zone compression. Although high-performance liquid chromatography (HPLC) is specifically treated in this work, the concepts are applicable to any elution chromatography method.

One of the tangible advantages of gradient elution is the reduction of the ca-

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pacity factor (k'), or increase in velocity of each zone as it leaves the column, with subsequent reduction in eluted peak volume. Typically terminal velocities of $k' \approx 2$ will be observed for zones exiting under gradient elution. Physical zone width is not usually reduced significantly[6] when separating small molecules under shallow gradient conditions, although it can be under the proper conditions[7]. Thus, the problem is how to reduce the elution volume, V_p, of a zone, in order to increase detection sensitivity and solute enrichment (preparative collection), or to reduce peak volume (multidimensional separations).

While gradient elution serves to compress and accelerate chromatographic zones, in theory it would be interesting if the peak compression and acceleration process could be made independent of the separation process. In this manner, decisions regarding separation optimization or other requirements could be made independently of zone concentration effects.

In order to achieve this condition, hypothetically one might picture a small tube inserted into the column some distance from the end. Strong mobile phase would be turned on just after each solute band passes the tube, compressing and eluting the zone with strong eluent, and achieving the desired reduction in eluted peak volume. For convenience, this process has been termed post-column acceleration (PCA) within this manuscript.

Instrumentally, this can be implemented by allowing the peak(s) to exit the primary analytical column onto a secondary acceleration column. Only one peak, or group of peaks is allowed onto the acceleration column at one time. Once on, the flow from the primary column is stopped, and a stronger mobile phase is switched onto the acceleration column. This step-gradient both physically compresses the zone and elutes it off the column at a high velocity, thus achieving enrichment.

THEORY

Under isocratic conditions, for the precision needed in this application, it is usually possible to assume that each solute reaches any point in the column with approximately the same physical zone width. As long as this assumption holds, then the physical width of any peak (in distance units) as it exits the primary analytical column (W_{pf}), can be approximated by;

$$W_{pf} = 4\sigma_p = 4L_p/\sqrt{N_p} \tag{1}$$

where σ_p is the peak standard deviation on the primary column in distance units, L_p is the primary column length and N_p is the number of theoretical plates generated by the primary column. The final volume of peak as it is eluted from the primary column (V_{pf}) is

$$V_{pf} = f_{vp} \cdot t_p = f_{vp} \cdot W_{pf}/u_{mp} = (\varepsilon_p \pi d_{cp}^2/4) \cdot W_{pf}\,(1+k'_{pf}) \tag{2}$$

where f_{vp} is the volumetric flow-rate of the primary mobile phase, t_p the time it takes the peak to elute off the primary column, and u_{mp} is the linear velocity of the primary mobile phase. ε_p is the porosity of the primary (analytical) column with diameter d_{cp}, and k'_{pf} is the capacity factor of the solute as it exits the primary column. Throughout

the following derivation, a subscript p will refer to the main (primary) separation column, and subscript a to the secondary (acceleration) column. A second subscript of i will refer to initial conditions, and subscript f to final conditions (see List of symbols).

As summarized in eqn. 2, the eluted volume of a peak depends upon two independent factors (i) its physical width and (ii), its exit velocity. Both these effects can be accomplished experimentally by shunting the solute zone onto a short secondary column and eluting the peak under stronger mobile phase conditions. The passage of a hypothetical step gradient over a solute zone on the acceleration column is shown in Fig. 1. The peak volume as it elutes from this final column, V_{af}, will be given by

$$V_{af} = \left(\frac{f_{va}}{u_{ma}}\right) W_{af} \cdot (1 + k'_{af}) \tag{3}$$

where f_{va} is the volumetric flow-rate of the acceleration mobile phase, u_{ma} is the linear velocity of the acceleration mobile phase and k'_{af} is the capacity factor of the zone as it leaves the acceleration column with a physical width of W_{af}.

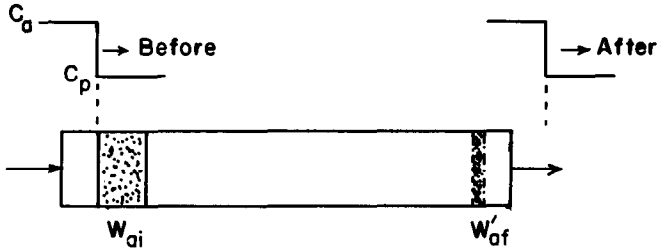

Fig. 1. Passage of a hypothetical mobile phase step-function over the solute zone on the acceleration column. W_{ai} and W'_{af} represent the initial width of the zone on the accelerator column prior to passage of the step-function and the width of the solute zone just after passage of the step-function, but before exit from the acceleration column. C_p and C_a are the mobile phase compositions of the primary and acceleration column effluents respectively.

