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DEVICE FOR LIQUID CHROMATOGRAPHY WITH A DYNAMIC TEMPERATURE GRADIENT

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SUMMARY

A device for liquid chromatography with a dynamic temperature gradient is described. The significance and technical solution of the selection of the temperature course in the column are discussed.

INTRODUCTION

The gradient technique is effective in liquid chromatography mainly for the separation of complex mixtures in liquid–solid systems and in ion-exchange chromatography. The composition of the mobile phase[1–3] is programmed most frequently, although programming of the stationary phase and the temperature of the chromatographic system has also been tested[2]. The gradient technique is particularly useful if, owing to a high capacity ratio, zone broadening occurs or if it is necessary to prevent irreversible sorption of the solute in the column or to reduce the time required for the analysis. In some instances the properties of the stationary phase are changed irreversibly.

The method involving the use of a dynamic temperature gradient[4] has been described for liquid–solid systems. The temperature gradient is established by means of an oven that advances along the column in the direction of a stream of a single-component mobile phase, the composition of which does not change during the experiment. The temperature in the oven decreases in the direction of movement of the oven. Experimental conditions were selected such that the linear velocity of the mobile phase, u, was always higher than the advance speed of the oven, w. Under these conditions, the components of the mixture under analysis can be divided into two groups. The first group comprises the components that proceed through the column more quickly than does the temperature gradient, and the second consists of the components whose translational velocity in the column is the same as the speed of the temperature gradient. The latter condition is provided for a component, i, even then when a characteristic temperature, $T_{i\ \text{char.}}$, exists in the gradient at which the retention time of the component, t_{Ri}, is the same as the time required for the passage of the temperature gradient through the column[5]. Component i occurs in the gradient at a distance x_i from its end having the maximal temperature $T_{\text{max.}}$, which is dependent on the distribution of temperatures in the gradient.

The following relationship[4] was derived for the characteristic temperature:

$$T_{i\ \text{char.}} = \frac{-Q}{R \ln \left[\dfrac{A}{\dfrac{u}{w}\left(1 + \dfrac{l - x_i}{L}\right) - 1} \right]} \tag{1}$$

where Q is the heat of adsorption, A is a constant, R is the gas constant, l is the length of the temperature gradient, L is the column length, u is the linear velocity of the mobile phase and w is the translational speed of the temperature gradient in the column. For the capacity ratio of component i, which is desorbed in the dynamic gradient, the following equation is valid:

$$k_i = \frac{u}{w} \cdot \frac{l - x_i}{L} + \frac{u}{w} - 1 \tag{2}$$

The resolution for the substances desorbed in the gradient can be expressed by the relationship

$$R_s = \frac{2\,(x_i - x_j)}{w\,(d_i + d_j)} \tag{3}$$

where d_i and d_j are the widths of the elution peaks at the base expressed in time units, and it holds simultaneously that $x_i > x_j$. In order to obtain optimal values of R_s, it is advantageous if the resolution can be controlled. This can be effected by varying the value of the difference $x_i - x_j$ by modifying some parameters of the temperature gradient, while the widths of the peaks do not change in proportion to the values of x. The relationship between the values of $T_{i\ \text{char.}}$, u/w and x_i is evident from eqn. 1.

The values of x_i can be changed by varying either u/w or the distribution of temperatures in the dynamic gradient. If u/w is changed, $T_{i\ \text{char.}}$ and x_i vary simultaneously for the components that are desorbed in the gradient. In a given temperature gradient, x_i and x_j cannot be varied in this way independently as u/w is identical for both the components.

A possible means of varying x_i and x_j independently is to modify the distribution of temperature in the dynamic gradient at a constant value of u/w. Characteristic temperatures of individual components also change in this instance but, in contrast to the previous situation, it is possible to vary the difference $x_i - x_j$.

