

CHROM. 7285

## GRADIENT ELUTION IN LIQUID CHROMATOGRAPHY

### II. RETENTION CHARACTERISTICS (RETENTION VOLUME, BAND WIDTH, RESOLUTION, PLATE NUMBER) IN SOLVENT-PROGRAMMED CHROMATOGRAPHY—THEORETICAL CONSIDERATIONS

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#### SUMMARY

A theory is presented for solvent-programmed chromatography involving gradient-elution chromatography and combined elution using subsequent steps with different forms of the concentration–time function. The theory is based on two simple equations describing the relationship between the capacity ratio and the concentration of the more efficient eluting component in the binary mobile phase. The first equation is valid in adsorption and ion-exchange chromatography and the second applies to partition chromatography, including salting-out chromatography on ion-exchange columns. Equations for retention volume, retention ratio (separation factor), peak width and resolution are derived for all the elution techniques used in chromatography where one of the two basic equations can be applied.

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#### INTRODUCTION

In Part I of this series<sup>1</sup>, a detailed theoretical discussion was given of the relationships between the capacity ratio of a sample compound and the composition of binary mobile phases in different modes of chromatography—adsorption, partition and ion-exchange. It was shown that, in a certain limited range, two simple equations could be written relating the capacity ratio ( $k'$ ) to the molal concentration ( $c$ ) of the component of the binary mobile phase that has the more efficient eluting strength. The equation

$$k' \approx k'_0 \cdot c^{-n} \quad (1)$$

applies in both adsorption and ion-exchange chromatography, often even in the presence of coincident equilibria (acid–base, complex formation) in the mobile phase, and the relationship

$$k' \approx k'_0 \cdot 10^{-n \cdot c} \quad (2)$$

can be used in partition chromatography (liquid-liquid, salting-out and solubilization chromatography on ion-exchange resins in mixed aqueous-organic media), where  $k'_0$  and  $n$  are constants. The limitations of the validity of eqns. 1 and 2 were discussed in the first part of this series<sup>1</sup>.

The equations derived form a reasonable foundation for theoretical calculations of important characteristics (retention volume, peak width, resolution and plate number) in solvent-programmed chromatography. In the present communication, the development of a theory is presented that enables prediction of these characteristics in gradient elution, stepwise and combined stepwise-gradient-elution chromatography. The results of practical verifications of the relationships derived will be published in the next part of this series.

### ASSUMPTIONS

In the present derivation, it is assumed that a gradient-generating device can be used capable of mixing two different liquids so as to produce a concentration gradient according to any mathematical function of concentration over time. Such devices are commercially available, e.g. those based on a photoelectric curve follower. Of the two liquids mixed in this device, one is assumed to be a much stronger eluting agent than the other.

It is further assumed that there is no concentration change in the mobile phase (gradient distortion) during the transport from the outlet of the gradient-generating device to the outlet from the column. To meet this requirement, the geometry of the instrument should be carefully designed in order to avoid any off-line void spaces and excessive volume of the connecting tubing. Additional changes in the composition of the mobile phase in adsorption chromatography may be caused by a preferential adsorption of the more efficient eluting component of the binary solvent mixture (greater  $\epsilon^0$  value), but these changes are assumed to have a minor importance, especially when partially deactivated adsorbents are used.

Gradient-elution techniques have not been widely used in partition chromatography because of serious washing of the stationary liquid phase out the column by the changing miscibility of the stationary and mobile phases in the course of a concentration gradient in the mobile phase. It is hardly practical nor possible to develop any quantitative theory for this. The introduction of new packing materials with permanently (chemically) bonded stationary phases, which remain unchanged on the support during gradient elution, makes possible the use of gradient-elution techniques in partition chromatography without the above difficulties, and a quantitative treatment is therefore possible, as in salting-out chromatography on ion-exchange columns.

In solubilization chromatography on ion exchangers in mixed aqueous-organic media, the composition of the solution in the "inner" exchanger phase changes with the changing composition of the "outer" solution (mobile phase). For this reason, the derived equations for elution characteristics can be expected to be valid only for gradients using rather limited concentration ranges.

The theory presented is further limited if the assumptions involved in the derivation of eqns. 1 and 2 are no longer valid. Detailed discussion of this is presented in the first part of this series<sup>1</sup>.

