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EFFECT OF A MIXER ON THE COURSE OF GRADIENT ELUTION AND METHODS FOR ITS ELIMINATION

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SUMMARY

The effect of a mixer in the gradient elution device on the variations in the concentration of eluents has been studied. The functions that characterize real concentration changes have been derived and compared with those describing programmed changes in concentration. On this basis the magnitudes of errors have been determined as a function of the mixer volume and the flow-rate. A method has been developed that permits one to correct the functions programming the operations of gradient elution devices containing mixers in such a manner as actually to obtain eluents of programmed composition. The correctness of the derived relationships has been verified experimentally.

INTRODUCTION

The composition of a mobile phase can be adjusted by three basic methods: (1) the inflowing solvent is mixed with the solvent in the tank and the tank contents are pumped simultaneously into the chromatographic column[1-5]; (2) each of the mobile phase components is pumped through a separate displacement pump of suitable capacity[6]; or (3) the proportions of the mobile phase components are determined by use of proportioning valves[6-11] and pumped with a single pump. The most promising is the third method, owing to its accuracy and low cost. Mixing of liquids after the proportioning valve is necessary in this method owing to fluctuations in concentration resulting from the operation of the valve. Mixing may be accomplished in a pump (e.g., in Varian devices), Alternatively, a mixing device is frequently employed for this purpose.

The presence of an inert mixer in a chromatographic system leads to distortion of concentration changes of the mobile phase with respect to those programmed. The distortions will depend on the operating conditions of a device such as (3) mentioned above[12]. However, the relationships derived are of little practical value as they are implicit functions which can be used for calculations only when a digital computer is used.

This paper describes a method for eliminating distortions introduced by a mixer through modifications of the changes in concentration of the mobile phase. When

considering the manner of generation of concentration changes or positioning the valve relative to the pump (on the suction or the forcing side), it is important that the mixer is positioned after the gradient-generating system. So far, it has been neglected in practice that chromatograms have been developed using gradients other than those programmed. This led to lower precision of the results when using different devices (various mixer volumes) or even with the same device but with various volume flow-rates. Of course, correction of the concentration gradient programme eliminates only the type of errors described above. Other sources of errors, such as concentration fluctuations due to valve operation, the effect of synchronization of the pump with the operation of the valve[8,9]. mixing in the pump, contraction and compressibility remain.

THEORETICAL

A schematic diagram of a mixer with the parameters used in further considerations is shown in Fig. 1.
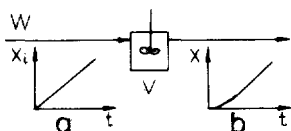

Fig. 1. Schematic diagram of a mixer with parameters used in theoretical analysis.

For the purpose of mathematical analysis, the following assumptions have been made: (1) the mixer is ideal, i.e., the liquid has a uniform composition in the entire volume of the mixer; (2) the mixer volume V and the volumetric flow-rate w are constant: and (3) the concentrations are expressed as volume fractions and concern an arbitrarily chosen component of a multi-component mixture. With these assumptions and from Fig. 1. the following balance for a chosen component of a mixture of liquids leaving the mixer. X(t). can be written:

$$X_i(t)w\mathrm{d}t - X(t)w\mathrm{d}t = \mathrm{d}XV \tag{1}$$

Introducing

$$TA = \frac{V}{w} \tag{2}$$

into eqn 1 and applying a Laplace transformation, one obtains

$$X(s) = \frac{X_i(s)}{sTA + 1} + \frac{X(0)TA}{sTA + 1} \tag{3}$$

where $X(0)$ is the initial concentration of liquid in the mixer, and in the time domain

$$X(t) = \frac{1}{TA} \int_0^t X_i(p)\exp\left[\frac{-(t-p)}{TA}\right] dp + X(0)\exp\left(\frac{-t}{TA}\right)$$

$$= \frac{1}{TA} \exp\left(\frac{-t}{TA}\right) * X_i(t) + X(0) \exp\left(\frac{-t}{TA}\right) \tag{4}$$

