Journal of Chromatography, 153 (1978) 309–319

CHROM. 10,755

GRADIENT OPTIMIZATION IN ELUTION LIQUID CHROMATOGRAPHY

I. THEORETICAL CONSIDERATIONS CONNECTED WITH EVALUATION OF THE CONCENTRATION–TIME FUNCTION FOR STEPWISE ELUTION

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(First received June 13th, 1977; revised manuscript received November 18th, 1977)

SUMMARY

Using the theoretical treatment of Jandera and Churáček, problems connected with evaluation of the concentration–time function are discussed. It is shown that the linear relationships of the type capacity factor *versus* concentration of the more efficient eluting component in the binary-solvent mobile phase, measured for different chromatographed compounds, can be used to predict the concentration–time function for stepwise elution with a mobile phase of constant composition in each step.

INTRODUCTION

Liteanu and Gocan wrote[1], "Gradient chromatography has developed in the general context of evolution of chromatography and is in full progress owing to the possibility of automation in the programming of certain parameters". The use of gradients is a basic means of optimizing the process of chromatographic separation, *i.e.*, achievement of the best resolution in as short a time as possible. Of many known types of gradients, the greatest experimental possibilities are offered by mobile phase gradients.

The theory of isocratic and gradient elution chromatography has been discussed by many workers[1–5]. The most advanced studies, both theoretical and experimental, have been carried out by Snyder[2] on adsorption liquid chromatography. Jandera and Churáček[6,7], using the fundamental relationships of Snyder defining distribution coefficients in adsorption chromatography for single- and binary-solvent mobile phases, derived theoretically the relationship between the capacity factor and the concentration of the more efficient eluting component in a binary-solvent mobile phase. A simplified version of this relationship is

$$\log k'_{(AB)i} = \log a_i - n_i \log x_B \qquad (1)$$

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where $k'_{(AB)i}$ is the capacity ratio for the ith component of the mixture in the binary-solvent mobile phase A–B, x_B is the molar fraction (the term "concentration" will subsequently be used) of the more efficient eluting component B in the binary-solvent mobile phase A–B and a_i and n_i are constants. It has also been shown[6] that eqn. 1 is valid for ion-exchange chromatography. However, for partition mechanisms (liquid–liquid chromatography, salting-out chromatography and solubilization chromatography on ion exchangers in mixed aqueous–organic media), a slightly different relationship has been derived[6]:

$$\log k'_{(AB)i} = \log b_i - m_i x_B \tag{2}$$

where b_i and m_i are constants. The constants a_i, n_i and b_i, m_i can either be calculated theoretically by means of the parameters that characterize the sample being chromatographed, the components of the mobile phase and the stationary phase, or can be determined directly from experimental data.

Eqns. 1 and 2, derived theoretically by Jandera and Churáček[6], have been obtained experimentally by several workers[8–11]. The systematic experimental studies of Bieganowska and Soczewiński[12] showed that the classification of chromatographic systems, as suggested by Jandera and Churáček[6], is not sufficiently accurate. The experimental dependences of $\log k'_{(AB)i}$ *versus* $\log x_B$ and $\log k'_{(AB)i}$ *versus* x_B, published in the present literature, have been summarized[12] and on this basis the types of chromatographic systems to which eqns. 1 and 2 apply have been distinguished.

Eqns. 1 and 2 describe satisfactorily a large number of experimental relationships between the capacity ratio and the concentration of component B. Hence the functions $\log k'_{(AB)i}$ *versus* $\log x_B$ and $\log k'_{(AB)i}$ *versus* x_B, measured for different compounds, may be very useful in evaluating the optimal concentration–time function for the separation of a mixture. In this paper, we discuss the problems connected with the numerical evaluation of the concentration–time functions in chromatography using elution with a mobile phase (binary-solvent mixture) with a constant composition in each step (stepwise elution chromatography). Such a type of gradient (stepwise function) is more effective than a continuous function in many chromatographic separations[13,14].