## ADSORPTION AND ION-EXCHANGE CHROMATOGRAPHY

*Simple gradient-elution chromatography*

The form of the concentration–time function should be chosen so as to be applicable to a great variety of curves. However, the polynomial form would introduce an excessively large number of independent parameters, the real meaning of which would not be clear. In addition, the mathematical solution to such a function would be difficult and the resulting relationships would be very complex. Therefore, the following form of the concentration–time function was chosen.

$$c = (A^{\frac{1}{\kappa}} + B' \cdot t)^{\kappa} \quad (3)$$

To obtain the equation describing the relationship between the concentration of the stronger eluting agent in the mobile phase at the outlet of the gradient-generating device and the volume  $V$  of the mobile phase delivered by this device at any time,  $t$ , the relationship  $t = V/u$  has to be considered, where  $u$  is flow velocity of the mobile phase in ml/min.

Then, the concentration–volume function can be written as

$$c = (A^{\frac{1}{\kappa}} + B \cdot V)^{\kappa} \quad (4)$$

where  $B = B'/u$ .

The real meaning of the parameters  $A$  and  $B$  can be determined as follows. At the beginning of the gradient elution (injection time)  $V = 0$  and  $c = c_0$ . Consequently,  $A = c_0$ , the initial concentration of the stronger eluting agent in the binary mobile phase.

Let concentration  $c$  be  $c_k$  for a volume  $V_y$  delivered by the gradient-forming device.  $V_y$  can be that volume of the mobile phase which should be pumped on to the column until pure second solution will be delivered, containing the more efficient eluting component at a concentration  $c_k$ . Then, the parameter  $B$  is defined as

$$B = \frac{c_k^{\frac{1}{\kappa}} - c_0^{\frac{1}{\kappa}}}{V_y} \quad (5)$$

Thus, parameter  $A$  relates to the concentration of the stronger eluant at the beginning of the gradient elution. Parameter  $B$  can be used as a measure of the slope of the total concentration change during the course of the gradient elution. As long as the concentration of the stronger eluting component increases in the binary mobile phase,  $B > 0$  and, on the contrary,  $B < 0$  as this concentration is diminishing.  $B = 0$  in isocratic elution.

Parameter  $\kappa$  characterizes the functional shape of the concentration gradient. Positive values of  $\kappa$  are sufficient for defining various gradient functions. As long as  $\kappa = 1$ , the change in concentration of the efficient component in the mobile phase is linear, while the concentration gradient is concave for  $\kappa > 1$  and convex for  $\kappa < 1$ .

Substituting concentration–volume function 4 in the equation for the capacity ratio (eqn. 1), the following relationship is obtained between the capacity ratio of a

sample compound in the solution leaving the outlet of the gradient-generating device and the volume of the mobile phase,  $V$ , delivered by this device at any time,  $t$ .

$$k' \approx k'_0 \cdot (A^{\frac{1}{\alpha}} + B \cdot V)^{-\alpha \cdot n} \quad (6)$$

The derivation of the equation for retention volume in gradient-elution chromatography is based on the following considerations.

A differential increase in the volume of the mobile phase  $dV$  delivered from the gradient-generating device on to the column causes a migration of the band of a sample compound in the column through a distance corresponding to a differential part of the column void volume,  $dV_m$ . The composition of the mobile phase and, consequently, the  $k'$  value remain constant in the course of this differential change. Therefore, an equation analogous to that valid for isocratic elution ( $k' = V'_R/V_m$ ) can be written for this differential change in volume in gradient elution chromatography.

$$k' = \frac{dV}{dV_m} \quad (7)$$

The relationship for retention volume in gradient-elution chromatography,  $V'_{R(\theta)}$ , can be obtained as a solution to the equation

$$\int_0^{V_m} dV_m = \int_0^{V'_{R(\theta)}} \frac{1}{k'} \cdot dV \quad (8)$$

(The volume of the connecting tubing between the outlet of the gradient-generating device and the column is assumed to be negligible in the present derivation. Otherwise, the retention volume in gradient-elution chromatography,  $V'_{R(\theta)}$ , can be calculated as in combined chromatography using isocratic elution in the first step (the volume of the mobile phase equals the volume of the connecting tubing,  $V_z$ ) followed by gradient elution in the second step, as in eqn. 36.)