Eqns. 3 and 4 describe the dependence between the output and input for any inertial system of the first order. The form of eqn. 4 is very inconvenient for calculations (convolution) and therefore in further considerations eqn. 3 will be employed. The advantage of eqn. 4 is the possibility of using arbitrary functions $X_i(t)$, whereas eqn. 3 can be employed only when $X_i(t)$ is transformable into the s domain. As the functions of changes of input concentration $X_i(t)$ used in gradient chromatography fulfil this condition, the limitations given above can be neglected. It should be noted that eqns. 3 and 4 are valid for both continuous and discrete functions. Hence, they can be applied for a description of concentration changes of liquids also behind a proportioning valve and a mixer when the analytical function of concentration changes at the mixer input has been determined. Eqns. 3 and 4 describe in a general way the course of concentration changes of a chosen component in liquid flowing out of the mixer of a gradient device. Hence they can form the basis for calculations of the magnitude of deviation of X(t) from $X_i(t)$, *i.e.,* from the desired (programmed) changes.

Principle of correction of concentration changes at the mixer inlet

The form of eqn. 3 suggests such a modification of the function $X_i(t)$ by rearrangement to the form $X_i'(t)$, so as to obtain at the mixer outlet the desired function $X_i(t)$. The method seems to be simple: $X_i(t)$ should be inserted in eqn. 3 as the output function and the input function $X_i'(t)$ should be sought. This procedure is the reverse of that described above. Thus,

$$X_i(s) = \frac{X_i'(s)}{sTA + 1} + \frac{X(0) TA}{sTA + 1} \tag{5}$$

and

$$X_i'(s) = X_i(s) + TA[sX_i(s) - X(O)] \tag{6}$$

After transformation to the time domain

$$X_i'(t) = X_i(t) + TA \frac{d}{dt} [Xi(t)] + TA[X_i(0) - X(0)]\delta(t) \tag{7}$$

where Xi(O) is the required initial concentration of a chosen component at time $t = 0.$

The method of programme correction represented by eqn. 7 is simple as it is sufficient to add to the function of interest the term $TA\frac{d}{dt} [Xi(t)]$. At the same time,

TABLE I

EXAMPLES OF FUNCTIONS DESCRIBING GRADIENT ELUTION

$X_i(t)$	$X(t)$	$X_i'(t)$
$at + b$	$at + b + \exp\left(\dfrac{-t}{TA}\right)[aTA - b - X(0)] - aTA$	$at + b + TAa$
$a\exp(kt) + b$	$a\left[\exp(kt) \dfrac{- \exp(-t/TA)]}{kTA + 1}\right] +$ $b[1 - \exp(-t/TA)] + X(0)\exp(-t/TA)$	$a\exp(kt)(1 + TAk) + b$
$a\ln(t + t_0) + b$	$a\exp(-t/TA)\{\exp(-t_0/TA)$ $\left[E_i(t_0/TA) - E_i\left(\dfrac{t + t_0}{TA}\right)\right] +$ $\exp(t/TA)\ln(t + t_0)\} +$ $b[1 - \exp(-t/TA)] +$ $X(0)\exp(-t/TA) + a\exp(-t/TA)\ln t_0$ $E_i(t) = \displaystyle\int_{-\infty}^{\infty} \dfrac{\exp X}{x}\,dx$	$a\ln(t + t_0) + b +$ $\dfrac{TAa}{t + t_0}$

the method is valid for any function $X_i(t)$, provided that it can be differentiated. Realization of the third term of eqn. 7 is impossible [the Dirac delta function, $\delta(t)$, cannot be realized in practice]. However, this term vanishes when $X_i(0) = X(O)$, *i.e.,* when the initial concentration of a selected component in a mixer equals the desired initial concentration of the component at the mixer outlet. This condition is obvious, as it is impossible to achieve a jump concentration change of liquid in the mixer shown in Fig. 1. In addition, another problem may occur. With an extremely rapid increase in elution gradient, the corrected function $X_i'(t)$ will require concentrations with volume fraction higher than 1 or lower than 0, which is physically impossible. Then, the possibilities of exact correction disappear and the mixer response to a unit jump begins. However, by a skilful extension of the time of delivery of one component, the disadvantageous effect of the mixer on programmed concentration changes of liquids can be substantially diminished also in this instance.

It can be seen from Table I that the modification of programme with linear concentration changes is uncomplicated. The values of programmed concentrations should be increased by the term TAa, that is. the straight line representing concentration changes should be shifted parallel.

Examples of concentration changes of a mixture component obtained at the outlet of a mixer are shown in Fig. 2.