EQUATIONS CHARACTERIZING OPTIMAL SEPARATION OF THE CHROMATOGRAPHED SAMPLE

The theoretical determination of resolution for a multi-component mixture is difficult[3,15]. In order to describe the efficiency of separation fully, the resolution for each successive pair of compounds, i and $i+1$, must be calculated. If the resolution of each such pair were at least greater than unity, then the separation of the given mixture would be good. Another problem is the time of analysis. It may happen that the resolution is very high for a pair of compounds i and $i+1$ which indicates a large distance between the maxima of their chromatographic peaks, and consequently a considerable increase in the time of analysis. The optimal time for the separation of a multi-component mixture can be obtained if the resolutions of consecutive pairs of components[3] are the numbers from the interval (1, 1.5). Taking into consideration

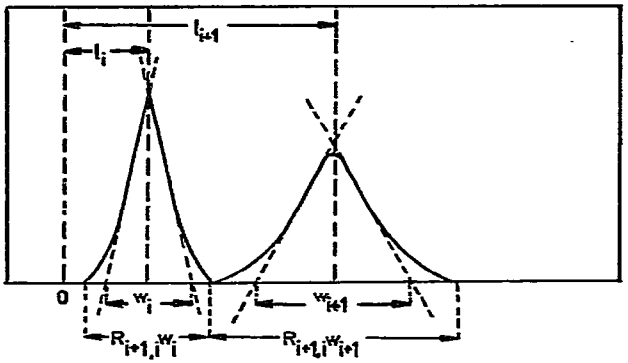

Fig. 1. Schematic diagram for eqn. 3; for $R_{i+1,i}$ the value 1.5 is assumed.

these two aspects (good resolution and a short time of analysis), a condition can be written that should be satisfied for two successive symmetrical peaks:

$$\Delta l_{i+1,i} = l_{i+1} - l_i = 0.5\, R_{i+1,i}\,(w_i + w_{i+1}) \tag{3}$$

where l_i is the distance of the peak maximum from the start for the ith component, w_i is the peak width for the ith component and $R_{i+1,i}$ is the optimal resolution for two successive peaks i and $i+1$. The optimal resolution, $R_{i+1,i}$, should be chosen so that the distances $R_{i+1,i}\cdot w_i$ and $R_{i+1,i}\cdot w_{i+1}$ are the maximal widths of the peaks of i and $i+1$, respectively (Fig. 1). Eqn. 3 can be re-written in a slightly different form:

$$\Delta V_{R_{i+1,i}} = V_{R_{i+1}} - V_{R_i} = D\, R_{i+1,i}\,(V_{R_i} + V_{R_{i+1}}) \tag{4}$$

where $D = 2/\sqrt{N}$, V_{R_i} is the retention volume of the ith component in the mixture and N is the total number of plates in the column. N is assumed to be independent of the type of compound and the composition of the mobile phase. Expressing the retention volume by means of the free volume of the column, V_m, and the capacity factor, k_i':

$$V_{R_i} = V_m\,(1 + k_i') \tag{5}$$

from eqn. 4 we obtain:

$$\Delta k'_{i+1,i} = k'_{i+1} - k'_i = D\, R_{i+1,i}\,(k'_i + k'_{i+1} + 2) \tag{6}$$

where k_i' and k_{i+1}' denote the capacity factors of the ith and $(i+1)$th component, respectively, and refer to the binary-solvent mobile phase A–B, *i.e.*, $k_i' = k'_{(AB)i}$ and $k_{i+1}' = k'_{(AB)i+1}$.

Now, we shall show the usefulness of eqn. 6 in the determination of the concentration–time function for two-step elution chromatography. Eqns. 1 and 2 will be used.