Substitution of eqn. 6 in eqn. 8 gives the equation

$$V_m \approx \frac{1}{k'_0} \cdot \int_0^{V'_{R(\theta)}} (A^{\frac{1}{\alpha}} + B \cdot V)^{\alpha \cdot n} \cdot dV \quad (9)$$

the solution of which gives the required equation for retention volume in gradient elution chromatography.

$$V'_{R(\theta)} \approx \frac{1}{B} \cdot \left[ (\alpha \cdot n + 1) \cdot B \cdot k'_0 \cdot V_m + A^{\frac{\alpha \cdot n + 1}{\alpha}} \right]^{\frac{1}{\alpha \cdot n + 1}} - \frac{A^{\frac{1}{\alpha}}}{B} \quad (10)$$

If the concentration gradient function is chosen so that the concentration of the efficient eluting component in the mobile phase equals zero at the beginning of the elution,  $A = c_0 = 0$ , the equation for  $V'_{R(\theta)}$  has the simple form

$$V'_{R(\theta)} \approx \frac{1}{B} \cdot \left[ (\alpha \cdot n + 1) \cdot B \cdot k'_0 \cdot V_m \right]^{\frac{1}{\alpha \cdot n + 1}} \quad (11)$$

The equation for the retention ratio (separation factor),  $\alpha_{(g)}$ , in gradient-elution chromatography can be obtained by a simple substitution.

$$\alpha_{(g)} \approx \frac{V'_{R(g)2}}{V'_{R(g)1}} = \frac{\left[ (\kappa \cdot n_2 + 1) \cdot B \cdot k'_{02} \cdot V_m + A \frac{\kappa \cdot n_2 + 1}{\kappa} \right] \frac{1}{\kappa \cdot n_2 + 1} - A \frac{1}{\kappa}}{\left[ (\kappa \cdot n_1 + 1) \cdot B \cdot k'_{01} \cdot V_m + A \frac{\kappa \cdot n_1 + 1}{\kappa} \right] \frac{1}{\kappa \cdot n_1 + 1} - A \frac{1}{\kappa}} \quad (12)$$

If  $A = 0$  and  $n_1 \approx n_2 \approx n$ , this equation reduces to a very simple form,

$$\alpha_{(g)} \approx \left( \frac{k'_{02}}{k'_{01}} \right)^{\frac{1}{\kappa \cdot n + 1}} \approx \alpha_0 \frac{1}{\kappa \cdot n + 1} \quad (13)$$

where  $\alpha_0 = k'_{02}/k'_{01}$ .

It can be seen that the retention ratio (separation factor) can be influenced by the choice of parameter  $\kappa$  in gradient-elution chromatography, in contrast to isocratic elution chromatography where  $\alpha$  is constant if  $n_1 \approx n_2$  (ref. 1).

The exact solution of peak width in gradient-elution chromatography using an approach analogous to that for retention volume would lead to an integral whose solution would imply numerical or approximation methods resulting in rather complicated equations. Another, simplified solution was therefore preferred. It is assumed that the width of an elution peak in gradient-elution chromatography equals approximately that in isocratic elution chromatography using the mobile phase of the composition corresponding to the composition of the mobile phase that elutes the peak maximum in the work with a concentration gradient. This simplified assumption is illustrated by Fig. 1, showing an elution peak with retention volume  $V'_{R(g)}$  and width  $w_{(g)}$ . Curve a indicates the change in concentration of the more efficient eluting component in the mobile phase during the elution of the peak and the straight line b shows the concentration corresponding to the elution of the peak maximum,  $c_{(g)}$ . A symmetrical (Gaussian) peak shape is assumed and the areas on both sides of line b limited by curve a are assumed to be approximately equal. Then the broadening of the width on that side of the peak corresponding to lower concentration of the mobile phase will be (at least partially) compensated by the suppression of the peak width on the higher concentration side. This assumption will be more valid as the actual change in the concentration of the more efficient eluting component decreases in the mobile phase during the elution of the peak, *i.e.*, the narrower will be the peak.

If no gradient distortion occurs in the column, the mobile phase that elutes the maximum of a peak has the same composition as the solution at the outlet of the gradient-generating device at the moment when the total volume of the mobile phase delivered to the column from the beginning of the elution (the volume of the eluate) was

$$V = V_{R(g)} - V_m - V_z = V'_{R(g)} - V_z \quad (14)$$

Here,  $V_z$  is the volume of the connecting tubing between the outlet of the gradient-generating device and the top of the column.

