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PROGRAMMING THE ELUTION GRADIENT IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY BY VARYING THE VOLUME OF THE MIXING CHAMBERS

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SUMMARY

An apparatus is described for gradient elution in high-performance liquid chromatography that operates by programming the volume of the mixing chambers. General equations determine the volume or weight fraction changes in any component of a multi-component mobile phase flowing out of any chosen tank constituting the *i*th element of an *n*-element battery of tanks connected in series.

Equations were solved for the simplest cases, which are actually the most important ones. Dependences were verified experimentally and good agreements with predicted theoretical dependences were obtained. Chromatograms representing several modes of use of the method are presented. This method of programming the composition of mobile phases in liquid column chromatography has been found to provide a simple and sufficiently accurate means of obtaining a large variety of programmes for the gradient of eluent composition. The tank system described is applicable to all types of pumping devices used in liquid chromatography.

INTRODUCTION

According to Snyder[1], the most convenient of the five techniques used to solve the general elution problem in liquid chromatography is the programming of composition changes in the mobile phase of the chromatographic column during the elution process. The mobile phase composition can be programmed by three basic modes:

(1) the inflowing solvent is mixed with the solvent in the tank and the tank contents are pumped simultaneously into the chromatographic column[2–4];

(2) each of the mobile phase components is pumped through a separate displacement pump of adequately programmed capacity[5];

(3) the proportions of the mobile phase components are determined by use of proportioning valves[6] and pumped with a single pump.

The first method is the simplest to apply, as it neither calls for a pump with an accurately regulated capacity[7], nor is its use hampered by uneven concentration changes resulting from inertia of the proportioning valves[6]. The gradient tank devices

previously used are, however, not only difficult to operate but also of limited applicability because linear and exponential composition changes in the mobile phase can only be obtained within the limited concentration ranges of the two components[3]. Moreover, the composition of the mobile phase, which flows through vessels of varying shapes and containing varying levels of liquid, can only be programmed with the aid of apparatus equipped with a micro-pump and suitable programmes are very difficult to select[8].

The purpose of this work was to improve the usefulness of gradient tank devices through programming volume changes in that part of a tank where the components of the mobile phase are being mixed.

PRINCIPLE OF OPERATION OF A GRADIENT DEVICE WITH VARYING MIXING CHAMBER VOLUMES

Fig. 1 shows the mode of operation of a device where the tank may be the ith element of an n-element battery of tanks connected in series. An essential part of the device is the closed tank (1) which contains a magnetic stirring bar (2) and the movable volume limiter (3) for the mixing chamber, *i.e.*, for the part of the tank where the liquid undergoes mixing. At the bottom of the tank are the outlet of a pipe (4),
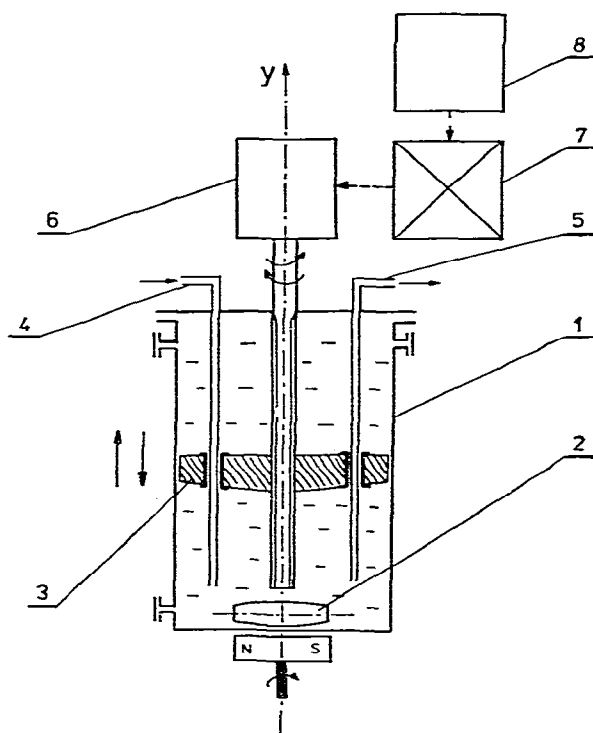

Fig. 1. Schematic diagram of programming device (Polish Patent No. 97357). 1 = Tank with liquid A; 2 = magnetic stirring bar; 3 = movable mixing chamber volume limiter; 4 = supply tube for liquid B; 5 = outlet tube for mixture of liquids A and B; 6 = gear; 7 = motor; 8 = rotational speed programmer.

designed to supply liquid B, and the inlet of a pipe (5), which drains the A–B mixture into the chromatographic column or into the next tank of the same type.