Firstly, we shall discuss eqn. 1. If we wish to use the concentration–time function to perform the separation of a given mixture, it is essential to know the concentration of the solvent B at which the elution of the first two components guarantees a resolution $R_{2,1}$ within the range 1–1.5. This concentration can be defined numerically from the equation that is obtained on substituting eqn. 1 in eqn. 6:

$$a_2 x_1^{-n_2}(1 - D R_{2,1}) - a_1 x_1^{-n_1}(1 + D R_{2,1}) = 2 D R_{2,1} \tag{7}$$

where x_1 is the molar fraction at which components 1 and 2 are eluted. When $n_1 = n_2$, eqn. 7 has an analytical solution[6]:

$$x_1 = \left(\frac{a_2}{2}\right)^{\frac{1}{n_1}} \cdot \left(\frac{\alpha_{2,1} - 1}{D R_{2,1} \alpha_{2,1}} - \frac{\alpha_{2,1} + 1}{\alpha_{2,1}}\right)^{\frac{1}{n_1}} \tag{8}$$

where

$$\alpha_{2,1} = a_2/a_1 \tag{9}$$

The next step is often necessary in order to shorten the retention time of a component, *i.e.*, to approximate the peak of component $i+1$ to that of component i. This effect can be achieved by increasing the concentration of the solvent B directly after elution of component i. The concentration at which component $i+1$ should be eluted can be calculated from a modified eqn. 6 and eqn. 1. For this purpose, eqn. 6 should be re-written in the form

$$k'_{i+1} - k'_i = D R_{i+1,i}(k'_i + \bar{k}'_{i+1} + 2) \tag{10}$$

where $\bar{k}'_{i+1}$ is an average capacity factor for the $(i+1)$th component which was initially eluting at the concentration x_1 and subsequently at the concentration x_2.

Let us consider migration of component $i+1$ through a chromatographic column of length L (see Fig. 2b). The elution time of the component $i+1$ is given by

$$\bar{t}_{R_{i+1}} = \gamma_{i+1,i}\, t_{R(1)i+1} + (1 - \gamma_{i+1,i})\, t_{R(2)i+1} \tag{11}$$

where

$$\gamma_{i+1,i} = \frac{L_{(1)i+1}}{L} \tag{12}$$

$t_{R_{(1)i+1}}$ and $t_{R_{(2)i+1}}$ are the retention times of component $i+1$ at concentrations x_1 and x_2, respectively and $\bar{t}_{R_{i+1}}$ is the time of elution of component $i+1$ corresponding to the capacity factor $\bar{k}'_{i+1}$. Substituting into eqn. 11 the fundamental relationship

$$k'_i = \frac{t_{R_i} - t_{R_0}}{t_{R_0}} \tag{13}$$

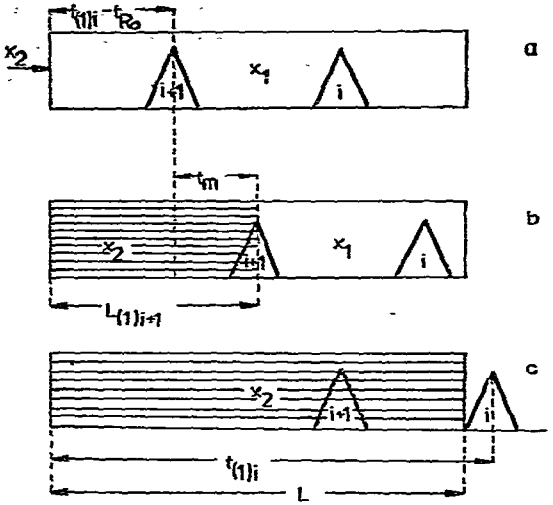

Fig. 2. Illustration of migration of components i and $i+1$ in solvent programming.

we obtain

$$t_{R_{t+1}} = t_{R_0}(1 + \bar{k}'_{t+1}) \tag{14}$$

where

$$\bar{k}'_{t+1} = \gamma_{t+1,t}\, k'_{(1)t+1} + (1 - \gamma_{t+1,t})\, k'_{(2)t+1} \tag{15}$$