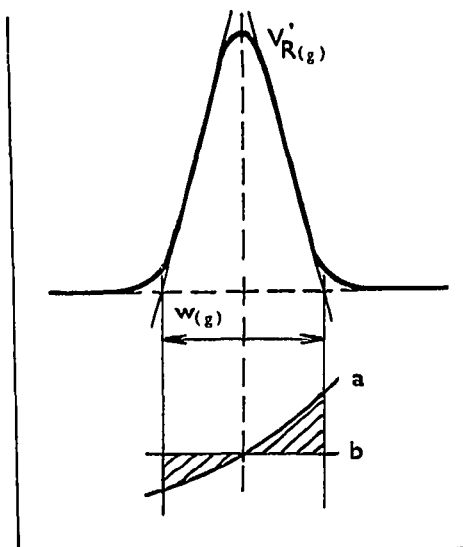


Fig. 1. Peak width in gradient-elution chromatography.  $V'_{R(g)}$  = Retention volume;  $w_{(g)}$  = peak width in gradient-elution chromatography. Curve a shows the change in concentration of the more efficient eluting component in the binary mobile phase during the course of the elution; the straight line b shows the concentration at the elution of the peak maximum,  $c_{(g)}$ .

The concentration of the more efficient component in the binary mobile phase eluting the peak maximum in gradient-elution chromatography,  $c_{(g)}$ , can be calculated by substituting eqn. 14 in eqn. 4.

$$c_{(g)} = [A^{\frac{1}{n}} + B \cdot (V'_{R(g)} - V_z)]^n \quad (15)$$

The retention volume of the sample compound in isocratic elution chromatography using the mobile phase containing the more efficient component in concentration  $c_{(g)}$  would have the value

$$V_R \approx V_m \cdot (k'_0 \cdot c_{(g)}^{-n} + 1) \quad (16)$$

According to the above assumptions, the peak width is approximately the same as in gradient-elution chromatography,  $w_{(g)}$ , and can be calculated by combining eqns. 15 and 16 with the defining relationship for plate number,  $N$ .

$$w_{(g)} \approx w = \frac{4 V_R}{\sqrt{N}} = \frac{4 V_m}{\sqrt{N}} \cdot \left\{ 1 + k'_0 \cdot [A^{\frac{1}{n}} + B \cdot (V'_{R(g)} - V_z)]^{-n \cdot n} \right\} \quad (17)$$

If a chromatogram obtained by gradient-elution chromatography is evaluated for plate number in the ordinary way, the plate number in gradient-elution chromatography,  $N_{(g)}$ , is obtained, which is related to the plate number in isocratic elution chromatography,  $N$ , by the following equation.

$$N_{(g)} = 16 \left( \frac{V'_{R(g)} + V_m}{W_{(g)}} \right)^2 \approx N \cdot \left( \frac{V'_{R(g)}}{V_m} + 1 \right)^2 \cdot \frac{1}{\left\{ 1 + k'_0 \cdot \left[ A^{\frac{1}{\alpha}} + B \cdot (V'_{R(g)} - V_z) \right]^{-\alpha \cdot n} \right\}^2} \quad (18)$$

The resolution ( $R_{s(g)}$ ) of the two sample compounds 1 and 2 in gradient-elution chromatography can be calculated by analogy with isocratic elution chromatography.

$$R_{s(g)} = \frac{2 \cdot (V'_{R(g)2} - V'_{R(g)1})}{W_{(g)2} + W_{(g)1}} \quad (19)$$

### *Combined elution using subsequent steps with different forms of the concentration-time function*

The derivation takes into consideration  $n$  steps with different mathematical functions describing the relationship between the concentration of the more efficient eluting component in the mobile phase and time (or volume of eluate). The sample compound is eluted in the  $n$ th step. If the concentration is changed continuously with time in each step, an equation analogous to eqn. 8 can be written for each step,  $i$ , corresponding to the total volume,  $V_i$ , of the eluate (mobile phase delivered by a gradient-generating device)

$$\int_0^{V_{mi}} dV_{mi} = \int_0^{V_i - V_{mi}} \frac{1}{k'_i} \cdot dV \quad (20)$$

Here  $V_{mi}$  is that part of the column void volume (volume of the mobile phase in the column,  $V_m$ ) through which the centre of the band of the sample compound migrated in step  $i$ .  $V_m$  represents the sum of all the  $V_{mi}$  partial contributions and the retention volume  $V_R$  is the sum of all volumes  $V_i$ .

$$\sum_{i=1}^n V_{mi} = V_m; \quad \sum_{i=1}^n V_i = V_R; \quad \sum_{i=1}^n (V_i - V_{mi}) = V'_R \quad (21-23)$$

The volumes of the mobile phase passing through the column in steps 1 to  $(n-1)$ ,  $V_1$  to  $V_{n-1}$ , are known and the volume of the eluate in the last step,  $V_n$ , can be calculated from the relationship

$$V_n = V_R - \sum_{i=1}^{n-1} V_i = V'_R - \sum_{i=1}^{n-1} V_i + V_m \quad (24)$$

This volume is of course dependent on the required value of the retention volume,  $V'_R$  and this value can be obtained by solving eqns. 20-24.