The distribution of temperatures and the position of the component in the dynamic gradient also affect the width of the zone. The tail of the zone is desorbed at a higher temperature, and a steeper increase in temperature at the position of the desorption causes a greater narrowing of the zone. Therefore, the original hyperbolic course of the temperature in the dynamic gradient

$$x_i = C/T_i \tag{4}$$

which was obtained by using an oven sliding along the column was replaced by a system of direct electrical resistance heating. In this type of heating, the sliding device is divided into sections and advances along the column and, at the same time, the heat input provided to the column in each section is optional. A moving field of temperature is thus established in the column.

The shape of the temperature gradient obtained in this way is obvious from Fig. 1. The gradient is formed by several sections within which the temperature increase is approximately linear. The speed of the temperature increase, given by the slopes of the straight lines, can be varied by changing the lengths of the individual sections. Let x_1, x_2, x_3, x_4, x_5 and x_6 indicate the distances of the boundary points of the sections from the end of the gradient ($x_1 = l$, where l is the length of the temperature gradient, and $x_6 = 0$), and $K_1, K_2, \cdots, K_5$ be the slopes of the straight lines. Then, for the temperatures in the individual sections, we obtain:

1st section:

$$T = T_I + K_1 (l - x)$$

for $x_2 \leqslant x \leqslant x_1$ (5a)

2nd section:

$$T = T_I + K_1 (l - x_2) + K_2 (l - x)$$

for $x_3 \leqslant x \leqslant x_2$ (5b)

3rd section:

$$T = T_I + K_1 (l - x_2) + K_2 (l - x_3) + K_3 (l - x)$$

for $x_4 \leqslant x \leqslant x_3$ (5c)

4th section:

$$T = T_I + K_1 (l - x_2) + K_2 (l - x_3) + K_3 (l - x_4) + K_4 (l - x)$$

for $x_5 \leqslant x \leqslant x_4$ (5d)

5th section:

$$T = T_I + K_1 (l - x_2) + K_2 (l - x_3) + K_3 (l - x_4) + K_4 (l - x_5) + \\ + K_5 (l - x)$$

for $0 \leqslant x \leqslant x_5$ (5e)

where T_I is the temperature of the section of the column working at an isothermal regime.

The dynamic temperature gradient thus not only speeds up the analysis, permits the control of the capacity ratio and increases the peak capacity, but also makes possible the control of distinction of significant pairs of peaks.

DESCRIPTION OF THE DEVICE AND RESULTS

The mobile phase is pumped from the container by an Orlita (Giessen, G.F.R.) DMP 1515 diaphragm pump. A damping device according to Locke[6] is attached behind the pump. The mobile phase then passes through a stabilizing column packed with silica gel, which is used to remove the remaining water from the mobile phase, into an analytical column. A UV analyzer operating at 254 nm (Development Workshops of the Czechoslovak Academy of Sciences, Prague) was used to detect the components of mixtures under analysis. The original cell of the UV analyzer was replaced with a quartz capillary with a volume of 27.2 μl and an optical pathlength of 1 mm.

The analytical column is heated by a resistive wire, which is wound round it. Sliding contacts, fed from a control transformer, advance along the column and heat a defined section of the column. The column is made of stainless steel of length 650 mm, O.D. 4 mm and I.D. 2 mm. A 600-mm stainless-steel tube with the same diameter is welded to the lower end of the column. At the position of welding, the column is closed with a plug, z, through which goes a capillary, k (see Fig. 1b), which serves as the column outlet. An insulated constantan wire, 0.3 mm in diameter, is wound round the column as well as round the welded tube and both of them are insulated with epoxide resin. Along the whole length of the winding, two grooves are ground opposite each other, along which the contacts move. Two of the contacts always face each other (see Fig. 1a), individual pairs being attached to copper rings. The rings are slid on a polyethylene tube and fastened with screws. The distance between the contact pairs and their number on the polyethylene tube can be varied; the length and the course of the temperature gradient in the heated section of the column are thus also changed. An example of a temperature course in the heated section of the column and of the electrical connection of the contacts are shown in Fig. 1b.