Before beginning the elution process, tank 1 is filled with liquid A, volume limiter 3 is placed at a determined height and the magnetic stirring bar 2 is started to stir the liquid. Liquid B is then released and the volume limiter is put into motion either to increase or to decrease the space in which the liquid is being stirred. An increased mixing chamber volume (i.e., when the volume limiter moves upwards along axis y in Fig. 1) makes liquid A flow from above the volume limiter into the space below it. However, if the limiter moves in the opposite direction, i.e., towards the tank bottom, the A–B mixture flows into the space above it. In both instances of course, the A–B mixture flows simultaneously into the chromatographic column or into the next tank of the same type.

Unless there is a volume interaction between the two compounds during mixing, the liquid flows out of tank 1 as rapidly as liquid B flows into that tank.

Liquids A and B may consist of several components that differ only in concentration. Liquid B should, as a rule, have a greater concentration of components with a higher elution strength. If thorough blending of liquids A and B in the mixing chamber i is assumed, we can use a differential equation to determine the dependence of the instantaneous concentration of the jth component of the A–B mixture flowing out of tank i into tank $i+1$ or into the chromatographic column, which depends on the initial concentration, time and other parameters of the process.

If the motion of limiter 3 decreases the mixing chamber volume, the rate of volume change, Z_i, is negative and we have the dependence

$$C_i \, dx_{j,i} + w[x_{j,i} - x_{j,(i-1)}] \, d\tau = 0 \tag{1}$$

whereas if the motion of the limiter increases the mixing chamber volume, the rate of volume change, Z_i, is positive and we have the dependence

$$C_i \, dx_{j,i} + w[x_{j,i} - x_{j,(i-1)}] \, d\tau + Z_i(x_{j,i} - x^0_{j,i}) \, d\tau = 0 \tag{2}$$

Eqns. 1 and 2 are applicable after the following definitions are taken into consideration:

$$Z_i = Z_i(\tau) = \frac{dC_i}{d\tau} = F_i \cdot \frac{dy}{d\tau} \tag{3}$$

$$C_i = C_i(\tau) = C^0_i + F_i \int_0^\tau \left(\frac{dy}{d\tau}\right) d\tau \in \langle 0; C^1_i \rangle \tag{4}$$

$$w = w(\tau) = \frac{dV}{d\tau} \geqslant 0 \tag{5}$$

$$x_{j,i} = x_{j,i}(\tau) \tag{6}$$

$$i = 1,2,3,\dots,n; \quad j = 1,2,3,\dots,m \tag{7}$$

Consideration must also be given to the boundary condition defined by the expression

$$x_{j,i} = x^0_{j,i} \text{ for } \tau = 0 \tag{8}$$

Considering dependences 3–8, the solution of eqns. 1 and 2 will present no difficulties, provided that C^0_i, $x^0_{j,i}$, Z_i, $x_{j,0}$ and w, are known. If, however, there are more then three mixing tanks to consider it might prove difficult to obtain the final solution.