The physical zone compression achievable under a step-function mobile phase strength increase can be derived as

$$\text{compression factor } (C) = \frac{k'_{af} - k'_{ai}}{k'_{ai} + k'_{ai}k'_{af}} \tag{4}$$

where k'_{ai} is the initial k' value of the solute on the accelerator column under the primary mobile phase. The compression factor, C, ranges from 0 to -1. This term is essentially the same as the G_1 term previously derived by Snyder and Saunders[5], in that $C = G_1 - 1$. When $k'_{af} \rightarrow k'_{ai}$, $C \rightarrow 0$, which is to say that no physical zone compression will result if no mobile phase change is introduced. At the other extreme, $C \rightarrow -1$ as $k'_{af} \rightarrow 0$, corresponding to the approach of the initial peak width to a delta function.

Eqn. 4 describes only the compression effect of a step-gradient moving the rear

of the band faster than the head over the time it takes the gradient to overtake the band. After this event, no further, solvent induced compression occurs. Another factor must be accounted for however. During the physical transfer of the zone from the primary to the acceleration column, physical zone compression (or expansion) can occur due to zone velocity differences on the two columns. This change will be proportional to $(1 + k'_{pf})/(1 + k'_{ai})$, where k'_{pf} and k'_{ai} are the capacity factors of the zone in the primary and acceleration columns respectively, while still in the same primary column mobile phase.

After transfer is complete, a new mobile phase is switched on, producing the effect described in eqn. 4. Combining eqn. 4 with the stationary phase compression effect, and accounting for differences in primary mobile phase linear velocity between the primary and acceleration columns, results in

$$W'_a = W_{pf}\left(\frac{u_{mp}'}{u_{mp}}\right)\left(\frac{1 + k'_{pf}}{1 + k'_{ai}}\right)\left(\frac{k'_{af} - k'_{ai}}{k'_{ai} + k'_{ai}k'_{af}}\right) \tag{5}$$

where W'_a is the physical width of the zone on the accelerator column just after being overtaken by the step gradient, but not necessarily eluted from the secondary column. The primary mobile phase linear velocity on the acceleration volumn is given by u_{mp}'. The ratio of linear velocities in eqn. 5 is fixed by the porosities and diameters of the two columns, since the same mobile phase is still flowing through both, *i.e.*, the strong mobile phase, which may be at a different velocity, has not yet been switched in.

Since the sole purpose of the accelerator column is to allow passage of the step gradient over the solute zone, this second column should be as short as possible, to avoid adding variance to the zone. This length is given by the distance travelled by the step gradient in just overtaking the front of the solute zone, which is travelling with a velocity of $u_{ma}/(1 + k'_{ai})$, thus yielding

$$L_{min} = W_{ai}\left(\frac{1 + k'_{ai}}{k'_{ai}}\right) \tag{6}$$

For reference, a 25-cm column producing $N = 8000$ would require an acceleration column 1.7 cm in length, for a peak eluting from the first column with a $k'_p = 2$ (assuming a peak width of 4σ).

In practice columns shorter than 2 cm are difficult to work with for two reasons: (i) these short lengths approach the minimum length needed for column end fittings and (ii) as the column gets shorter the extra variance of the connectors and tubing is no longer negligible. Also L_{min} is different for each peak, with the fastest moving peaks requiring the longest acceleration columns. Thus, in practice longer than optimal acceleration columns will often be used. In such cases, the variance contribution of the acceleration column to the system can be significant, and must be taken into account. The accelerator column variance contribution can be expressed as:

$$\sigma_a^2 = L_a h_a d_{pa} \tag{7}$$

Where σ_a is the peak standard deviation on the accelerator column in distance units, and L_a, h_a, d_{pa} are the length, reduced plate height and particle size of the accelerator column. Combining the dispersion effect of the accelerator column with eqn. 5 yields the following expression for the physical zone width, W_{af}, of a band as it exits the acceleration column

$$W_{af} = \left[(W_{pf})^2 \left(\frac{1 + k'_{pf}}{1 + k'_{ai}} \right)^2 \left(1 + \frac{k'_{af} - k'_{ai}}{k'_{ai} + k'_{ai}k'_{af}} \right)^2 + 16 L_a h_a d_{pa} \right]^{0.5} \quad (8)$$

Combining the effects of both physical zone compression, and final zone exit velocity (k'_{af}), yields the peak volume as it elutes off the accelerator column, V_{af}, assuming additivity of variances:

$$V_{af} = \frac{4 f_{va}(1 + k'_{af})}{u_{ma}} \left[L_p h_p d_{pp} \left(\frac{u_{ma}}{u_{mp}} \right)^2 \left(\frac{1 + k'_{pf}}{1 + k'_{ai}} \right)^2 \left(1 + \right. \right.$$

$$\left. \left. + \frac{k'_{af} - k'_{ai}}{k'_{ai} + k'_{ai}k'_{af}} \right)^2 + L_a h_a d_{pa} \right]^{0.5} \quad (9)$$

where h_p and d_{pp} are the reduced plate height and particle size of the primary column respectively. The constant 4 represents a peak width of 4 standard deviations.