In eqns. 13–15, $k'_{(1)i+1}$ and $k'_{(2)t+1}$ denote the capacity factors for the component $i+1$ at the concentrations x_1 and x_2, respectively; $t_{R_0} = L/v$, where v is the linear velocity of the mobile phase. Combination of eqns. 10 and 15 leads to

$$k'_{(2)t+1} = \frac{k'_{(1)t}(1 + D\,R_{t+1,t}) - \gamma_{t+1,t}\, k'_{(1)t+1}(1 - D\,R_{t+1,t}) + 2\,D\,R_{t+1,t}}{(1 - \gamma_{t+1,t})(1 - D\,R_{t+1,t})} \tag{16}$$

Substituting eqn. 1 into eqn. 16 and taking into account the definition of $\gamma_{t+1,t}$ (see eqn. 21), we obtain the expression for the concentration x_2 at which component $i+1$ should be eluted:

$$x_2 = \left[\frac{a_{t+1}(1 - \gamma^a_{t+1,t})(1 - D\,R_{t+1,t})}{2\,D\,R_{t+1,t}(1 + k'_{(1)t})}\right]^{\frac{1}{n_{t+1}}}$$

$$= \left[\frac{a_{t+1}(1 - \gamma^a_{t+1,t})(1 - D\,R_{t+1,t})}{2\,D\,R_{t+1,t}(1 + a_t\, x_1^{-n_t})}\right]^{\frac{1}{n_{t+1}}} \tag{17}$$

Now we derive an expression for the parameter $\gamma^a_{t+1,t}$, for which purpose the distance $L_{(1)t+1}$ should be calculated. Let us consider the migration of components i

and $i+1$ through the chromatographic column (see Fig. 2). Solvent of concentration x_2 should be introduced into the column at such a time that its front will reach the end of column just as the peak of component i has been eluted completely, *i.e.*, at the time $t_{(1)i} - t_{R_0}$. Hence the time of migration of component $i+1$ at concentration x_1, $t^*_{(1)i+1}$, is given by

$$t^*_{(1)i+1} = t_{(1)i} - t_{R0} + t_m$$

$$= \frac{1}{\vartheta}\left(1 + \frac{1}{k'_{(1)i+1}}\right)\left[(L + 0.5\, w_{(1)i})\, k'_{(1)i} + 0.5\, w_{(1)i}\right] \tag{18}$$

The distance $L_{(1)i+1}$ can be calculated from the expression

$$L_{(1)i+1} = t^*_{(1)i+1}\left[\frac{\vartheta}{1 + k'_{(1)i+1}}\right] \tag{19}$$

From eqns. 19, 18 and 12, we have

$$\gamma_{i+1,i} = \frac{1}{k'_{(1)i+1}} \cdot \left[\frac{0.5\, w_{(1)i}}{L} + k'_{(1)i}\left(1 + \frac{0.5\, w_{(1)i}}{L}\right)\right] \tag{20}$$

If $L \gg w_{(1)i}$, this expression can be reduced to

$$\gamma_{i+1,i} = \frac{k'_{(1)i}}{k'_{(1)i+1}} \tag{21}$$

Substituting eqn. 1 into eqn. 20, we obtain an exact numerical value of the parameter $\gamma_{i+1,i}$, which is necessary for calculating the concentration x_2 (see eqn. 17). A very simple expression for $\gamma_{i+1,i}$ can be obtained from eqns. 21 and 1:

$$\gamma^a_{i+1,i} = \frac{a_{(1)i}}{a_{(1)i+1}} \cdot x_1^{n_{i+1} - n_i} = \frac{1}{\alpha_{i+1,i}} \cdot x_1^{n_{i+1} - n_i} \tag{22}$$