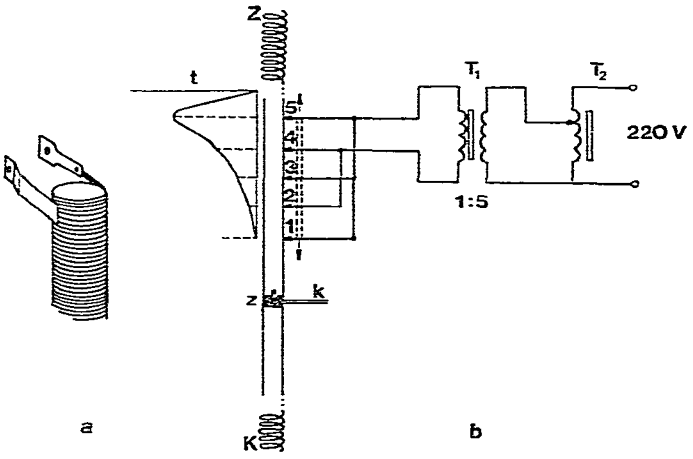

Fig. 1. (a) Pair of sliding contacts. (b) Diagram of electrical circuits for oven contacts. T_1 = separating transformer; T_2 = control transformer; Z, K = beginning and end of the winding of the resistive wire, respectively; 1–5 = sliding contacts; t = temperature; k = capillary; z = plug.

The column is fixed vertically, parallel to a brass rod 20 mm in diameter, on which the polyethylene tube with the contacts is slid. The tube with the contacts hangs on cables wound by a gear electric motor. The translational speed of the contact assembly along the column is determined by the speed of rotation of the shaft of the electric motor. The speed of its rotation is controlled and stabilized by a circuit which makes use of counter-electromotive force that is induced in the rotor of the motor and the intensity of which is proportional to the speed of rotation. A control signal is obtained by its comparison with a reference voltage, which, after being am-

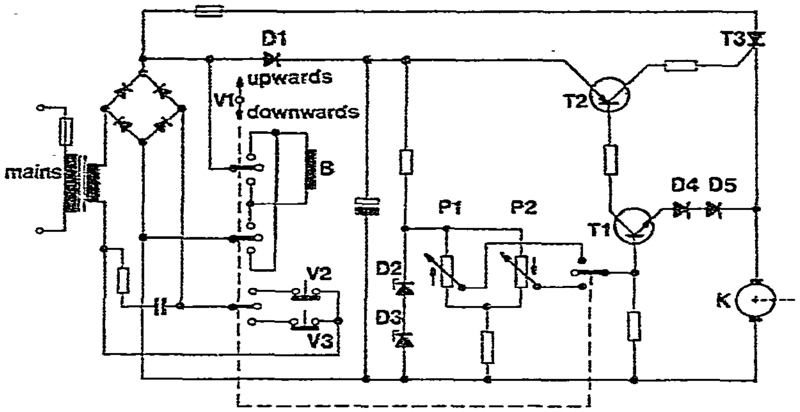

Fig. 2. Connection of the electromotor and the speed regulator of the contact advance.

plified, controls a thyristor in the motor circuit. The rotation speed can be adjusted by varying the reference voltage.

The function of the electric circuit can be seen from Fig. 2. The current from a bridge rectifier is fed to a stator, B, of the motor via a separating diode, D1, to a filtered source of the reference voltage with Zener diodes, D2 and D3, and potentiometers, P1 and P2. The voltage from the sliding contact of the potentiometer is compared with that of the rotor in a transistor, T1; the rotor voltage is led to an emittor, T1, via diodes, D4 and D5. The difference voltage, after being amplified in transistors T1 and T2, controls the thyristor, T3, in such a way that the speed of the motor is stabilized against fluctuations, whether caused by the change in mechanical load of the motor or by changes in the feeding voltage.