EXPERIMENTAL AND RESULTS

Materials

n-Hexane was obtained from Reachim (U.S.S.R.), non-absorbing in the UV region at 253.7 nm. It is sometimes designated as liquid A. n-Hexane + 0.3% by volume of acetone is sometimes designated as liquid B.

Acetone (analytical-reagent grade) was obtained from P.O.Ch. (Gliwice, Poland), absorbing in the UV region, $\lambda_{max.} = 279$ nm, log $\varepsilon = 1.16$.
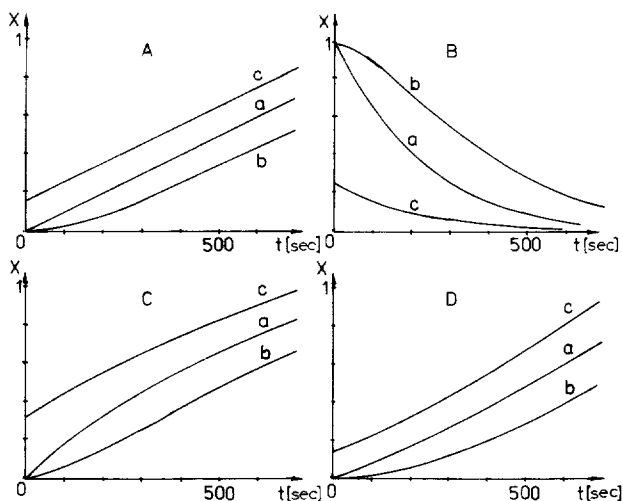
Fig. 2. Examples of curves of concentration changes of liquid B in a mixture. Curve a, desired concentration changes of liquid B in a mixture and, simultaneously, concentration changes of liquid B expected after correction of operation of the device described by curve c; curve b, expected concentration changes of liquid B in a mixture obtained as a result of operation of the device with uncorrected programme described by curve a; curve c, functions characterizing corrected programme of operation of the gradient elution device. Calculations were performed for the following desired functions of concentration changes of liquid B: (A) $0.001t$; (B) $\exp(-0.0044t)$; C $0.9712 \ln(t + 500) - 6.036$; (D) $\exp(0.00077\,t) - 1$. The relationships are valid for time t expressed in seconds, and the result is obtained as a volume fraction. Time constant of the gradient elution device: $TA = 169$ sec.

Apparatus

A schematic diagram of the apparatus is shown in Fig. 3. All parts, except (a) and (g), were made in the Institute of Inorganic Chemistry and Technology of the Technical University of Gdańsk.

Procedure

Experimental functions of changes in concentration of liquid B in liquid A were obtained employing the difference in the UV absorption of the two liquids at 253.7 nm. Under these conditions, when appropriate portions of liquid B were added in accordance with the programme to non-absorbing liquid A, then the curve obtained on a recorder connected to a UV detector (254 nm) determined the concentration changes of the mobile phase in the form of the function $A = f(t)$, reflecting reliably the experimental results, which could be compared directly with the programmed concentration changes.

The mixer volume was determined by a unit jump method. The absorption changes at the mixer outlet were recorded after a jump change in concentration of one of the components of the mixture. Assuming that the mixer is an inert object of the first order with a time constant $TA = V/w$, the recorded curve can be described by the relationship

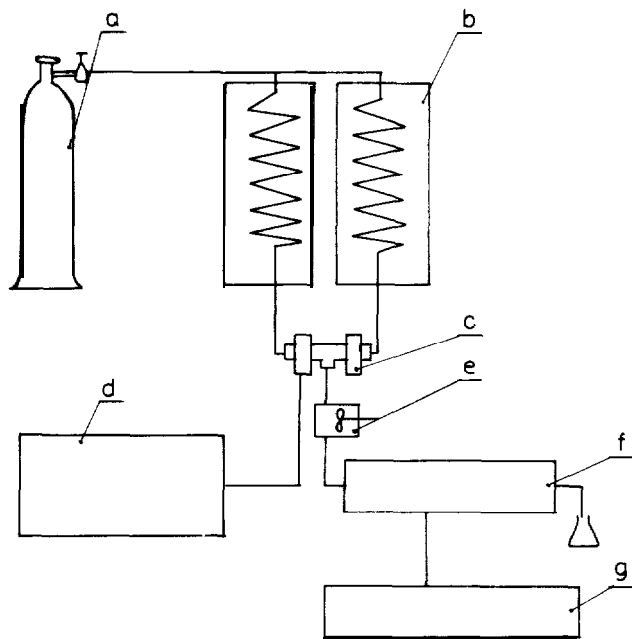$$X(t) = X_{max.} \left[1 - \exp\left(-\frac{t}{TA} \right) \right] \qquad (8)$$