If a general concentration-volume function (eqn. 4) controls the form of the concentration gradient in each step, the value of the capacity ratio,  $k'_i$ , in each step depends on the concentration of the more efficient eluting component in the binary mobile phase according to the relationship

$$k'_i \approx k'_0 \cdot \left( A_i^{\frac{1}{\alpha_i}} + B_i \cdot V \right)^{-\alpha_i \cdot n} \quad (25)$$

where  $A_i$ ,  $B_i$  and  $\alpha_i$  are the values of the parameters  $A$ ,  $B$  and  $\alpha$  in step  $i$ .

A mathematical solution gives an equation for the partial contribution to the total retention volume,  $V'_R$ , in each step,  $i$  as

$$V_i - V_{mi} \approx \frac{1}{B_i} \cdot \left[ (\alpha_i \cdot n + 1) \cdot B_i \cdot k'_0 \cdot V_{mi} + A_i \frac{\alpha_i \cdot n + 1}{\alpha_i} \right] \frac{1}{\alpha_i \cdot n + 1} - \frac{A_i}{B_i} \frac{1}{\alpha_i} \quad (26)$$

$V'_R$  can be calculated as the sum of all the partial contributions

$$V'_R = \sum_{i=1}^n (V_i - V_{mi}) \approx \sum_{i=1}^n \left\{ \frac{1}{B_i} \cdot \left[ (\alpha_i \cdot n + 1) \cdot B_i \cdot k'_0 \cdot V_{mi} + A_i \frac{\alpha_i \cdot n + 1}{\alpha_i} \right] \frac{1}{\alpha_i \cdot n + 1} - \frac{A_i}{B_i} \frac{1}{\alpha_i} \right\} \quad (27)$$

The values of the partial contributions,  $V_{mi}$ , in eqn. 27 can be calculated from eqn. 26, using numerical methods and a derivation of the relationship for retention volume in the explicit form is not possible in this instance.

This solution, however, can be presented in two more special instances which should be useful in practical chromatographic operations.

*Chromatography using elution with a mobile phase of constant composition in each step (stepwise elution chromatography).* The capacity ratio of a sample compound is constant in each step  $i$  ( $k'_i = \text{const.}$ ) and a partial contribution  $V_i$  to the retention volume can be obtained from the relationships

$$V_i = V_{mi} \cdot (1 + k'_i); V_i - V_{mi} = V_i \cdot \frac{k'_i}{1 + k'_i} \quad (28, 29)$$

The retention volume of the sample compound is given as the sum of these partial contributions,

$$V'_R = \sum_{i=1}^{n-1} (V_i - V_{mi}) + V_n - V_{mn} = \sum_{i=1}^{n-1} V_i \cdot \frac{k'_i}{1 + k'_i} + (V'_R + V_m - \sum_{i=1}^{n-1} V_i) \cdot \frac{k'_n}{1 + k'_n} \quad (30)$$

and can be calculated as

$$V'_R = \sum_{i=1}^{n-1} V_i \cdot \left( k'_i \cdot \frac{1 + k'_n}{1 + k'_i} - k'_n \right) + V_m \cdot k'_n \quad (31)$$

The width of a peak can be obtained from the following equation

$$w \approx \frac{4 V_m}{\sqrt{N}} \cdot (k'_0 \cdot c_n^{-n} + 1) \quad (32)$$

assuming that the peak is eluted in one,  $n$ th step. Here,  $k'_n$  and  $c_n$  are the values of  $k'$  and  $c$  in step  $n$ .



Resolution and plate number can be calculated from the definition equations using relationships 31 and 32.

*Chromatography using elution with a mobile phase of constant composition in the first step followed by gradient elution in the second step.* The capacity ratio of a sample compound  $k'_1$  is constant in the first step and eqn. 29 for the contribution to retention volume can be used in the form

$$V_1 - V_{m1} = V_1 \cdot \frac{k'_1}{1 + k'_1} \quad (33)$$

The concentration of the more efficient eluting component in the binary mobile phase is changed during the course of the gradient-elution in the second step according to eqn. 4. The contribution to the retention volume in the second step can then be expressed as

$$V_2 - V_{m2} \approx \frac{1}{B} \cdot \left[ (\alpha \cdot n + 1) \cdot B \cdot k'_0 \cdot V_{m2} + A \frac{\alpha \cdot n + 1}{\alpha} \right] \frac{1}{\alpha \cdot n + 1} - \frac{A}{B} \frac{1}{\alpha} \quad (34)$$

