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GRADIENT OPTIMIZATION IN ELUTION LIQUID CHROMATOGRAPHY

II. THEORY OF MULTI-STEP ELUTION WITH A MOBILE PHASE OF CONSTANT COMPOSITION IN EACH STEP

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SUMMARY

Equations describing multi-step elution with a mobile phase of constant composition in each step have been derived. These equations are very useful for calculating the concentration-time function if the experimental relationships between the capacity factors and the concentration of the more efficient eluting component in the binary-solvent mobile phase are known for different sample compounds.

INTRODUCTION

In Part I¹, theoretical problems connected with evaluation of the concentration-time function for two-step elution with a mobile phase were considered and a method for calculating two-step concentration-time functions was proposed. In this method, the experimental relationships of the type capacity factor *versus* concentration of the more efficient eluting component in the binary-solvent mobile phase, measured for different pure components of the chromatographed sample, were applied. These experimental dependences may be presented in a linear form. Knowing the parameters that characterize these linear functions, the capacity factor can be calculated for an arbitrary composition of the mobile phase.

In this paper, the procedure for calculating the concentration-time function for multi-step elution in liquid chromatography is discussed. The theoretical discussion relates to elution with a mobile phase of constant composition in each step.

GENERAL CONSIDERATIONS

Theoretical problems connected with the determination of the resolution of multi-component mixture were discussed in Part I¹. It was shown that the efficiency of separation of a given multi-component mixture can be characterized by means of the resolutions of successive pairs of components. Assuming optimal resolutions for each

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pair of components of the mixture (for the interval 1–1.5 or 1–2.0), the composition of the mobile phase can be calculated.

Here, chromatography of an s -component mixture is discussed. Let A–B denotes the mobile phase, where B is the more efficient eluting solvent. Let us assume an r -step elution, *i.e.*, in the i th step the molar fraction (concentration) of B is x_i and at this concentration the components from $j_{i-1} + 1$ to j_i will be eluted; thus, $j_r = s$ (see Fig. 1). The fundamental assumption, which makes the change of the concentration x_B at the optimal moment possible, is as follows: mobile phase with a concentration x_i of solvent solvent B should be introduced into the column at such a time that its front reaches the end of the column just as the peak of the component j_{i-1} has been eluted, *i.e.*, at the time $t_{R(i, \dots, i-1) j_{i-1}} - t_{R0}$, where $t_{R(i, \dots, i-1) j_{i-1}}$ is the retention time for the last component, which is eluted in the $(i-1)$ th step and $t_{R0} = L/v$ (L is the length of the column and v is the linear velocity of the mobile phase). Hence this component migrated through the column at $i-1$ different concentrations of x_B . In such a model, we assume that the time of elution of a given peak can be neglected in comparison with the retention time, *i.e.*, the width of the peak is very small. Furthermore, small concentrations of the chromatographed compounds and symmetrical peaks are considered.

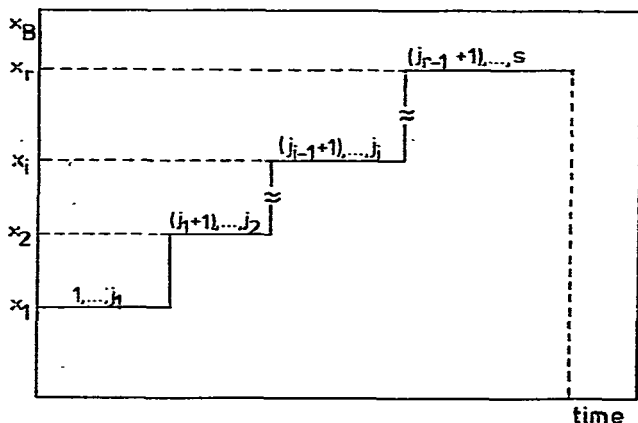


Fig. 1. Schematic diagram for r -step elution when an s -component mixture is separated.

EQUATIONS FOR RETENTION VOLUME AND RETENTION TIME IN STEPWISE ELUTION

In gradient elution chromatography, the reduced retention volume, $V'_{Rj} = V_{Rj} - V_m$ (V_{Rj} is the retention volume of the j th sample compound and V_m is the total volume of the mobile phase in the column) can be calculated by means of the following relationships²⁻⁴:

$$\int_0^{V_m} dV_m = \int_0^{V'_{Rj}} \frac{dV}{k'_j} \quad (1)$$

where k'_j is the capacity factor equal to the ratio of the total amount of the j th compound in the stationary phase to that in the mobile phase A-B under equilibrium conditions; k' is a function of the concentration of solvent B. For r -step elution with a mobile phase of constant composition in each step, eqn. 1 can be re-written as