Fig. 3. Schematic diagram of the apparatus for gradient elution. (a) Tank with compressed gas; (b) containers with mobile phase components; (c) two-way proportioning valve; (d) device controlling operation of the valve. (e) mixer of 3.1 cm^3 volume with electromagnetic stirrer; (f) UV detector (254 nm) with sensitivity, 0.01 a u.f.s., equipped with an 8-μl cell: (g) recorder.

where X_{max} is the maximum change in absorption at the mixer outlet. Hence, a plot of the dependence

$$\ln\left(1 - \frac{X}{X_{max.}}\right) = -\frac{t}{TA} \tag{9}$$

will be a straight line.

The time constant of such a mixer can be calculated by (1) measurement of time necessary to attain at the mixer outlet an absorption equal to $(1 - e^{-1})X_{max.}$ $= 0.632 \, X_{max}$; this time is equal to the time constant of the system; and (2) determination of the slope of the straight line given by eqn. 9; the reciprocal of this slope is equal to TA.

On the basis of the recorded curves, a plot of eqn. 9 was constructed. It was a straight line, which confirmed the assumption that a mixer may be described by equations valid for an inert material of the first order. Deviations from linearity were associated with the determination of time (for small values of time) and the ratio X/X_{max} (for values approaching unity). The time constant was determined according to (1) and (2) above, neglecting the dead time of the system. Subsequently, the mixer volume was calculated by means of eqn. 2. Gradient elution curves (exponential and logarithmic) were approximated by eight straight lines.
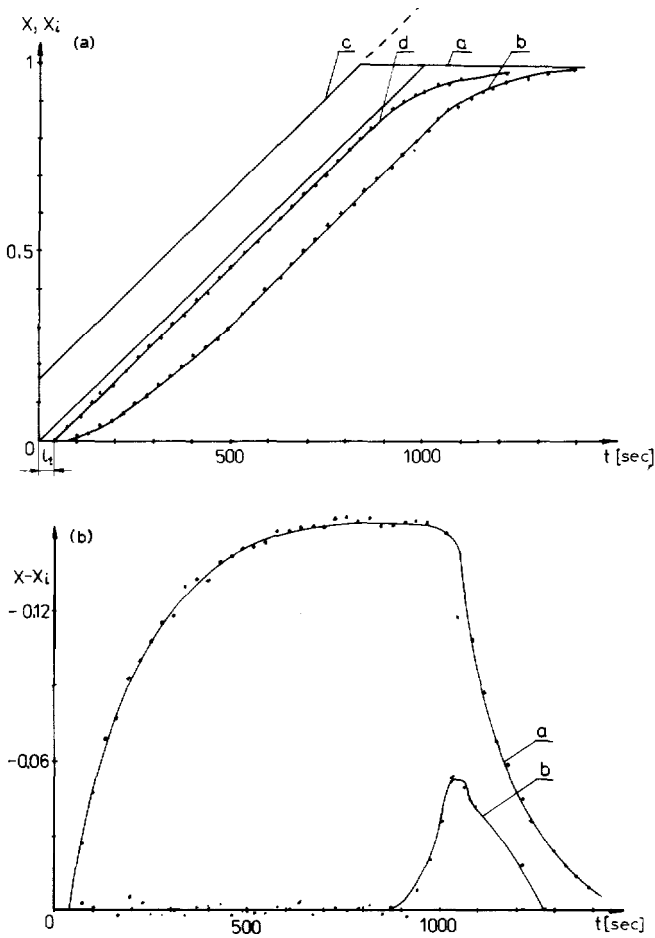
Fig. 4. (a) Family of curves representing programmed linear concentration changes and those obtained in practice (the same as shown in Fig. 2A, but shifted in time characterizing the transport delay of the programme. Desired concentration gradient $X_i(t) = 0.001t$; b. concentration gradient obtained when applying the programme described by curve a; c, corrected programme of operation of the gradient elution device; d, concentration changes obtained after correction of the operation described by curve c. Time constant of the gradient elution device; $TA = 169$ sec. (b) Curves representing the differences (errors) between the desired and experimental concentration changes during gradient elution when applying: curve a, uncorrected programme described by the function $X_i(t) = 0.001t$ (see Fig. 4a, curve a) and curve b, corrected programme eliminating the effect of the mixer during the operation of the gradient elution device (see Fig. 4a, curve c) Time constant of the gradient elution device: $TA = 169$ sec.