For chromatographic systems that satisfy eqn. 2, the following equations are analogous to eqns. 7, 8, 17 and 22:

$$b_2\, 10^{-m_2 x_1}(1 - D\, R_{2,1}) - b_1\, 10^{-m_1 x_1}(1 + D\, R_{2,1}) = 2\, D\, R_{2,1} \tag{23}$$

$$x_1 = \frac{1}{m_1} \cdot \log\left[\frac{b_2}{2}\left(\frac{\beta_{2,1} - 1}{D\, R_{2,1}\, \beta_{2,1}} - \frac{\beta_{2,1} + 1}{\beta_{2,1}}\right)\right] \tag{24}$$

for $m_1 = m_2$

$$x_2 = \frac{1}{m_{i+1}} \cdot \log\left[\frac{b_{i+1}(1 - \gamma^b_{i+1,i})(1 - D\, R_{i+1,i})}{2\, D\, R_{i+1,i}(1 + b_i\, 10^{-m_i x_1})}\right] \tag{25}$$

where

$$\beta_{i+1,i} = b_{i+1}/b_i \tag{26}$$

and

$$\gamma^b_{i+1,i} = \frac{b_i}{b_{i+1}} \cdot 10^{(m_{i+1}-m_i)x_1} = \frac{1}{\beta_{i+1,i}} \cdot 10^{(m_{i+1}-m_i)x_1} \tag{27}$$

The parameter $\gamma^b_{i+1,i}$ is evaluated from eqns. 2 and 21. Eqn. 24 has been also proposed by Jandera and Churáček[6].

EVALUATION OF THE CONCENTRATION–TIME FUNCTION

Let us now discuss the chromatographic separation of an n-component mixture. Assume that we know the relationships between the capacity factors and the concentration of solvent B in the mixture A–B for all components contained in the chromatographed sample. If the chromatographic system satisfies eqn. 1, then eqns. 7, 8, 17 and 22 are useful for programming the gradient. Eqns. 23–27 are used for programming the gradient in chromatographic systems that satisfy eqn. 2. For the sake of illustration, we shall discuss eqns. 7, 8, 17 and 22. Let the sequence of elution of the components be

$$l_1 < l_2 < \ldots < l_i < l_{i+1} < \ldots < l_n \tag{28}$$

Series 28 is equivalent to the following series of capacity factors:

$$k'_1 < k'_2 < \ldots < k'_i < k'_{i+1} < \ldots < k'_n \tag{29}$$

However, for the determined concentration of solvent B, if $n_1 = n_2 = \ldots = n_n$, the following inequality is satisfied:

$$a_1 < a_2 < \ldots < a_i < a_{i+1} < \ldots a_n \tag{30}$$

For the sake of simplification, let us consider a mixture of compounds for which $n_i = 1$ (from numerous experimental studies it appears that for many compounds n_i is close or equal to unity[16]). In Fig. 3, the capacity factor (k^1_i) *versus* molar fraction of solvent B, *i.e.*, $k^1_i = a_i/x$, are presented for different values of a_i. The initial concentration, x_1, at which compounds 1 and 2 should be eluted is calculated from eqn. 8, assuming $R_{2,1} = 1$. In order to obtain the resolution for the interval (1, 1.5) during separation of compounds i and $i+1$ (where $i \geqslant 2$), the distance between the curves i and $i+1$ at the point $x = x_1$ should satisfy the inequality

$$D[(a_i + a_{i+1})/x_1 + 2] \leqslant a_{i+1,i} \leqslant 1.5\, D[(a_i + a_{i+1})/x_1 + 2] \tag{31}$$

for $i = 1, 2, 3, \ldots$ This inequality is equivalent to

$$\frac{a_i(1 + D) + 2\,Dx_1}{1 - D} \leqslant a_{i+1} \leqslant \frac{a_i(1 + 1.5\,D) + 3\,Dx_1}{1 - 1.5\,D} \tag{32}$$