For practical purpose, one, or at most two, tanks of that type connected in series are enough to programme the composition of a mobile phase in liquid chromatography. The dependences 9 and 10 are solutions of eqns. 1 and 2 when a single tank with a moveable mixing chamber volume limiter is used:

for eqn. 1:

$$x_{j,1} = x_{j,0} - (x_{j,0} - x^0_{j,1}) \left(1 + \frac{w\tau}{a_1 C^0_1}\right)^{-a_1} \tag{9}$$

for eqn. 2:

$$x_{j,1} = \frac{x^0_{j,1} + a_1 x_{j,0}}{a_1 + 1} + \frac{a_1}{a_1 + 1}(x^0_{j,1} - x_{j,0})\left(\frac{1}{1 + \frac{w\tau}{a_1 C^0_1}}\right)^{1+a_1} \tag{10}$$

Eqns. 9 and 10 were obtained after assuming that the boundary condition (expression 8) was fulfilled and that $x_{j,0}$, $x^0_{j,1}$, Z_1 and w were constant. a_1 is the ratio of the rate of flow through the tank to the rate of its volume change and can be written as

$$a_1 = \frac{w}{Z_1} \tag{11}$$

If $Z_1 \to 0$, then when $a_1 \to \pm \infty$ eqns. 9 and 10 reach the boundary form, i.e., the following dependence for the concentration of components in each single tank can be formulated:

$$x_{j,1} = x_{j,0} - (x_{j,0} - x^0_{j,1})e^{-\frac{w\tau}{C^0_1}} \tag{12}$$

Eqn. 12 defines changes in the volumetric fraction of the component in the liquid flowing out of the tank. In this instance, the limiter remains stationary in such a position that the volume underneath it is C^0_1.

In gradient liquid chromatography, the concentration of the component j having the higher elution strength should increase during development of the chromatogram; thus, when the condition $x_{2,0} > x^0_{2,1}$ is satisfied, the elution strength of the two-component mobile phase will increase with increasing volume of liquid B flowing through tank 1 (see Fig. 1).

Fig. 2 shows some of the theoretical dependences defined by eqns. 8 an 10. The curve resulting from eqn. 12 is denoted by a broken line. The curves in Fig. 2 were plotted with the additional assumption that the concentration of component j at the inlet of the tank is larger than the initial concentration of this component in the tank:
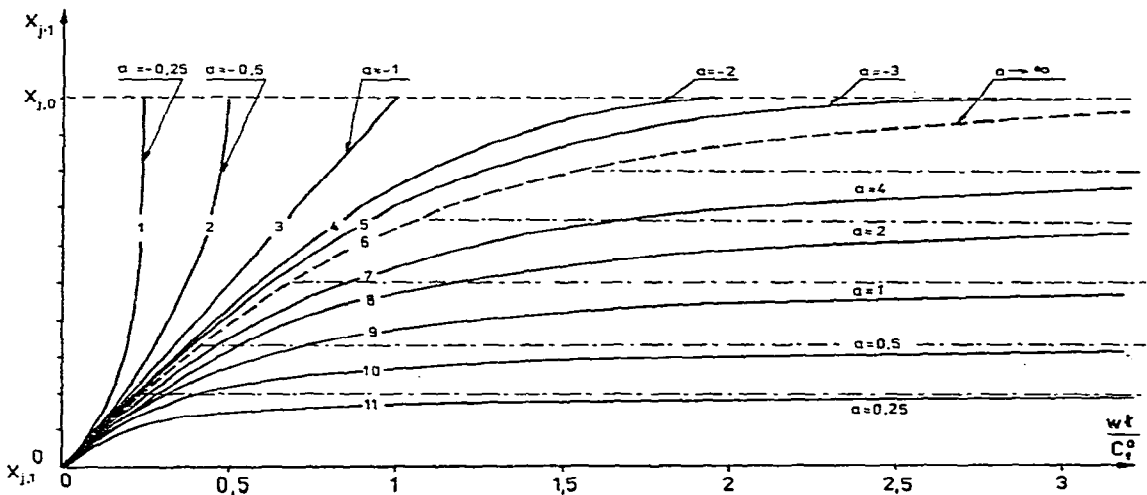
$$x_{j,0} > x_{j,1}^0 \tag{13}$$

Fig. 2. Theoretical plots of the volumetric or weight fraction of component j in the mixture of outflowing liquids according to Fig. 1:

$$x_{j,:} = f\left(\frac{w\tau}{C_1^0}\right)_{x_{j,0} > x_{j,1}^0}$$

Curves 1–5 show the steady decrease of mixing chamber volume and curves 7–11 its steady increase; curve 6 shows the case when the mixing chamber volume remains constant.

