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## PEAK WIDTH IN SOLVENT-PROGRAMMED CHROMATOGRAPHY

### I. GENERAL DESCRIPTION OF PEAK BROADENING IN SOLVENT-PROGRAMMED ELUTION

H. POPPE\*, J. PAANAKKER and M. BRONCKHORST

*Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam (The Netherlands)*

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#### SUMMARY

An equation describing the peak broadening or peak contraction in liquid chromatography under solvent-programmed or gradient conditions is derived from the mass-transport equation in the dispersion model. The results are applicable to all cases where the retention can be calculated in advance. The predictions obtained with the equation are equal to those obtained by other means, when available. This is the case for step gradients with the assumption of negligible dispersion, and for the linear solvent gradients.

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#### INTRODUCTION

Solvent programming in liquid chromatography is a powerful tool used to resolve some important problems arising in analytical applications. For example, continuous gradients have been employed<sup>1,2</sup> as an answer to the "general elution problem", and step gradients to circumvent poor detection sensitivity in on-column concentration procedures<sup>3-5</sup>.

Migration, or retention, of solutes under solvent-programmed conditions is well described. Snyder and Saunders<sup>6</sup> derived the fundamental equation and also gave a numerical treatment of the effect of the solvent program on the peak dispersion, *i.e.*, the contraction effect due to the fact that the peak front moves in a solvent of lesser strength than does the tail of the peak. More recent treatments of solvent-programmed elution<sup>2,7-9</sup>, inspired by the increasing popularity of gradients in high-performance liquid chromatography (HPLC), do not differ in essence from the approach employed by Snyder in 1964, although the mathematical tools used are sometimes much more sophisticated. No new treatments of the peak broadening were given. On the other hand, Snyder *et al.*<sup>2</sup> have recently given results of the use of the above numerical procedures for the specific case of the linear solvent strength (LSS) gradients they advocate strongly.

In the present paper a description of band broadening in solvent programming is given which applies to all slow gradients, and the results also seem to be valid for

step gradients. Experimental verification of the equations as well as a discussion of the implications for optimization will be given in subsequent papers.

## THEORETICAL

### *Mathematical description of the transport of a solute*

The differential equation used here to describe mass transport in the column is

$$\frac{\partial c_t}{\partial t} = D \frac{\partial^2 c_m}{\partial z^2} - v \frac{\partial c_m}{\partial z} \quad (1)$$

in which  $c_t$  is the "total concentration", defined as

$$c_t = c_m + q c_s = c_m(1 + \kappa)$$

$c_m$  is the concentration in the mobile phase,  $c_s$  is the concentration in the stationary phase,  $q$  is the volume ratio of the stationary and mobile phases,  $\kappa$  is the capacity factor,  $t$  is the time,  $z$  is the axial coordinate in the column,  $v$  is the migration velocity of the mobile phase and  $D$  is the dispersion coefficient, equal to  $Hv/2$ , where  $H$  is the plate height. Immediate equilibrium between mobile and stationary phase is assumed described by

$$c_s = K c_m \quad (2)$$

in which  $K$  is the partition or adsorption constant.

It should be noted that the use of eqns. 1 and 2 introduces certain limitations on the applicability of the results obtained. The most important of these are as follows.

The use of a dispersion model for chromatographic transport<sup>10,11</sup>, as implied by eqn. 1 groups together all kinds of dispersion mechanisms (normal and eddy diffusion, resistance to mass transfer in the various phases) into one dispersion coefficient,  $D$ . This is an adequate description of the physical reality, if the column length is large enough so that all dispersion mechanisms finally affect the elution function in the same way: a gaussian function results, being an asymptotic solution of the various differential equations, provided the column length (expressed, *e.g.*, as plate number) is large. Our results will therefore be valid only for reasonably large plate numbers (100–1000, depending on the required accuracy).

The capacity ratio,  $\kappa$ , changes with time in gradient elution. It is known<sup>12</sup> that the plate height,  $H$ , in LC is a function of  $\kappa$ . We do not include this effect in our treatment because it would lead to unacceptable mathematical complexity. In situations where resistance to mass transfer in the stationary phase plays a predominant rôle (*e.g.*, in classical ion-exchange chromatography) the results would be questionable. However, in most HPLC situations the dependence of  $H$  on  $\kappa$  is not so strong as to ruin the practical validity of our treatment.