$$V_m = \sum_{i=1}^{l-1} V'_{(i)j}/k'_{(i)j} + V'_{(l)j}/k'_{(l)j} \quad (2)$$

where

$$V'_{R(1,\dots,l)j} = \sum_{i=1}^{l-1} V'_{(i)} + V'_{(l)j} \quad (3)$$

for $l \leq r$; $V'_{(i)}$ is the volume of mobile phase of concentration x_i of $i > 1$; however, for $i = 1$ $V_{(1)} = V'_{(1)} + V_m$ and $k'_{(1)j}$ is the capacity factor of the j th component for mobile phase A-B with a concentration x_1 of solvent B. The volume $V'_{(i)}$ is proportional to the capacity factor $k_{(i)j}$:

$$V'_{(i)} = V_{m,j,j_i}^i \cdot k'_{(i)j} \quad (4)$$

for $j \geq j_i$ where V_{m,j,j_i}^i is the proportionality factor and j_i denotes the last component which is eluted in the i th step (see Fig. 1). $V_{(i)j}$ denotes the volume of mobile phase A-B with a concentration x_i in which the sample compound j migrated. Substituting eqn. 4 into eqn. 3, we obtain

$$V_{R(1,\dots,l)j} = V_m \left[1 + \sum_{i=1}^{l-1} \gamma_{j,j_i}^i \cdot k'_{(i)j} + \left(1 - \sum_{i=1}^{l-1} \gamma_{j,j_i}^i \right) k'_{(l)j} \right] \quad (5)$$

where

$$\gamma_{j,j_i}^i = \frac{V_{m,j,j_i}^i}{V_m} \quad (6)$$

for $i < l$ and $j > j_i$ and l is the last step of elution for the j th compound. Eqn. 5 can be re-written in the following form:

$$V_{R(1,\dots,l)j} = V_m (1 + k'_{(1,\dots,l)j}) \quad (7)$$

or

$$t_{R(1,\dots,l)j} = t_{R0} (1 + k'_{(1,\dots,l)j}) \quad (8)$$

where

$$k'_{(1,\dots,l)j} = \sum_{i=1}^{l-1} \gamma_{j,j_i}^i k'_{(i)j} + \left(1 - \sum_{i=1}^{l-1} \gamma_{j,j_i}^i \right) k'_{(l)j} \quad (9)$$

The symbol $k'_{(1,\dots,i)j}$ denotes the capacity factor of the j th sample compound, which migrated through the column with the mobile phase of different concentrations x_B from x_1 to x_i inclusive. The capacity factor $k'_{(1,\dots,i)j}$ is an average of the capacity factors $k_{(i)j}$ for $i = 1, 2, \dots, l$.

DETERMINATION OF THE PARAMETERS γ

For the purpose of calculating the parameter γ_{j,j_i}^i the volume V_{m,j,j_i}^i should be evaluated. Considering the definition of $V'_{(i)}$ (see eqn. 3) and eqn. 4, we obtain

$$V_{m,j,j_i}^i = \frac{V'_{(i)}}{k'_{(i)j}} = \frac{V_{R(1,\dots,i)j_i} - V_{R(1,\dots,i-1)j_{i-1}}}{k'_{(i)j}} \quad (10)$$

Substitution of eqns. 7 and 10 in eqn. 6 leads to

$$\gamma_{j,j_i}^i = \frac{k'_{(1,\dots,i)j_i} - k'_{(1,\dots,i-1)j_{i-1}}}{k'_{(i)j}} \quad (11)$$

$$\begin{aligned} & \frac{\sum_{p=1}^i \gamma_{j_{i-p}^p}^p k'_{(p)j_i} - \sum_{p=1}^{i-1} \gamma_{j_{i-1-p}^p}^p k'_{(p)j_{i-1}}}{k'_{(i)j}} \\ &= C + \frac{\gamma_{j_{i-i}^i}^i k'_{(i)j_i}}{k'_{(i)j}} \end{aligned}$$

where

$$C = \frac{1}{k'_{(i)j}} \sum_{p=1}^{i-1} \left[\gamma_{j_{i-p}^p}^p k'_{(p)j_i} - \gamma_{j_{i-1-p}^p}^p k'_{(p)j_{i-1}} \right] \quad (12)$$

It follows from eqn. 4 that

$$V'_{(p)}/V_m = \gamma_{j_{i-p}^p}^p k'_{(p)j_i} = \gamma_{j_{i-1-p}^p}^p k'_{(p)j_{i-1}} \quad (13)$$

for $p \leq i-1$. According to eqn. 13, $C = 0$. Then, from eqn. 11 we obtain

$$\gamma_{j,j_i}^i = \frac{\gamma_{j_{i-i}^i}^i k'_{(i)j_i}}{k'_{(i)j}} = \frac{k'_{(i)j_i}}{k'_{(i)j}} \left(1 - \sum_{p=1}^{i-1} \gamma_{j_{i-p}^p}^p \right) \quad (14)$$