The value of the contribution  $V_{m2}$  can be found from the relationship

$$V_{m2} = V_m - V_{m1} = V_m - \frac{V_1}{1 + k'_1} \quad (35)$$

The equation for the retention volume,  $V'_{R(\sigma)}$ , of the compound eluted in the second step is given as the sum of both contributions ( $V_1 - V_{m1}$ ) and ( $V_2 - V_{m2}$ ).

$$V'_{R(\sigma)} \approx \frac{V_1 \cdot k'_1}{1 + k'_1} + \frac{1}{B} \cdot \left[ (\alpha \cdot n + 1) \cdot B \cdot k'_0 \cdot \left( V_m - \frac{V_1}{1 + k'_1} \right) + A \frac{\alpha \cdot n + 1}{\alpha} \right] \frac{1}{\alpha \cdot n + 1} - \frac{A}{B} \frac{1}{\alpha} \quad (36)$$

The relationship for peak width can be derived analogously to simple gradient elution (eqn. 17), but the real contribution to retention volume in the second step must be considered instead of the term ( $V'_{R(\sigma)} - V_2$ ).

$$V'_{R(\sigma)} - V_1 + V_{m1} = V'_{R(\sigma)} - V_1 \cdot \frac{k'_1}{1 + k'_1} \quad (37)$$

With this correction, the following equation for peak width is obtained:

$$w_{(\sigma)} \approx \frac{4 \cdot V'_m}{\sqrt{N}} \cdot \left\{ 1 + k'_0 \cdot \left[ A \frac{1}{\alpha} + B \cdot \left( V'_{R(\sigma)} - V_1 \cdot \frac{k'_1}{1 + k'_1} \right) \right]^{-\alpha \cdot n} \right\} \quad (38)$$

The number of theoretical plates in this mode of elution,  $N_{(g)}$ , can be calculated using the relationship

$$N_{(g)} \approx N \cdot \left( \frac{V'_{R(g)}}{V_m} + 1 \right)^2 \cdot \frac{1}{\left\{ 1 + k'_0 \cdot \left[ A^{\frac{1}{\alpha}} + B \cdot \left( V'_{R(g)} - V_1 \cdot \frac{k'_1}{1 + k'_1} \right) \right]^{-\alpha \cdot n} \right\}^2} \quad (39)$$

and eqn. 19 can be used for calculation of the resolution,  $R_{s(g)}$ , of two sample compounds 1 and 2.

If the volume  $V_z$ , of the connecting tubing between the outlet of the gradient-generating device and the top of the column, cannot be neglected, this value must be added to the volume of the eluate in the first step,  $V_1$ , in the above calculations concerning both stepwise and combined two-step isocratic-gradient elution. In elution involving subsequent steps with different concentration gradient functions in each step, elution with a constant composition of the mobile phase in volume  $V_z$  of the eluate should be added as the first step.

#### PARTITION CHROMATOGRAPHY, INCLUDING SALTING-OUT CHROMATOGRAPHY ON ION-EXCHANGE COLUMNS

The present derivation is based on eqn. 2 which can be applied in partition chromatography.

The assumptions concerning the gradient-generating device are the same as in the derivation for adsorption and ion-exchange chromatography. The general mathematical form of the concentration gradient (eqns. 3 and 4) would introduce an integral whose solution would involve numerical methods, and therefore a derivation considering only the linear change in concentration of the more efficient component in the binary mobile phase is presented here. This change is expressed by the equation

$$c = A + B' \cdot t = A + B \cdot V \quad (40)$$

where  $t = V/u$  and the meaning of the constants  $A$ ,  $B'$  and  $B$  is the same as in eqns. 3 and 4.