Changes in  $\kappa$  are accomplished by changes in eluent composition. A number of second-order effects are therefore to be expected: the velocity,  $v$ , and the dispersion coefficient  $D$  can change because of changes in the viscosity and in the (related) values of the diffusion coefficients. We neglect these effects. In order to avoid complications

in the formulae, we express the influence of solvent strength on the distribution by giving the capacity factor,  $\kappa$ , rather than the distribution constant,  $K$ , as a function of  $z$  and  $t$ :

$$\kappa = \kappa(z, t)$$

Description of the peak width is first carried out by using the second normalized centralized place moment

$$\mu_2 = \frac{\int_{-\infty}^{+\infty} x^2 c_t(x) dx}{\int_{-\infty}^{+\infty} c_t(x) dx} \quad (3)$$

where  $x = z - \mu$ , the axial coordinate, relative to the centre of gravity,  $\mu$ , of the  $c_t(z)$  function:

$$\mu_1 = \frac{\int_{-\infty}^{+\infty} z c_t(z) dz}{\int_{-\infty}^{+\infty} c_t(z) dz} \quad (4)$$

Again, some comment is required, in order to indicate the limitations of the treatment. Characterization of local and temporal distribution functions by the use of moments has a number of advantages, among which the most important are the direct relation of the moments with physicochemical parameters in the column, and (connected with this) the mathematical simplification obtained. On the other hand it should be recognized that for odd, non-gaussian peak shapes, expression of the peak width as the second moment,  $\mu_2$ , gives little information about overlap of adjacent peaks, which is of prime concern to an analytical chemist. Only for known peak shapes, *e.g.*, block functions, first-order responses, gaussian shapes, can we infer from the first and second moments how severe will be the peak overlap. It follows that our treatment will give valuable results for the analytical chemist only for cases where the peak shape is known in advance: gaussians for columns with high plate numbers ( $>100$ ) and small injection profiles, or, *e.g.*, block functions when these are expected because of the injection conditions. Fortunately these extremes are exactly the cases where solvent programs are important.

A second comment concerns the use of  $c_t$  rather than  $c_m$  in eqns. 3 and 4. It could be argued that in elution chromatography  $c_m(t)$  at the column end is a more relevant function than  $c_t(z)$  at a particular moment. The choice of  $c_t(z)$  in our treatment is based purely on mathematical reasons. It can be shown that for slowly varying capacity factors (in the case of slow gradients, or in a region of constant  $\kappa$  after a steeper gradient), the second moments based on  $c_m$  and  $c_t$  are the same. The use of local distributions, prior to the temporal distribution, is widespread in treatments of chromatographic transport. The translation of the results into elution peak widths in time units is straightforward and valid for all columns with large plate numbers.

The denominator of eqns. 3 and 4 is constant and equal to  $Q/A_m$ , where  $Q$  is the total amount of the solute and  $A_m$  is the area of the part of the cross section of the column which is occupied by the mobile phase. Therefore:

$$\mu_2 = \frac{A_m}{Q} \int_{-\infty}^{+\infty} x^2 c_t(x) dx \quad (5)$$

and

$$\mu_1 = \frac{A_m}{Q} \int_{-\infty}^{+\infty} z c_t(z) dz \quad (6)$$

As we need expressions for the retention parameters for manipulating and interpreting the dispersion formulae, we will repeat known<sup>6-9</sup> derivations of these in our terms. Thus, differentiating eqn. 6, and interchanging integration and differentiating, one obtains:

$$\frac{d\mu_1}{dt} = \frac{A_m}{Q} \int_{-\infty}^{+\infty} z \frac{\partial c_t}{\partial t} dz \quad (7)$$

Substituting from eqn. 1 leads to:

$$\frac{d\mu_1}{dt} = \frac{A_m}{Q} \int_{-\infty}^{+\infty} \left( zD \frac{\partial^2 c_m}{\partial z^2} - zv \frac{\partial c_m}{\partial z} \right) dz \quad (8)$$