Knowing the parameters $\gamma_{j,j_1}^k, \gamma_{j,j_2}^k, \dots, \gamma_{j,j_{i-1}}^k$, the capacity factor $k'_{(1, \dots, j)}$ can be calculated by means of eqn. 9. These parameters are as follows:

$$\gamma_{j,j_1}^k = \frac{k'_{(1)j_1}}{k'_{(1)j}} \quad (15)$$

$$\gamma_{j,j_2}^k = \frac{k'_{(2)j_2}}{k'_{(2)j}} \left(1 - \frac{k'_{(1)j_1}}{k'_{(1)j_2}}\right)$$

$$\gamma_{j,j_3}^k = \frac{k'_{(3)j_3}}{k'_{(3)j}} \left(1 - \frac{k'_{(1)j_1}}{k'_{(1)j_3}} - \frac{k'_{(2)j_2}}{k'_{(2)j_3}} \left[1 - \frac{k'_{(1)j_1}}{k'_{(1)j_2}}\right]\right)$$

... etc.

EVALUATION OF THE CONCENTRATION-TIME FUNCTION

As Part I¹, we applied the following equations for the capacity factors k'_j :

$$k'_j = a_j x^{-n_j} \quad (16a)$$

or

$$k'_j = b_j \cdot 10^{-m_j x} \quad (16b)$$

where a_j, n_j, b_j and m_j are constants, which can be calculated from the experimental values of k'_j presented in a linear form⁵⁻⁷.

The concentration of solvent B at which the elution of two arbitrary components guarantees the optimal resolution $R_{j+1,j}$ (for example, from 1.0 to 1.5) can be calculated from eqn. 16a or 16b and the following expression:

$$k'_{(1,2,\dots,j+1)} - k'_{(1,2,\dots,j)} = DR_{j+1,j} (k'_{(1,2,\dots,j+1)} + k'_{(1,2,\dots,j)} + 2) \quad (17)$$

where $D = 2/\sqrt{N}$, N is the total number of plates in the column, which is assumed to be independent of the type of compound and the composition of the mobile phase, and j and $j+1$ denote two arbitrary successive sample compounds.

The first step in the evaluation of the concentration-time function is the calculation of the first concentration, x_1 , at which sample compounds 1 and 2 can be eluted with optimal resolution, $R_{2,1}$. This concentration can be evaluated numerically from the following equations:

$$a_2 x_1^{-n_2} (1 - DR_{2,1}) - a_1 x_1^{-n_1} (1 + DR_{2,1}) = 2DR_{2,1} \quad (18a)$$

or

$$b_2 10^{-m_2 x_1} (1 - DR_{2,1}) - b_1 \cdot 10^{-m_1 x_1} (1 + DR_{2,1}) = 2DR_{2,1} \quad (18b)$$

It follows from Fig. 1 that the sample compounds from 1 to j_i will be eluted at the concentration x_i . The number of compounds that can be eluted at the concentration x_i is calculated so as to give optimal resolutions for two successive peaks.

The next step is the calculation of a new concentration, x_i , at which the sample compounds from $j_{i-1} + 1$ to j_i will be eluted. The concentration x_i can be evaluated from the following equation:

$$k'_{(1)j_{i-1}+1} = \left[\frac{k'_{(1,\dots,i-1)j_{i-1}} (1 + DR_{j_{i-1}+1,j_{i-1}}) + 2DR_{j_{i-1}+1,j_{i-1}}}{1 - DR_{j_{i-1}+1,j_{i-1}}} - \sum_{p=1}^{i-1} \gamma_{j_{i-1}+1,j_p}^p k'_{(p)j_{i-1}+1} \right] / \left(1 - \sum_{p=1}^{i-1} \gamma_{j_{i-1}+1,j_p}^p \right) \quad (19)$$

Substituting in eqn. 9 for $k_{(1)j_{i-1}+1}$, from eqns. 16a and 16b we obtain

$$x_i = \left\{ a_{j_{i-1}+1} \left(1 - \sum_{p=1}^{i-1} \gamma_{j_{i-1}+1,j_p}^p \right) / \left[\frac{k'_{(1,\dots,i-1)j_{i-1}} (1 + DR_{j_{i-1}+1,j_{i-1}}) + 2DR_{j_{i-1}+1,j_{i-1}}}{1 - DR_{j_{i-1}+1,j_{i-1}}} - \sum_{p=1}^{i-1} \gamma_{j_{i-1}+1,j_p}^p k'_{(p)j_{i-1}+1} \right] \right\}^{1/n_{j_{i-1}+1}} \quad (20a)$$

or

$$x_i = \frac{1}{m_{j_{i-1}+1}} \log \left\{ b_{j_{i-1}+1} \left(1 - \sum_{p=1}^{i-1} \gamma_{j_{i-1}+1,j_p}^p \right) / \left[\frac{k'_{(1,\dots,i-1)j_{i-1}} (1 + DR_{j_{i-1}+1,j_{i-1}}) + 2DR_{j_{i-1}+1,j_{i-1}}}{1 - DR_{j_{i-1}+1,j_{i-1}}} - \sum_{p=1}^{i-1} \gamma_{j_{i-1}+1,j_p}^p k'_{(p)j_{i-1}+1} \right] \right\} \quad (20b)$$