Studies of the effect of a mixer on the concentration changes of liquids during gradient elution

      The results of the investigations are shown in Figs. 4-7. It can be seen that the theoretical curves shown in Fig. 2 and those obtained experimentally are generally close, confirming the validity of the assumptions and derived relationships. The observed small differences between the theoretical and experimental curves are due to the dead time in the system, the errors in concentration measurements and inaccuracy
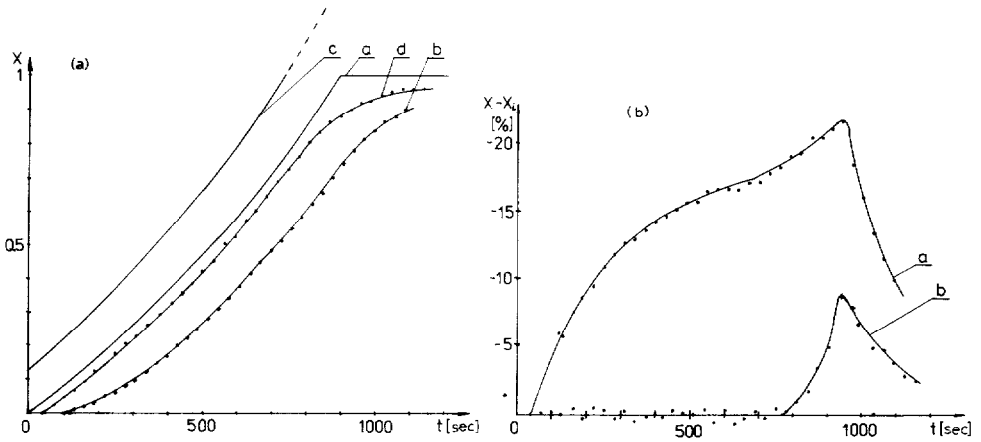
Fig. 5. (a) Family of curves representing programmed exponential concentration changes and those ob-
tained experimentally (similar to the predicted ones, see Fig. 2D). Curve a, desired concentration gradient
$X_i(t) = \exp(0.00077t) - 1$; curve b, concentration gradient obtained when applying the programme de-
scribed by curve a. curve c, corrected programme of operation of the gradient elution device; curve d,
concentration changes obtained after correction of the operation described by curve c. Time constant of
the gradient elution device: $TA = 169$ sec. (b) Curves representing the differences (errors) between the
desired and experimental concentration changes during gradient elution when applying: curve a, uncor-
rected programme described by the function $X_i(t) = \exp(0.00077t) - 1$ (see Fig. 5a, curve a); curve b,
corrected programme eliminating the effect of the mixer during the operation of the gradient elution device
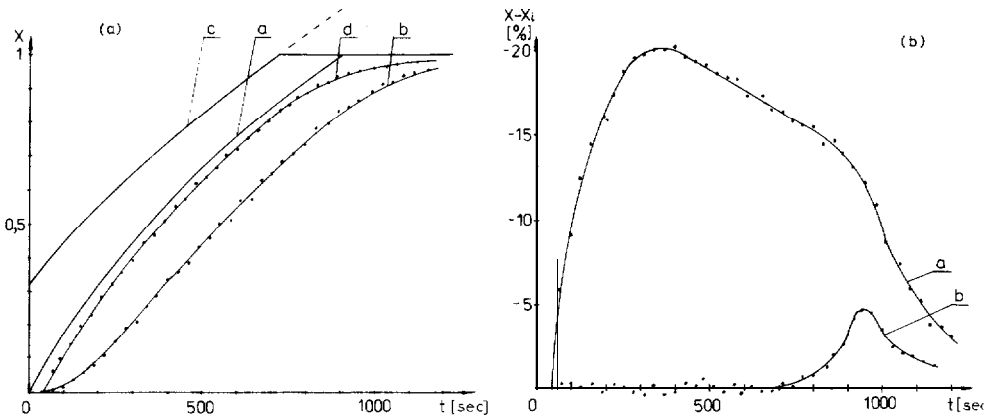(see Fig. 5a, curve c). Time constant of the gradient elution device: $TA = 169$ sec.