IMPLEMENTATION OF POST-COLUMN ACCELERATION

Acceleration of emerging solute zones can be implemented experimentally through the use of a simple switching valve. Just after entrance of the entire solute zone onto the accelerator column flow through the accelerator column is then switched to the stronger mobile phase. Fig. 2 shows a typical post-column acceleration (PCA) valving arrangement. Ideally, two detectors should be used, with the first one prior to the PCA column sensing the eluting zones for accurate valve timing. With a single detector, the acceleration column can be placed either before or after the detector, depending upon the needs of the analyst. If detection after acceleration is used, then either prior calibration of the switching times for the valve must be performed, or a second detector added to the system just before the switching valve.

Fig. 3 shows the application of PCA on a test mixture of small molecules separated on a reversed-phase system. Under isocratic elution, at 45% methanol, the last peak (*p*-propylphenol) emerges with a retention time of over 80 min (k'_{pf} *ca.* 30). A second, stronger mobile phase of 80% methanol was used in conjunction with a switching valve and a short (15 cm) column packed with C_{18} material. The accelerated zone exited with a k'_{af} value of less than 1.0. The accelerated band shown in Fig. 3 displays a strong refractive index (RI) peak in the dead volume, arising from the heat of mixing of methanol with the water. This RI disturbance was essentially constant in magnitude, regardless of the solute zone accelerated. The presence of this disturbance zone limited the k'_{af} that could be used, and thus the enrichment factor, since if bands were eluted within this zone, quantitation became difficult.

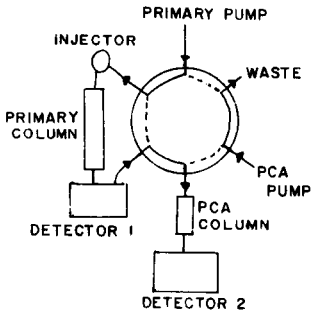

Fig. 2. A typical valving arrangement used for PCA. The solid lines indicate the position of the switching valve for shunting the zone of interest to the accelerator column. The dashed lines indicate the position of the valve just after the zone has been totally transferred to the PCA column.

The peak enrichment can be calculated by the ratio of the zone volume as it exits the primary column (V_{pf}) to the zone volume as it exits the accelerator column (V_{af}). Table I lists the theoretical and observed enrichment factors for the test solutes. Only peaks with k' values higher than about 9 were useful with the PCA method, due to overlap with the disturbance zone. Propylphenol, with a k'_{pf} value of 33.1, was concentrated by a factor of 12:1 when using post-column acceleration (17.3:1 theoretical), when compared to simply collecting the peak directly from the primary column. Methylbenzoate, with a k'_{pf} of 17.3, was enriched about 9:1 (12.3:1 theo-