EXPERIMENTAL

Apparatus

Container devices for gradient elution. In order to verify the theoretical dependences given above and to confirm that the method described can be used for separations, we constructed the device shown in Fig. 1. The actual tank volume was 7–85 ml, depending on the position of the limiter. The sliding up-and-down motion of the volume limiter was assured by attaching it to a threaded shaft which could be put into circular motion at a controlled velocity. The limiter was driven by a direct-current motor through a transmission gear. Circular motion of the limiter was prevented by the fact that it was sliding on two fixed rods (see Fig. 1).

Liquid chromatograph. Investigations were carried out on a liquid chromatograph made in our laboratory. It was equipped with a membrane pump with a controlled yield for each head within the range 0–35 ml/min at 0–350 bar. A septum injector and 150 × 2 mm I.D. stainless-steel columns were used. A UV absorption detector operating at 254 nm with an 8-μl cell volume was employed. The detector sensitivity was 0.01 absorbance unit at 10 mV for full-scale deflection.

Materials

The column was packed by the wet method[9] with LiChrosorb Si 60 of particle size 7–12 μm (Merck, Darmstadt, G.F.R.).

In order to check whether the gradient tank device operated properly, we used methanol as liquid A and methanol containing 0.01 % of benzene as liquid B. Subsequently, a mixture of azobenzene, *o*-, *m*- an *p*-nitroaniline and 2,4-dinitroaniline (the concentration of each component was 0.2 mg/ml in dioxane) was separated with *n*-hexane as liquid A and *n*-hexane–dioxane (85:15) as liquid B.

Methods

Before starting the work we checked the linearity of the detector response depending on the volume fraction of benzene–methanol solution (liquid B) in the mixture of liquids A and B.

Experimental test of theoretical dependences. The concept of programming the composition of the liquid was tested for correctness and proper operation of the gradient device as follows. The tank was filled with methanol, which was then pumped through the detector cell in order to establish a baseline. At the same time the volume limiter (element 3, Fig. 1) was placed in its initial position and mixing of the liquids in the tank was started. The solution of benzene in methanol was then introduced into the mixing chamber and the volume limiter was moved in the chosen direction at a constant, pre-determined speed. At that stage the liquid was flowing through the tank at a rate of 4 ml/min. From the tank the mixture flowed through the cell of the UV detector, which operated at a sensitivity setting of 0.04 a.u.f.s. Thus, the absorbance of the liquid flowing through the detector was shown on the recorder tape as a function of time.

The plots of varying concentrations of liquid B in the mixture leaving the tank are shown in Fig. 3 .

Each test was carried out several times. The reproducibility was about 2%.

The points in Fig. 3 denote theoretically predicted concentrations. Curve segments represented by broken lines in Fig. 3 are related to runs obtained without moving the volume limiter from one of the ultimate positions.

Separation of substances. Chromatographic separations were carried out in a similar manner, with the injection valve and chromatographic column placed between gradient tank and detector. The tank was initially filled with *n*-hexane. After establishing the detector baseline during the *n*-hexane flow, the sample was injected into the column and the *n*-hexane–dioxane mixture was pumped into the tank. The upwards or downwards movement of the volume limiter was maintained at a steady rate. The flow-rate through the chromatographic column was 2 ml/min for gradient separation and 1 ml/min for isocratic separation. Some of the chromatograms are shown in Fig. 4. The broken lines denote varying dioxane concentrations.