Fig. 6. (a) Family of curves representing programmed logarithmic concentration changes and those ob-
tained in practice (similar to the predicted ones. see Fig. 2C). Curve a, desired concentration gradient $X_i(t)$
$= 0.9712 \ln(t + 500) - 6.036$; curve b, concentration gradient obtained when applying the program
described by curve a; curve c, corrected programme of operation of the gradient elution device; curve d,
concentration changes obtained after correction of the operation described by curve c. Time constant of
the gradient elution device: $TA = 169$ sec. (b) Curves representing the differences (errors) between the
desired and obtained concentration changes during gradient elution when applying: curve a, uncorrected
programme described by the function $X_i(t) = 0.9712 \ln(t + 500) - 6.036$ (see Fig. 6a, curve a); curve b,
corrected programme eliminating the effect of the mixer during the operation of the gradient elution device
(see Fig. 6a. curve c). Time constant of the gradient elution device: $TA = 169$ sec.
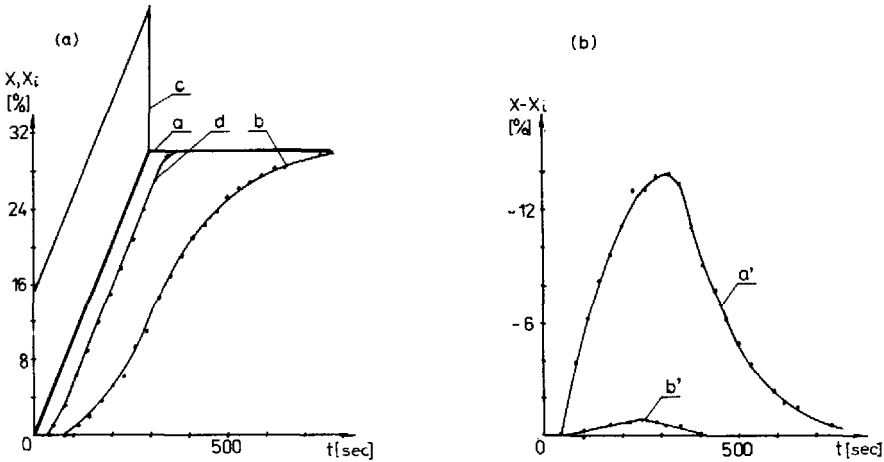
Fig. 7. (a) Family of curves representing the desired and experimental concentration changes and (b) the differences (errors) between them. Curve a, desired concentration changes: $X_i(t) = 0.0011$, $X_i < 0.3$; $X_i(t) = 0.3$, $X_i \geqslant 0.3$. Curves b and a', obtained concentration changes and the difference between theoretical and experimental concentration changes after application of the programme described by curve a. Curve c, corrected programme of the operation of the gradient elution device. Curves d and b', experimental concentration changes and the errors in their realization after application of the programme described by curve c.

of the determination of the effective volume of mixing. Fig. 5b shows the difference (errors) between the programmed concentration changes of liquid B and those obtained. In these calculations, the dead time introduced by the gradient elution device was not considered to be an error. The dead time cannot be reduced to zero (some time is required for the liquid to flow through all the tubes and connecting pieces).
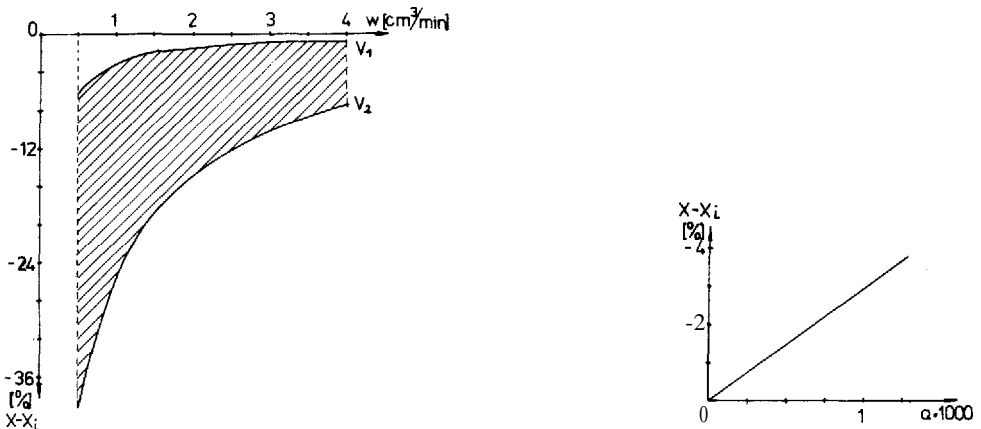