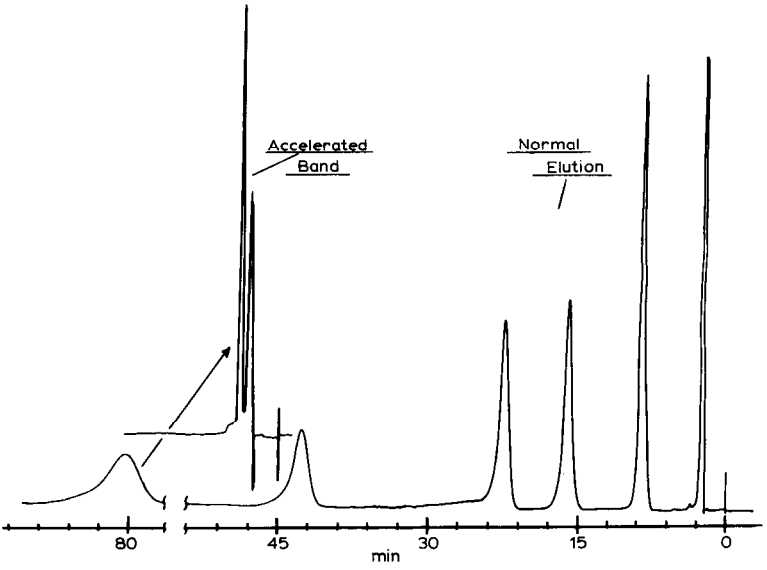

Fig. 3. A test mixture of small molecules on a 25-cm C_{18} column eluted with 35% methanol, 1 ml/min, showing PCA performed on the last peak, *p*-propylphenol, which had a retention of over 80 min on the primary column. The zone was accelerated off a second 15-cm, C_{18} column with 80% methanol, 1 ml/min. The inset shows the accelerated zone eluting at a k'_{af} of *ca.* 1.0. The refractive index change in the dead volume is due to the heat of mixing of the methanol and water, and in this system, restricted the application of PCA methods to solutes with a k'_{pf} of about 9 or greater. The identity of the peaks are listed in Table I.

TABLE I

THEORETICAL AND OBSERVED ENRICHMENT FACTORS FOR TEST SOLUTES

Note: all volumes are listed in milliliters. na = Not available.

Compound	t_R	k'_{pf}	V_{pf}	k'_{af}	k'_{af}	V_{af}	*Enrichment factor:* V_{pf}/V_{af}	
							Theory	*Obs.*
Phenol	8.35	2.55	0.24	2.54	0.11	0.057	4.2:1	na
p-Cresol	16.0	5.79	0.46	5.75	0.28	0.077	6.0:1	na
Nitrobenzene	22.5	8.55	0.64	8.50	0.37	0.065	9.8:1	7:1
Methylbenzoate	43.1	17.3	1.23	17.3	0.49	0.10	12.3:1	9:1
p-Propylphenol	80.2	33.1	2.29	33.0	1.00	0.13	17.3:1	12:1

retical), while nitrobenzene, $k'_{pf} = 8.55$, was enriched by 7:1 (9.8:1 theoretical). Peaks with k'_{pf} values less than this were merged with the disturbance zone, and were not measurable.

In practice, early eluting peaks should not be run through the PCA column, but switched directly to the detector. This is not a problem however, since such peaks are fairly concentrated as they leave the first column. The main reason for employing PCA techniques is to enhance the concentration of long retained zones, without gradient elution.

Several factors could account for the difference in observed and theoretical enrichment factors. For one, it is difficult to produce a perfect step-function. Any rounding of the step due to tubing and valving variance will diminish the impact of eqn. 6. Secondly, as a sharp change in mobile phase passes over a zone, it is possible that disturbances due to mixing of the two solvents could add variance to the zone. Finally, increased zone variance due to valving and tubing was not taken into account in eqn. 6.

The increase in either detection sensitivity, or in concentration enrichment upon collection, makes the PCA technique an attractive option to consider in trace analysis, or in preparative separations. PCA techniques, though similar in principle to on-column concentration and gradient elution, differ in that with PCA methods a short column is used, the sole purpose of which is to collect, hold and then discharge a band, in a manner similar to that of a capacitor in electronics. Using PCA methods, isocratic elution can be used to achieve separations with no penalty in detectability or collection efficiency. While not used in this study, PCA could be readily automated, with electronic switching valves and peak sensing, so that automatic, unattended operation is possible.

LIST OF SYMBOLS

C = compressibility factor
d_{cp} = diameter of primary column
d_{pa} = particle size of the accelerator column
d_{pp} = particle size of the primary column

ε_p = porosity of primary column
f_{va} = volumetric flow-rate of the accelerator mobile phase
f_{vp} = volumetric flow-rate of the primary mobile phase
h_a = reduced plate height of the accelerator column
h_p = reduced plate height of the primary column
k'_{af} = capacity factor of peak as it exits the accelerator column
k'_{ai} = initial capacity factor of peak on accelerator column in the primary mobile phase
k'_{pf} = capacity factor of peak as it exits the primary column
L_a = length of accelerator column
L_{min} = length of accelerator column needed for the step-function to just overtake the peak
L_p = length of primary column
N_p = number of theoretical plates of the primary column
N_a = number of theoretical plates of the acceleration column
σ_p = peak standard deviation on primary column in cm
σ_a = peak standard deviation on accelerator column in cm
t_p = peak width in seconds as it elutes off the primary column
u_{ma} = linear velocity of acceleration column mobile phase
u_{mp} = linear velocity of primary mobile phase on the primary column
u_{mp}' = linear velocity of primary mobile phase on the acceleration column
V_{af} = peak volume as it elutes from the acceleration column
V_{pf} = volume of peak as it elutes from the primary column
W_{af} = width of zone as it elutes off the acceleration column
W_{ai} = initial zone width on the acceleration column
W_{af}' = width of zone just after being overtaken by the step-function on the accelerator column
W_{pf} = Width of peak as it exits the primary column

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