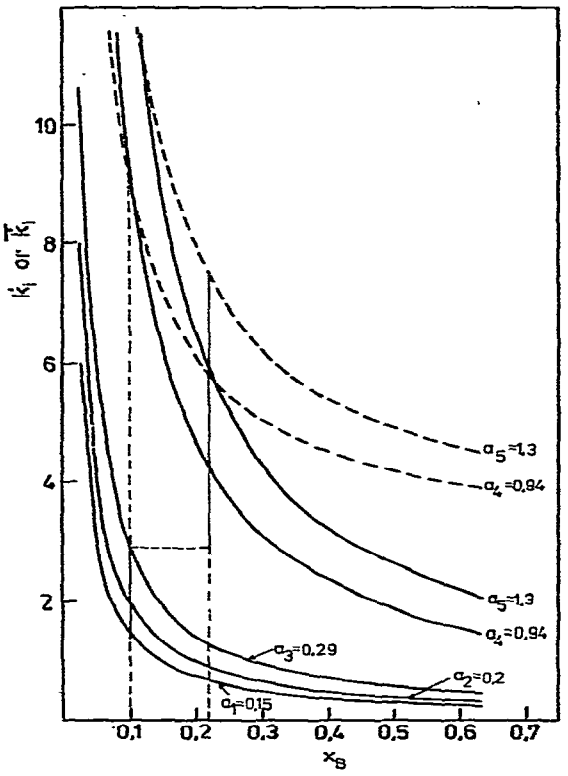

Fig. 3. Dependences between the capacity factor and the molar fraction of the more efficient eluting component calculated for different values of a_i. The solid lines denote the functions $k' = a_i/x$ (i = 1,2,3,4,5) and the broken lines denote the functions $\bar{k}'_j = k'_{1)3} + (1 - \gamma_{j,3})\, a_j/x$ (i = 4,5). The subscripts i and j denote a given component of the chromatographed sample.

for $i = 1, 2, 3, \ldots$ If the distances between the successive curves at the point $x = x_1$, beginning from $i \geqslant 2$, satisfy inequality 31, then these compounds will be separated at concentration x_1. From Fig. 3, it appears that inequality 31 is satisfied by compounds from 1 to 3 inclusive. The distance between the curves 3 and 4 at the point x_1 already exceeds the upper limit of the distance calculated for a resolution of 1.5. Hence the concentration x_2 at which component 4 will be eluted should be determined. This concentration is determined by means of eqn. 17, assuming the optimal value of the resolution $R_{4,3}$, which should be greater than unity but approximate to unity if the concentration x_2 increases. The capacity ratio of each successive peak j, for $j \geqslant 4$, is given by the equation

$$\bar{k}'_j = \gamma^a_{j,3}\, k'_{(1)j} + (1 - \gamma^a_{j,3})\, k'_{(2)j}$$

$$= k'_{(1)3} + \left[1 - \frac{k'_{(1)3}}{k'_{(1)j}}\right] a_j\, x_2^{-n_j} \qquad (33)$$

for $j \geqslant 4$. In Fig. 3, the broken lines present the functions $\bar{k}'_j$ *versus* x_2 for $j \geqslant 4$ and $n_j = 1$.

In order to obtain the resolution for the interval (1, 1.5) for components with $j \geqslant 4$, the distances $d_{j+1,j}$ between the curves j and $j+1$ at the point $x = x_2$ should be in the following range:

$$D\,(\bar{k}'_j + \bar{k}'_{j+1} + 2) \leqslant d_{j+1,j} \leqslant 1.5\,D\,(\bar{k}'_j + \bar{k}'_{j+1} + 2) \tag{34}$$

$j = 4,5, \ldots$ From eqn. 34, an interesting inequality can be deduced:

$$\frac{\bar{k}'_j\,(1 + D) + 2\,D}{1 - D} \leqslant \bar{k}'_{j+1} \leqslant \frac{\bar{k}'_j\,(1 + 1.5\,D) + 3\,D}{1 - 1.5\,D} \tag{35}$$

or

$$\left[\frac{\bar{k}'_j\,(1 + D) + 2\,D}{1 - D} - k'_{(1)3}\right] \frac{x_2}{1 - \gamma^a_{j+1,3}} \leqslant a_{j+1} \leqslant$$

$$\leqslant \left[\frac{\bar{k}'_j\,(1 + 1.5\,D) + 3\,D}{1 - 1.5\,D} - k'_{(1)3}\right] \frac{x_2}{1 - \gamma^a_{j+1,3}} \tag{36}$$