The control circuit also contains other elements. The sense of rotation is switched by means of a three-position switchboard key, V1, in the circuit of the stator, B; in the middle position, the switchboard key is off. The outlets of the potentiometers are switched by the switchboard key simultaneously, so that the speed of an upward movement of the contacts along the column is adjusted by the potentiometer, P1, and that of downward movement by the potentiometer, P2. Terminal switches, V2, and V3, stop the contacts moving on the upper and lower ends of the column.

The speed can be adjusted independently in both directions of movement within the range 10–50 rev/min, which corresponds, behind a worm gear on a winding drum, to a linear speed of the contacts and the heat field of ca. 30–150 mm/min.

Fluctuations of the mains voltage (200–240 V) and simultaneous changes in the load within a range greater than in common operation cause fluctuations of the speed that will not exceed ± 4.5% in most of the operative ranges. Only for an extreme decrease in the mains voltage to 200 V and simultaneous overload will the maximal adjusted speed decrease by 11.4%.

The dynamic temperature gradient was used in a liquid–solid system, with silica gel as adsorbent and *n*-heptane as mobile phase. A mixture to be analyzed contained benzene, anisole and nitrobenzene. The distance between the individual pairs of contacts was varied during the measurements and the effect of these changes on the course of the temperature gradient was studied. Examples of measurements are

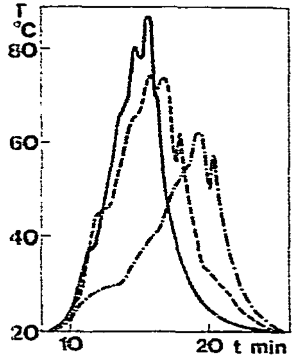

Fig. 3. Effect of the distance between individual pairs of sliding contacts on the course of temperature in the dynamic temperature gradient. Column, length = 65 cm, I.D. = 2 mm; column packing, silica gel CH, 50–63 μm; mobile phase, n-heptane; flow-rate, 1.06 ml/min; advance speed of the oven, 0.06 cm/sec. Distance between the pairs of contacts: (——) 7.1, 4.3, 3.5, 3.3, 2.5 cm; (– – –) 6.1, 4.5, 3.6, 3.6, 2.7 cm; (–·–·–) 12.0, 7.9, 5.1, 3.5, 2.5 cm.

shown in Fig. 3. There are perceptible steps on the temperature dependence, which are caused by local cooling of the column by fins of the electrical contacts (see Fig. 1a). The course of temperature in the dynamic gradient that is most suitable for a particular mixture under analysis can be selected by varying the distance between the contacts. The effect of the temperature course in the dynamic gradient on the column efficiency and resolution, R_s, for anisole and nitrobenzene, which are desorbed in the dynamic gradient, are evident from Table I. The translational speed of the temperature gradient along the column was identical in all instances (w = 0.06 cm/sec) as well as the feeding voltage (3.2 V). It follows from Table I that for the separation of the mixture of benzene, anisole and nitrobenzene the course of temperature from the dynamic gradient No. 2 is most suitable, when R_s acquires the value

TABLE I

RESULTS OF MEASUREMENTS USING AN ISOTHERMAL REGIME AND A TEMPERATURE GRADIENT WITH VARIOUS COURSES OF TEMPERATURES IN THE GRADIENT