Fig. 8. Plots of errors due to uncorrected programme as a function of the volume flow-rate w for the extreme values of the mixer volume $V_1 = 0.5$ cm^3 and $V_2 = 4$ cm^3. Calculations were performed for the function $X_i(t) = 0.001t$ and $t = 600$ sec.

Fig. 9. Errors of realization of the programme for linear concentration changes $X_i(t) = at$ as a function of the slope a of the straight line. The plot was drawn for examples of values: $t = 600$ sec, $V = 0.5$ cm^3 and $w = 1$ cm^3/min.

This does not change the character of the concentration gradient but delays the expected changes. The delay can be measured and accounted for during the chromatographic process. It is evident that the application of a correction function describing the operation of a gradient elution device substantially reduces the error expressed as the difference between the programmed and experimental concentration changes of a liquid. Especially conclusive data were obtained for rapid changes of concentration gradient increase. As shown in Fig. 7, under these extreme conditions the programme correction was very useful.

Slight errors observed for concentrations lower than 80% presented in Figs. 4–7, depicting the effect of a correction programme for a gradient elution device resulted from the error in the determination of the mixer volume. On the other hand, at high concentrations the errors were due to the limited possibilities for correcting the operation of the valve in these concentration ranges, as it was impossible to obtain from the device a concentration of liquid B higher than 100% required by the programme. The response of the mixer to a unit jump was then begun. It should be pointed out that an analogous situation occurs when the valve should operate, according to the correction programme, at concentrations lower than zero. If in part of the programme the function values exceed the range (0–1), then the deviations from the desired concentration can be decreased by skilful extension of the period during which the concentration of liquid B is equal to 0 or 1. Fortunately, such cases seldom occur in chromatographic practice.

The experiments generally confirmed the conclusions resulting from the assumed model of a mixer. This result led us to plot generalized theoretical diagrams of differences (errors) between the concentration changes of liquid B in a mixture leaving the mixer and the desired concentration changes for practically limiting concentration gradients of an eluent component. This plot facilitates the prediction of maximum errors due to neglect of correction of the programme for the gradient elution device as a function of mixer volume and flow-rate of liquid.

The straight line $X_i(t) = 0.001t$ is the limiting case for the curves. The plot of errors for a linear elution gradient $X_i(t) = 0.001\ t$ is shown in Fig. 8. The difference $X(t) - X_i(t)$ did not reach the maximum value for a given time t and it was decided to perform the calculations for $t = 600$ sec. After an extended period of gradient elution the inaccuracy in the production of linear changes in eluent composition remains practically constant. The accuracy of production of a linear concentration gradient described by the equation $X_i(t) = at$ depends not only on the mixer volume V and the volume flow-rate w, but also on the rate of increase of concentration a. The dependence $X - X_i = f(a)$ is linear. The effect of a on the errors for fixed values of the remaining parameters (V and w) is shown in Fig. 9.

In previous considerations, only the effect of a mixer on the course of concentration changes was taken into account. However, in a chromatographic system mixing of liquids occurs not only in the mixer but also in other elements of the system, e.g., the pump, connecting pieces and proportioning valve. The contribution of these additional factors in liquid mixing may cause the gradient elution error to be larger than those depicted in Figs. 8 and 9. As we are interested in the concentration gradient of the mixture entering a chromatographic column, the influence of all these additional elements on the final effect of liquid mixing should be taken into account when correcting the programme for valve operation. Although in components other

than the mixer ideal mixing does not occur to a first approximation it can be assumed that the entire gradient elution system is a combination of an ideal mixer with an object delaying concentration changes. The mixing volume of such a system can be determined using methods (1) and (2) described above and then utilized for calculating the corrected function $X_i'(t)$ describing the concentration changes. It can be presumed that the correction of the desired function $X_i(t)$ will enable the errors of the concentration gradient relative to the procedure to be decreased, taking into account only the volume of mixing.

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