RESULTS AND DISCUSSION

Eqns. 9 and 10 describe changes in liquid composition, programmed by means of only one tank. The diagrams of these dependences (Fig. 2) indicate that the use of a single tank allows a large variety of possibilities for the programming of liquid phase compositions. The parameter *a* may vary within the ranges —0.1 to —10 and 10
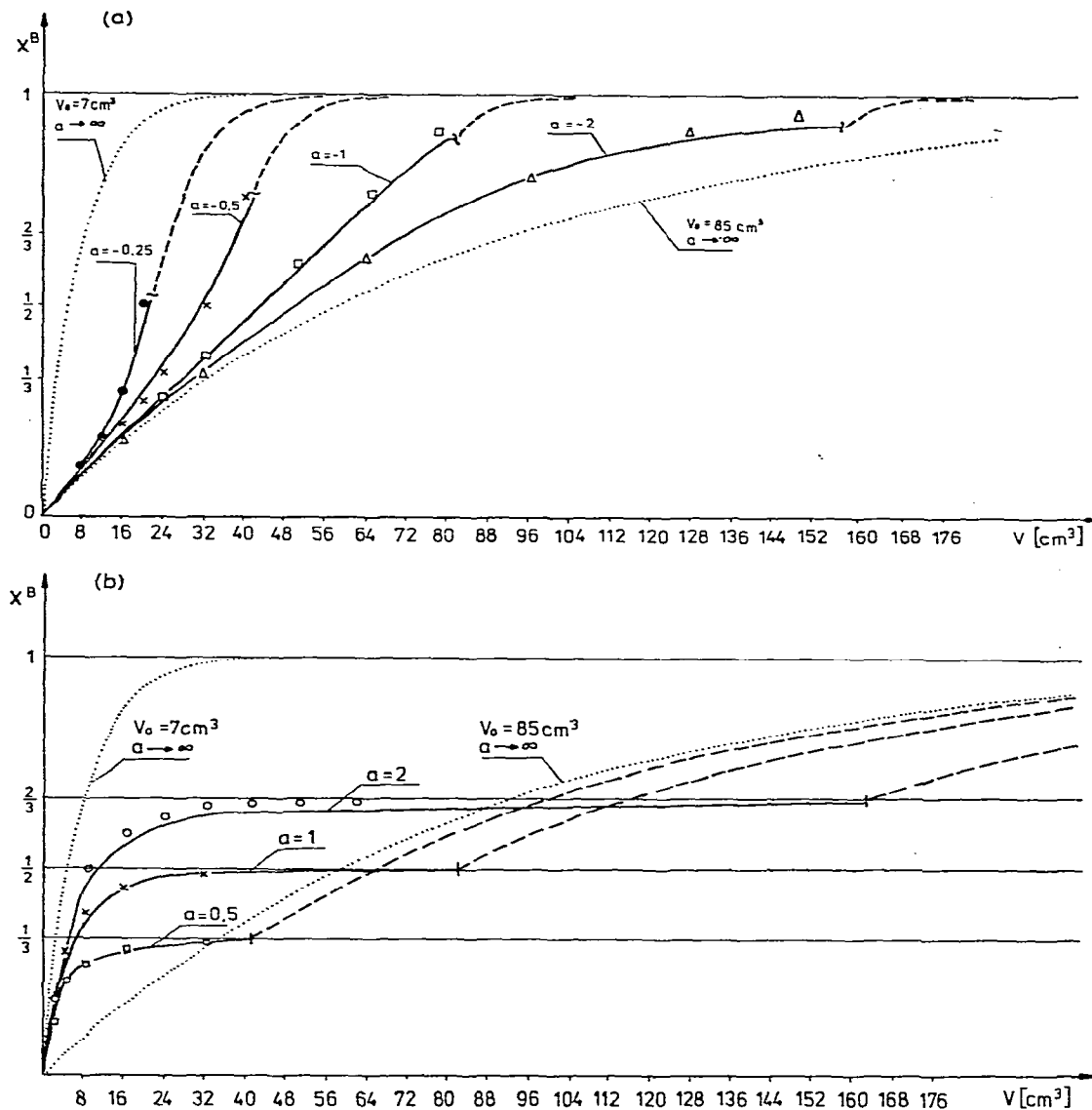
Fig. 3. Examples of changes in the volumetric fraction of liquid B in the liquid flowing out of the device, according to Fig. 1. (a) Steady decrease of mixing chamber volume from $C_1^0 = 85$ ml to $C_1^t = 7$ ml. (b) Steady increase of mixing chamber volume from $C_1^0 = 7$ ml to $C_1^t = 85$ ml. ————, Runs obtained during movement of volume limiter; — — —, runs obtained after the limiter has reached its final position; ...., runs obtained at the terminal positions of the limiter without moving it. Dots denote theoretically predicted concentrations $x_{j,i}$. Liquid A = methanol; liquid B = 0.01 % of benzene in methanol. Detector sensitivity, 0.04 a.u.; flow-rate of liquid, $w = 4$ ml/min.

to 0.1. An additional advantage of the device is the possibility of applying various values of the parameter C_1^0, the initial volume of the mixing chamber.