Partial integration of eqn. 8 using the physically obvious fact that for  $z \rightarrow \pm \infty$ ,  $c_m \rightarrow 0$ , leads to

$$\frac{d\mu_1}{dt} = \frac{A_m}{Q} \int_{-\infty}^{+\infty} v c_m dz \quad (9)$$

which indicates that only  $c_m$  contributes to migration;  $c_m$  is related to  $c_t$  via the varying value of  $\kappa + 1$  in the column:

$$c_m = c_t \cdot \frac{1}{\kappa + 1}$$

Thus:

$$\frac{d\mu_1}{dt} = \frac{A_m}{Q} \int_{-\infty}^{+\infty} v \frac{c_t}{\kappa + 1} dz \quad (10)$$

Assuming that the gradient is flat, *i.e.*,  $\kappa$  changes little within the  $z$  region of significant values of  $c_t$ , we can treat the  $\kappa$  value as a constant,  $\bar{\kappa}$ , the value it has for the centre of the peak  $z = \mu_1$ ;

$$\frac{d\mu_1}{dt} = \frac{A_m}{Q} \frac{v}{\bar{\kappa} + 1} \int_{-\infty}^{+\infty} c_t dz = \frac{v}{\bar{\kappa}(\mu_1, t) + 1} \quad (11)$$

Eqn. 11 shows the dependence of  $\bar{\kappa}$  on  $t$  and  $\mu_1$ . Now, this equation is more easily integrated by replacing the  $t$  coordinate by  $t - \mu_1/v = \xi$ . For gradients *which do not change their shape on migration through the column, i.e.*, those which are not distorted by dispersion or by selective uptake of one of the solvent components by the

stationary phase (solvent demixing), the capacity factor within the peak's centre of gravity,  $\bar{\kappa}$ , is simply

$$\bar{\kappa} = \kappa(\xi) \quad (12)$$

where  $\xi$  is proportional to the volume of solvent passed through the peak centre<sup>6</sup>, but expressed as a time coordinate. It can be shown that a linear dependence of  $\kappa$  on  $z$  does not impair the validity of this approximation. Expression of eqn. 11 in these new coordinates yields:

$$\frac{d\mu_1}{d\xi} = \frac{v}{\kappa(\xi)} \quad (13)$$

This equation, in different forms, has been put forward by Snyder and Saunders<sup>6</sup>, and used by Jandera and Churáček<sup>7</sup>, Schoenmakers *et al.*<sup>8</sup> and Liteanu and Gocan<sup>9</sup>. The  $\xi_e$  for elution ( $\mu_1 = L$ ) is found by

$$\int_0^{\xi_e} \frac{1}{\kappa(\xi)} d\xi = \frac{L}{v} = t_0 \quad (14)$$

Returning to the second moment, we obtain\*, when differentiating eqn. 5

$$\frac{d\mu_2}{dt} = \frac{A_m}{Q} \int_{-\infty}^{+\infty} x^2 \frac{\partial c_i}{\partial t} dx \quad (15)$$

and substitution of eqn. 1 in eqn. 15 yields:

$$\frac{d\mu_2}{dt} = \frac{A_m}{Q} \int_{-\infty}^{+\infty} \left( x^2 D \frac{\partial^2 c_m}{\partial x^2} - vx^2 \frac{\partial c_m}{\partial x} \right) dx \quad (16)$$

By repeated partial integration of eqn. 16 and using again that for  $x \rightarrow \pm \infty$ ,  $c_m = 0$ , it can be shown that:

$$\frac{d\mu_2}{dt} = \frac{A_m}{Q} \left[ 2D \int_{-\infty}^{+\infty} c_m dx + v \int_{-\infty}^{+\infty} 2xc_m dx \right] \quad (17)$$

Now we assume again that the gradient is flat within the range of  $x$  (the total peak width) in which  $c_m$  is significantly different from zero. Continuous gradients generally justify this assumption, step gradients obviously do not. However, the curious fact arises that the results obtained with this treatment, mathematically justified only for slowly varying capacity factors, exactly reproduce certain results obtained for transport functions for dispersionless situations and step gradients<sup>3,9</sup>. It therefore seems appropriate to hope for more general validity of the treatment, beyond the range of slow variation of  $\kappa$  with  $t$  and  $z$  for which the present calculation can be justified mathematically.