The capacity ratio of a sample compound at the outlet of the gradient-generating device depends on the volume of the eluate,  $V$  (volume of the mobile phase delivered by the gradient-generating device on to the column at any moment), according to the relationship

$$k' \approx k'_0 \cdot 10^{-n \cdot c} \approx k'_0 \cdot 10^{-n \cdot (A + B \cdot V)} \quad (41)$$

Thus, analogous to eqns. 8 and 9, we can write

$$\int_0^{V_m} dV_m = \int_0^{V'_{R(g)}} \frac{1}{k'} \cdot dV \approx \frac{1}{k'_0} \cdot \int_0^{V'_{R(g)}} 10^{n \cdot (A + B \cdot V)} \cdot dV \quad (42)$$

The retention volume,  $V'_{R(\theta)}$ , in gradient-elution partition chromatography can be obtained from the solution of eqn. 42 as

$$V'_{R(\theta)} \approx \frac{1}{n \cdot B} \cdot \log(2.3 n \cdot B \cdot V_m \cdot k'_0 + e^{2.3 n \cdot A}) - \frac{A}{B} \quad (43)$$

or, if the concentration of the more efficient eluting component in the binary mobile phase equals zero at the beginning of the elution,  $A = c_0 = 0$  as

$$V'_{R(\theta)} \approx \frac{1}{n \cdot B} \cdot \log(2.3 n \cdot B \cdot V_m \cdot k'_0 + 1) \quad (44)$$

Eqns. 43 and 44 are very similar in form to the solution for retention volumes in gradient-elution adsorption chromatography with a linear change in the solvent strength parameter,  $\varepsilon^\circ$ , which was derived by Snyder<sup>2</sup>.

The retention ratio  $\alpha_{(\theta)}$  (separation factor) of two sample compounds 1 and 2 in gradient-elution partition chromatography is

$$\alpha_{(\theta)} \approx \frac{n_1 \cdot \log(2.3 n_2 \cdot B \cdot V_m \cdot k'_{02} + e^{2.3 n_2 \cdot A}) - A \cdot n_1 \cdot n_2}{n_2 \cdot \log(2.3 n_1 \cdot B \cdot V_m \cdot k'_{01} + e^{2.3 n_1 \cdot A}) - A \cdot n_1 \cdot n_2} \quad (45)$$

The peak width in gradient-elution partition chromatography can be derived using a similar approach as in gradient-elution adsorption and ion-exchange chromatography. The concentration of the more efficient eluting component in the binary mobile phase that elutes the maximum of the peak is

$$c_{(\theta)} = A + B \cdot (V'_{R(\theta)} - V_z) \quad (46)$$

and the retention volume of the sample compound in isocratic elution using a binary mobile phase containing the more efficient component at concentration  $c_{(\theta)}$  would be

$$V_R \approx V_m \cdot (k'_0 \cdot 10^{-n \cdot c_{(\theta)}} + 1) \quad (47)$$

It is assumed that the peak width in gradient-elution chromatography approximately equals the width of the peak with this retention volume:

$$w_{(\theta)} \approx w = \frac{4 V_R}{\sqrt{N}} \approx \frac{4 V_m}{\sqrt{N}} \cdot \left\{ k'_0 \cdot 10^{-n \cdot [A + B \cdot (V'_{R(\theta)} - V_z)]} + 1 \right\} \quad (48)$$

The value of plate number evaluated from a chromatogram obtained in gradient-elution partition chromatography,  $N_{(\theta)}$ , can be estimated using the relationship

$$N_{(\theta)} \approx N \cdot \left( \frac{V'_{R(\theta)}}{V_m} + 1 \right)^2 \cdot \frac{1}{\left\{ 1 + k'_0 \cdot 10^{-n \cdot [A + B \cdot (V'_{R(\theta)} - V_z)]} \right\}^2} \quad (49)$$

It is possible to derive equations for the retention volume and peak width in combined elution using subsequent steps with different forms of concentration gradient by analogy with adsorption and ion-exchange chromatography. If the concen-

tration of the more efficient eluting component in the mobile phase follows eqn. 40 in each step, the relationship between the capacity ratio of a sample compound in step  $i$ ,  $k'_i$ , and the volume of the eluate,  $V$ , can be expressed by

$$k'_i \approx k'_0 \cdot 10^{-n \cdot (A_i + B_i \cdot V)} \quad (50)$$

where  $A_i$  and  $B_i$  are the constants  $A$  and  $B$  in step  $i$ . Retention volume is then given by

$$V'_R = \sum_{i=1}^n (V_i - V_{mi}) \approx \sum_{i=1}^n \left[ \frac{1}{n \cdot B_i} \cdot \log(2.3 \cdot n \cdot B_i \cdot V_{mi} \cdot k'_0 + e^{2.3n \cdot A_i}) - \frac{A_i}{B_i} \right] \quad (51)$$

The partial contribution,  $V_{mi}$ , in each step  $i$  must be calculated from

$$V_i - V_{mi} \approx \frac{1}{n \cdot B_i} \cdot \log(2.3 \cdot n \cdot B_i \cdot V_{mi} \cdot k'_0 + e^{2.3n \cdot A_i}) - \frac{A_i}{B_i} \quad (52)$$

using numerical methods as in adsorption and ion-exchange chromatography.