At a given concentration, x_i , of solvent B, the sample compounds from $j_{i-1} + 1$ to j_i should be eluted, if the following inequality is satisfied:

$$\frac{k'_{(1,\dots,i)p} (1 + DR_{p+1,p}^{\min}) + 2DR_{p+1,p}^{\min}}{1 - DR_{p+1,p}^{\min}} \leq k'_{(1,\dots,i)p+1} \leq \frac{k'_{(1,\dots,i)p} (1 + DR_{p+1,p}^{\max}) + 2DR_{p+1,p}^{\max}}{1 - DR_{p+1,p}^{\max}} \quad (21)$$

for $j_{i-1} + 1 \leq p \leq j_i - 1$, where R^{\min} and R^{\max} , the values for the interval (1.0, 2.0), can be assumed. This inequality can be presented in slightly different forms, which can be obtained from eqns. 21 and 16a or 16b (see Part I¹).

Knowing the concentrations x_i ($i = 1, 2, \dots, r$) in the successive steps of the elution and the time of these steps, the concentration-time function for an r -step elution can be presented in graphical form (in Part I¹, such a function for a two-step elution was calculated).

The elution time of the p -th compound, measured from the start of the analysis to the end of the p th peak, can be calculated as follows:

$$\begin{aligned} t_{(1, \dots, i)p} &= t_{R(1, \dots, i)p} + DR_{p+1, p} t_{R(i)p} \\ &\approx t_{R(1, \dots, i)p} (1 + DR_{p+1, p}) = t_{R0} (1 + k'_{(1, \dots, i)p}) (1 + DR_{p+1, p}) \\ &= t_{R0} G_{(1, \dots, i)p} \end{aligned} \quad (22)$$

for $j_{i-1} + 1 \leq p \leq j_i - 1$. It follows from eqn. 22 that the sample component p is eluted at the concentration x_i . The time t_{i+1} when mobile phase with a concentration x_{i+1} of solvent B enters the column is

$$t_{(i+1)}^{\text{in}} = t_{(1, 2, \dots, i)j_i} - t_{R0} = t_{R0} [G_{(1, 2, \dots, i)j_i} - 1] \quad (23)$$

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SYMBOLS

A	less efficient eluting component in the binary-solvent mobile phase;
B	more efficient eluting component in the binary-solvent mobile phase;
D	parameter equal to $2\sqrt{N}$;
L	length of the column;
N	number of theoretical plates in the column;
$R_{j+1, j}$	resolution of two compounds, j and $j+1$;
V_m	total volume of the mobile phase in the column;
$V_{R(1, \dots, i)j}$	retention volume of the j th sample compound, which migrated through the column with mobile phase having different concentrations, x_B , from x_i to x_i ;
V'_R	reduced retention volume for the j th sample compound;
$V'_{(i)}$	total volume of the mobile phase of concentration x_i of $i > 1$;
$V'_{(i)j}$	volume of mobile phase A-B of concentration x_i for the j th sample compound, where $j_{i-1} < j < j_i$;
$V^l_{m, j, i}$	proportionality factor in eqn. 4;
a, b	constants in eqns. 16a and 16b;

$k'_{(i)j}$	capacity factor of the j th component for mobile phase A–B with a concentration x_i of solvent B;
$k'_{(1,\dots,i)j}$	average capacity factor of the j th sample compound, which migrated through the column with mobile phase having different concentrations, x_B , from x_1 to x_i (see eqn. 9);
m	constant in eqn. 16b;
n	exponent in eqn. 16a;
r	total number of elution steps;
s	number of sample compounds;
t_{R_0}	equal to L/v ;
t_R	retention time of the j th compound;
$t_{(1,\dots,i)j}$	elution time of the j th compound, measured from the start of analysis to the end of the j th peak;
$\gamma_{j,i}^t$	parameter defined in eqn. 14;
v	linear velocity of the mobile phase.

Subscripts

$(i)j$	refers to j th compound migrating through the column with mobile phase of composition x_i ;
$(1,\dots,i)j$	refers to j th compound migrating through the column with mobile phase having different compositions from x_1 to x_i ;
j_i	last sample compound eluted at concentration x_i ;
p	summation index in eqns. 11–13, 19 and 20 or a component in the range $\langle j_{i-1} + 1, j_i - 1 \rangle$.

Superscript

i	refers to mobile phase of concentration x_i .
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