\* In eqn. 5,  $c$  is not the only variable which changes;  $x$ , taken relative to the peaks centre of gravity, also changes. It can be shown, however, that the result of a derivation which takes this into account is the same as eqn. 16.

Assuming such a slow variation of  $\kappa$  with  $x$ , and again denoting the  $\kappa$  at  $x = 0$  (the peak centre of gravity) by  $\bar{\kappa}$  (of course a time-dependent value), we can express  $\kappa$  as a function of  $x$  by first-order approximation:

$$\kappa(x) = \bar{\kappa}(1 + ax) \quad (18)$$

This leads to:

$$c_m(x) = c_t(x) \frac{1}{1 + \kappa(1 + ax)} \quad (19)$$

With the same assumption of slow variation of  $\kappa$ , this can be set equal to:

$$c_m(x) = c_t(x) \left[ \frac{1}{\bar{\kappa} + 1} - \frac{\bar{\kappa}}{(\bar{\kappa} + 1)^2} ax \right] \quad (20)$$

Insertion of this into eqn. 17 yields four terms, two of which vanish because  $x$  measures the distance relative to the centre of gravity, *i.e.*:

$$-\infty \int^{+\infty} xc_t(x) dx = 0$$

The two remaining terms yield:

$$\begin{aligned} \frac{d\mu_2}{dt} &= \frac{A_m}{Q} \left[ 2D \frac{1}{\bar{\kappa} + 1} \int_{-\infty}^{+\infty} c_t(x) dx - 2v \frac{a\bar{\kappa}}{(\bar{\kappa} + 1)^2} \int_{-\infty}^{+\infty} x^2 c_t(x) dx \right] \\ &= \frac{A_m}{Q} \left[ 2D \frac{1}{\bar{\kappa} + 1} \frac{Q}{A_m} - 2v \frac{\kappa}{(\bar{\kappa} + 1)^2} a\mu_2 \frac{Q}{A_m} \right] \\ &= 2D \frac{1}{\bar{\kappa} + 1} - 2v \frac{\bar{\kappa}}{(\bar{\kappa} + 1)^2} a\mu_2 \end{aligned} \quad (21)$$

Eqn. 21 describes the change in peak width with time. For comparison it should be noted that in the isocratic situation ( $a = 0$ ) the second term on the right-hand side vanishes.

For a number of cases it is easier to look at the change in peak width with the migrated distance,  $\mu_1$ . By using eqn. 11

$$d\mu_1 = \frac{v}{\bar{\kappa} + 1} dt$$

it follows that:

$$\frac{d\mu_2}{d\mu_1} = \frac{2D}{v} - 2 \frac{\bar{\kappa}}{\bar{\kappa} + 1} \mu_2 = H - 2 \frac{\bar{\kappa}}{\bar{\kappa} + 1} a\mu_2 \quad (22)$$

This equation gives very good insight, as it gives the increase of the second moment with the migrated distance as a sum of the normal dispersion effect ( $H$  term) and the band compression effect (last term), proportional to  $\mu_2$  and  $a$ . However, for a general

solution of eqn. 11 it is convenient to replace the dependent variable  $\mu_2$  by  $\mu_2[(1 + \bar{\kappa})/\bar{\kappa}]^2 = y$ . This gives:

$$\begin{aligned} \frac{dy}{d\mu_1} &= \frac{(1 + \bar{\kappa})^2}{\bar{\kappa}^2} H - \frac{2(\bar{\kappa} + 1)}{\bar{\kappa}^3} \frac{d\bar{\kappa}}{d\mu_1} \mu_2 - 2a \frac{\bar{\kappa} + 1}{\bar{\kappa}} \mu_2 \\ &= \frac{(1 + \bar{\kappa})^2}{\bar{\kappa}^2} H + 2 \frac{\bar{\kappa} + 1}{\bar{\kappa}^3} \mu_2 \left( -\frac{d\bar{\kappa}}{d\mu_1} - a\bar{\kappa}^2 \right) \end{aligned} \quad (23)$$