The latter inequality is especially important in determining the resolutions for further pairs of components. From eqn. 36, the boundary parameters a_{j+1} ($j \geqslant 4$) are obtained, which guarantee the resolution of further compounds for the interval (1, 1.5). From Fig. 3, it follows that at concentration x_2 component 5 can be eluted. Proceeding in this way, elution concentrations can be found for other sample components. Knowing these concentrations as well as parameters a and n, the elution time (from the start to the end of the peak) for individual components can be calculated. They are

$$t_{(1)i} = t_{R(1)i} + D\,R_{i+1,i}\,t_{R(1)i} = t_{R_0}\,(1 + k'_{(1)i})\,(1 + D\,R_{i+1,i}) = t_{R_0}\,G_{(1)i} \tag{37}$$

for $i = 1, 2, 3$ and (see Appendix)

$$t_{(1,2)j} = t_{R(1,2)j} + D\,R_{j+1,j}\,t_{R(2)j}$$

$$\approx t_{R_0}\,(1 + \bar{k}'_j)\,(1 + D\,R_{j+1,j}) = t_{R_0}\,G_{(1,2)j} \tag{38}$$

Thus, the concentration–time function presented in Fig. 4 corresponds to Fig. 3. In the above discussion, we assumed the resolutions for successive pairs of the components at a given concentration for the interval (1, 1.5). The upper limit of this interval may be higher than 1.5, depending on the particular mixture being chromatographed.

The evaluation of the optimal concentration–time function may be difficult for many real chromatographic systems. Then, the position of successive peaks can

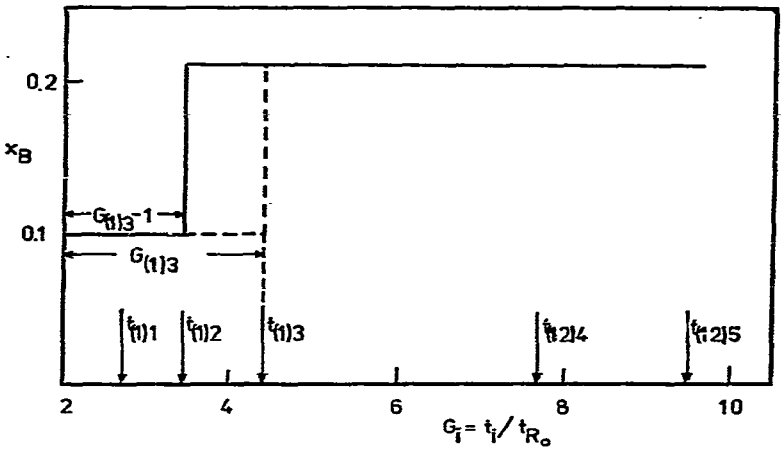

Fig. 4. Two-step gradient corresponding to Fig. 3.

be regulated by changing the concentration x or by changing the solvents in the mobile phase.

APPENDIX

Eqn. 38 defines approximately the elution time $t_{(1,2)j}$, *i.e.*, the time from the start to the end of the jth peak, for two-step elution. The first term of this equation denotes the retention time of the jth component, *i.e.*, the time from the start to the peak maximum, and it is defined by means of the average capacity factor, $\bar{k}'_j$. The other term in eqn. 38 defines approximately half of the peak width for the jth component and it is usually small in comparison with the first term. Although the jth peak is formed during all steps of elution, it is formed mainly in the last step. Therefore, the peak width for stepwise elution may be equal to or greater than that for isocratic elution at concentration x_2. Thus, the second term in eqn. 38 can be determined by means of $t_{R(2)j}$ or $t_{R(1,2)j}$.

ACKNOWLEDGEMENTS

We thank Professor E. Soczewiński and Dr. W. Gołkiewicz (Department of Inorganic and Analytical Chemistry, Medical Academy, Lublin, Poland) and Dr. A. Stołyhwo (Polytechnic School, Gdańsk, Poland) for many helpful discussions. This research was supported by the Polish Academy of Sciences, problem No. 03.10.6.03.03.

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