In stepwise elution using a constant composition of the mobile phase in each step  $i$  the retention volume and peak width will satisfy

$$V'_R \approx \sum_{i=1}^{n-1} V_i \cdot \left( k'_0 \cdot 10^{-n \cdot c_i} \cdot \frac{1 + k'_0 \cdot 10^{-n \cdot c_n}}{1 + k'_0 \cdot 10^{-n \cdot c_i}} - k'_0 \cdot 10^{-n \cdot c_n} \right) + V_m \cdot k'_0 \cdot 10^{-n \cdot c_n} \quad (53)$$

and

$$w \approx \frac{4 V_m}{\sqrt{N}} \cdot (k'_0 \cdot 10^{-n \cdot c_n} + 1) \quad (54)$$

where  $c_i$  and  $c_n$  are the concentrations of the more efficient eluting component in the mobile phase for any step  $i$  and the last step  $n$ , respectively.

In two-step elution chromatography using isocratic elution in the first step followed by gradient elution in the second step (with a concentration gradient function given by eqn. 40) the retention volume and peak width of a sample compound eluted in the second step can be found from the following relationships:

$$V'_{R(2)} \approx \frac{V_1 \cdot k'_1}{1 + k'_1} + \frac{1}{n \cdot B} \cdot \log \left[ 2.3 \cdot n \cdot B \cdot \left( V_m - \frac{V_1}{1 + k'_1} \right) \cdot k'_0 + e^{2.3n \cdot A} \right] - \frac{A}{B} \quad (55)$$

$$w_{(2)} \approx \frac{4 V_m}{\sqrt{N}} \cdot \left\{ k'_0 \cdot 10^{-n \cdot [A + B \cdot (V'_{R(2)} - V_1 \cdot \frac{k'_1}{1 + k'_1})]} + 1 \right\} \quad (56)$$

Here,  $k'_1$  is the capacity ratio of the sample compound in the first, isocratic elution step with a total volume  $V_1$  of eluate. Other assumptions concerning eqns. 51–56 are the same as those made for adsorption and ion-exchange chromatography (eqns. 27–38).

## SYMBOLS

$A, B, B'$	parameters characterizing the shape of the concentration gradient
$A_i, B_i$	$A$ and $B$ in step $i$ ( $i = 1, \dots, n$ ) in combined elution
$N$	number of theoretical plates in a column
$N_{(g)}$	$N$ in gradient-elution chromatography
$R_s$	resolution of two compounds, 1 and 2
$R_{s(g)}$	resolution in gradient-elution chromatography
$V$	volume of eluate from the beginning of gradient elution
$V_i$	volume of eluate in step $i$ ( $i = 1, \dots, n$ ) in stepwise or combined elution chromatography with steps differing in concentration gradient function
$V_m$	total volume of the mobile phase in the column
$V_{mi}$	partial contribution to $V_m$ in step $i$
$V_R$	retention volume
$V'_R = V_R - V_m$	reduced retention volume
$V_{R(g)}, V'_{R(g)}$	$V_R$ and $V'_R$ in gradient-elution chromatography
$V_y$	$V$ at the end of gradient elution
$V_z$	volume of the connecting tubing between the outlet of the gradient-generating device and the top of the column
$c$	concentration of the more efficient eluting component in the binary mobile phase
$c_0, c_k$	$c$ at beginning and at end of gradient elution
$c_{(g)}$	$c$ at the elution of the peak maximum in gradient-elution chromatography
$k'$	capacity ratio
$k'_i$	$k'$ in step $i$ ( $i = 1, \dots, n$ )
$k'_0$	constant in eqns. 1 and 2, the capacity ratio of the sample compound in the mobile phase where $c = 1$ (eqn. 1) or $c = 0$ (eqn. 2)
$n$	exponential constant in eqns. 1 and 2
$t$	time
$u$	flow-rate of the mobile phase through the column
$w$	peak width
$w_{(g)}$	peak width in gradient-elution chromatography
$\alpha = k'_2/k'_1$	retention ratio (separation factor) of compounds 2 and 1
$\alpha_{(g)}$	$\alpha$ in gradient-elution chromatography
$\alpha_0$	$\alpha$ in the mobile phase where $c = 1$
$\epsilon^\circ$	solvent strength parameter
$\varkappa$	parameter characterizing the slope of the concentration gradient.

## REFERENCES

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