The value of  $d\kappa/d\mu_1$  can be derived, again for gradients which *do not change their shape on migration through the column, i.e.*, which are not distorted by dispersion or by selective uptake of one of the solvent components by the stationary phase (solvent demixing). For these cases  $\kappa = \kappa(z, t) = \kappa(\xi)$  and

$$\frac{d\bar{\kappa}}{d\mu_1} = \frac{d\bar{\kappa}}{d\xi} \frac{d\xi}{d\mu_1} = \bar{\kappa} a (-v) \frac{\kappa(\xi)}{v} = -\bar{\kappa}^2 a \quad (24)$$

from eqns. 13 and 18. For those gradients, which are the only ones for which analytical mathematical considerations give insight into transport properties, eqn. 12 becomes simply:

$$\frac{dy}{d\mu_1} = \frac{d\mu_2 \left( \frac{\bar{\kappa} + 1}{\bar{\kappa}} \right)^2}{d\mu_1} = \left( H \frac{\bar{\kappa} + 1}{\bar{\kappa}} \right)^2 \quad (25)$$

As an example, we treat first the LSS gradient. Here

$$\kappa(\xi) = \kappa_0 e^{-b\xi} \quad (26)$$

describes the dependence of the capacity factor on time. The result for the net retention time is<sup>1</sup>

$$\xi_e = (1/b) \ln(\kappa_0 b t_0 + 1) \quad (27)$$

and the capacity factor on elution,  $\kappa_e$ , is:

$$\kappa_e = \frac{\kappa_0}{\kappa_0 b t_0 + 1} = \frac{\kappa_0}{\kappa_0 b L/v + 1} \quad (28)$$

This expression can be used to find  $\kappa$  as a function of the peak position,  $\mu_1$ , in the column. Then the right-hand side of eqn. 25 is known and this equation can be integrated. The result is:

$$y = HL(1 + p + \frac{1}{3}p^2) \quad (29)$$

where

$$p = \frac{\kappa_0}{\kappa_0 + 1} b t_0$$

The second place moment is

$$\mu_2 = HL \left[ \frac{1 + p + \frac{1}{3}p^2}{(1 + p)^2} \right] \quad (30)$$

in which the bracketed factor in the right-hand side is equal to  $G^2$  as defined by Snyder *et al.*<sup>2</sup>. A plot of  $G$  versus the slope of the gradient for large  $\kappa_0$  according to this equation is to be found in ref. 2, and these numerical results are equal to those predicted by eqn. 29.

A second example is the step gradient. The solution of eqn. 24 for a finite  $H$  value depends of course on the dependence of  $\kappa$  on  $\mu_1$ , *i.e.*, on the moment where the peak is overtaken by the step, and a complex expression will result. However, if we take  $H = 0$  (ideal, dispersionless chromatography), we should find the same result as that obtained with more elementary reasoning. This is indeed the case. Eqn. 24 predicts that for  $H = 0$ ,  $y$  is invariant. Thus the peak widths before (b) and after (a) the passage of the step have a ratio:

$$\frac{\kappa_b + 1}{\kappa_b} \cdot \frac{\kappa_a}{\kappa_a + 1} \quad (31)$$

This is exactly what has been derived<sup>1,3</sup>. The ratio of the concentrations in the mobile phase after and before the passage of the step is:

$$\frac{c_{ma}}{c_{mb}} = \frac{\kappa_b}{\kappa_a} \quad (32)$$

However, with eqn. 25 it is possible to calculate how dispersion affects the validity of eqn. 32, especially when the absolute values of  $\kappa$  are small.

## CONCLUSIONS

A general differential equation of relatively simple form can be derived which predicts the peak broadening, or contraction, under solvent-programmed conditions. The predictions coincide with numerical results obtained for LSS gradients, and with derivations for step gradients when dispersion is neglected. A discussion of the effect of the contraction on optimal gradient shape, as well on the choice of optimal conditions for on-column concentration procedures, is now possible. Future papers will be devoted to these subjects.

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