It is important that the minimal value of the mixing chamber volume, C_{min}^0, should be as small as possible. In the investigations described here it was about 7 ml.
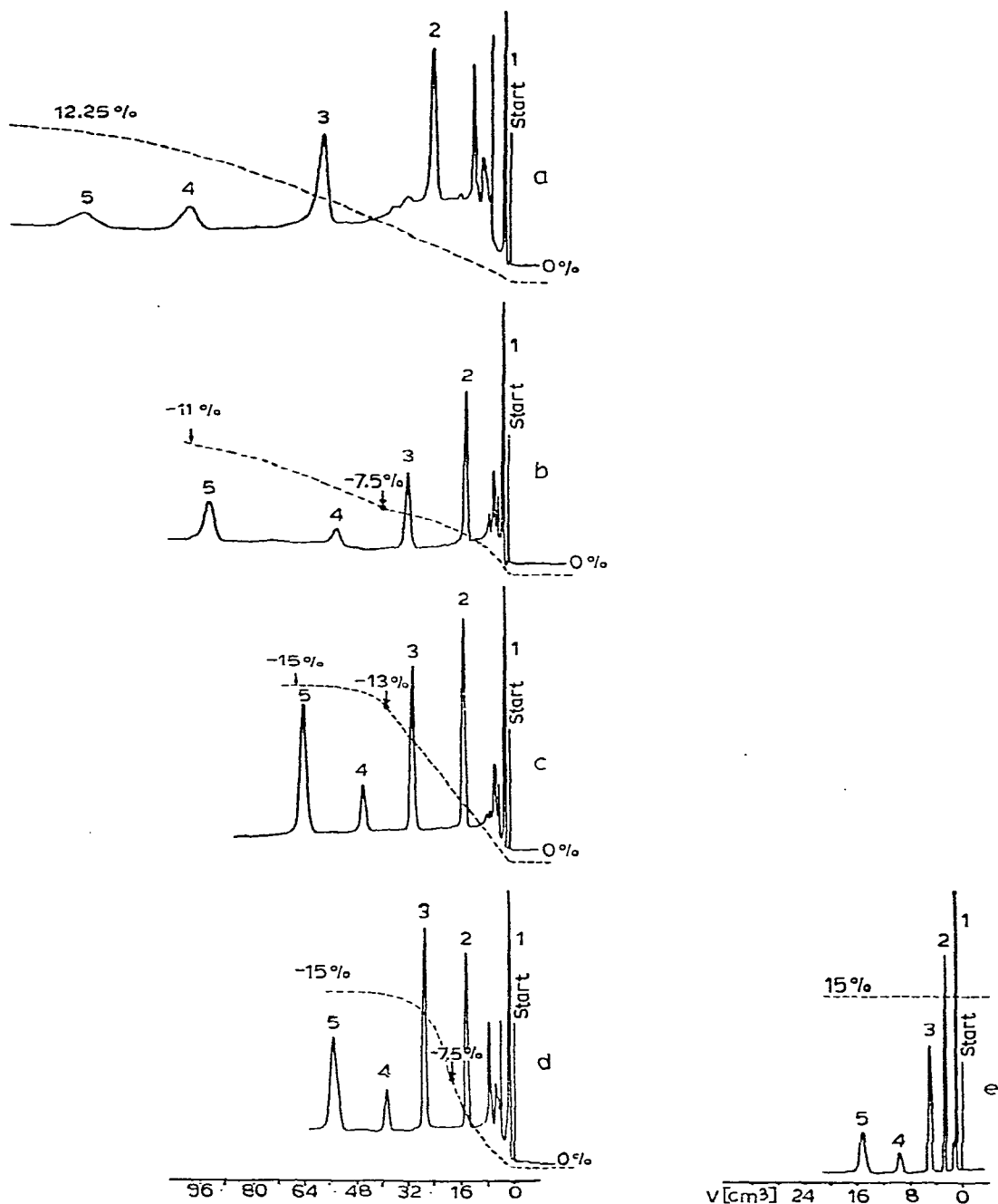
Fig. 4. Chromatograms of a model mixture using several gradient elution programmes by means of the device shown in Fig. 1. Composition of sample: 1 = azobenzene; 2 = o-nitroaniline; 3 = m-nitro-aniline; 4 = p-nitroaniline; 5 = 2,4-dinitroaniline. Concentration of sample components = 0.2 mg/ml. Sample size: 20 μl for chromatograms a and d, 10 μl for chromatogram e. Column: 250 × 2 mm I.D. Stationary phase: LiChrosorb Si 60 (10 μm). Mobile phase: for gradient separations (chromatograms a and d), A = n-hexane and B = n-hexane–dioxane (85:15); for isocratic separation (chromatogram e), n-hexane–dioxane (85:15). UV detector (254 nm), sensitivity 0.16 a.u. Operating parameters of gradient device: (a) C_1 = constant = 85 ml, $a = \infty$, w = 2 ml/min; (b) C_1^0 = 45 ml; C_1^1 = 85 ml, $a = 1$, w = 2 ml/min; (c) C_1^0 = 45 ml, C_1^1 = 7 ml; $a = -1$, w = 2 ml/min; (d) C_1^0 = 85 ml, C_1^1 = 7 ml, $a = -0.25$, w = 2 ml/min; (e) $C_1^0 = C_1^1$ = 0 ml, $a = \infty$, w = 1 ml/min.

It might be further decreased if the magnetic stirring bar is of a suitable size and design.

If this volume is zero ($C^0_{min} = 0$) then the changes in liquid composition shown in Fig. 3 would be horizontal within the range from $w\tau/C^0_1 = 0$ to $w\tau/C^0_1 = \infty$; and the following equation for $x_{j,1}$ would be satisfied:

$$x_{j,1} = \frac{x^0_{j,1} + a_1 x_{j,0}}{a_1 + 1} \qquad (14)$$

The curves shown in Fig. 3a would then be consistent with the corresponding curves in Fig. 2.

An advantage of this device is the possibility of forming linear gradients within a wide range of eluent concentrations. Linear gradients are now often employed in liquid chromatography.

As can be seen from Fig. 3, the experimentally obtained concentration changes in the liquid flowing out of the tank do not differ substantially from those theoretically predicted. Deviations may have been brought about by inaccurate measurement of the C^0_{min} value which was determined experimentally to be 7 ± 0.5 ml.

It is possible to use more than one tank with a movable mixing chamber volume limiter. This would extend the applicability of the device further but also make its use technically more difficult and its mathematical description too complicated for ready use.

The method described here for programming the gradient elution in liquid chromatography is suitable for all types of pumping devices employed in high-performance liquid chromatography. The tank must then be resistant to high pressures and placed between the pump and the chromatographic column.

The method can also be used in a chromatographic apparatus equipped with micro-pumps. The tank can be of the low-pressure type, e.g., made of glass or thin iron sheet, and be placed on the sucking side of the pumping system.

SYMBOLS

C_i	transient volume of mixing chamber in the ith tank;
C^0_i	initial volume of mixing chamber in the ith tank;
C^1_i	final volume of mixing chamber in the ith tank;
F_i	surface of transversal section of the ith tank;
V	volume of liquid flowing through the tanks;
w	flow-rate through the tanks;
$x_{j,i}$	transient concentration of the jth component in the liquid leaving the ith tank;
$x_{j,(i-1)}$	transient concentration of the jth component in the liquid leaving the $(i-1)$th tank and flowing into the ith tank;
$x^0_{j,i}$	concentration of the jth component in the liquid in the ith tank before the beginning of elution;
y	direction of tank volume increase (see Fig. 1);
i,j	see eqn. 7;
τ	time;

Z_i rate of volume change in the mixing chamber of the ith tank;

a_i ratio of the flow-rate through the tank to the rate of volume change in the mixing chamber of the ith tank; $a_i = w/Z_i$.

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