Regime	*Component*	*V_R (ml)*	*k*	*H (mm)*	*N_{eff}*	*N_{eff}/sec*	*$T_{l\ char.}$ (°C)*	*R_s*
Isothermal regime	Benzene	3.10	1.00	3.73	43.5	0.259		
	Anisole	29.81	18.23	16.09	39.1	0.024		
	Nitrobenzene	73.23	46.24	5.22	119.3	0.030		
Dynamic temperature gradient* No. 1	Benzene	3.10	1.00	3.03	53.7	0.319		
	Anisole	18.22	10.75	0.84	644.7	0.651	34.8	
	Nitrobenzene	20.98	12.51	0.36	1557.2	1.366	58.0	1.16
Dynamic temperature gradient* No. 2	Benzene	3.10	1.00	3.03	53.7	0.319		
	Anisole	17.46	10.26	0.45	1203.3	1.240	29.1	
	Nitrobenzene	20.34	12.12	0.21	2622.5	2.321	52.7	1.73
Dynamic temperature gradient* No. 3	Benzene	3.10	1.00	3.03	53.7	0.319		
	Anisole	18.75	11.10	1.55	350.4	0.336	35.6	
	Nitrobenzene	22.40	13.45	0.13	4224.2	3.385	55.0	1.44

* For the parameters of the gradients, see caption to Fig. 3.

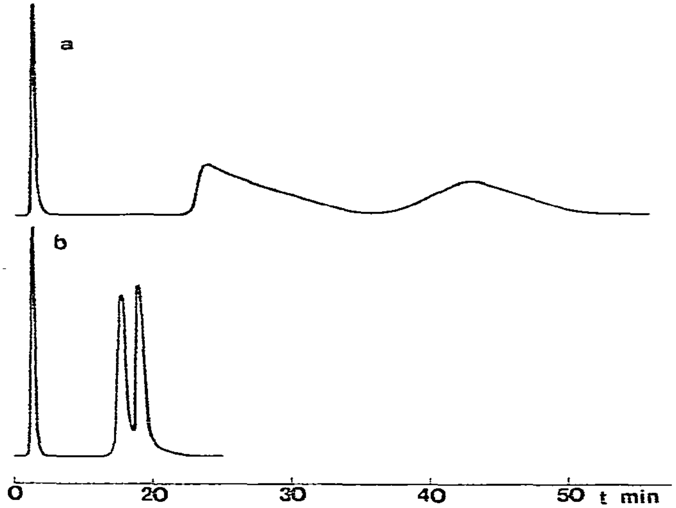

Fig. 4. Chromatogram of a mixture of benzene, anisole and nitrobenzene. Column length = 65 cm, I.D. = 2 mm; column packing, silica gel CH, 50–63 μm; mobile phase, *n*-heptane; flow-rate, 1.1 ml/min. (a) Isothermal run, $T = 21°$; (b) experiment at the dynamic temperature gradient; advance speed of the oven, 0.10 cm/sec.

1.73 and the height equivalent to a theoretical plate decreases, compared with the isothermal regime, 36-fold for anisole and 25-fold for nitrobenzene.

When using the dynamic temperature gradient, the capacity ratio, k, for the components desorbed in the gradient decreases and the time required for the analysis of the mixture is reduced compared with the isothermal regime. At the same time, the column efficiency increases. The results of the measurements on the mixture of benzene, anisole and nitrobenzene are listed in Table I and in Fig. 4. The number of effective plates per second, N_{eff}/sec, characterizes the reduction in the analysis time. With the use of temperature gradient No. 2, this value increases 52-fold for anisole and 77-fold for nitrobenzene. The peak capacity, n, with regard to the number of peaks that can be eluted between benzene and nitrobenzene increases with the use of the dynamic temperature gradient whereas the capacity ratio of nitrobenzene decreases. While under an isothermal regime $n = 9.4$, for the dynamic temperature gradient No. 2 $n = 13.7$. The values of n were calculated according to the following equations[4]. For an isothermal regime:

$$n = 1 + (N^{\frac{1}{2}}/m) \ln (V_n/V_1) \tag{6}$$

where N is the number of theoretical plates, m is a constant ($m = 4$ for peak resolution 4), V_n is the retention volume of the most retained component and V_1 is the retention volume of the least retained component. When using the dynamic temperature gradient, the value of n is the sum of two contributions: n_G, the contribution for the components eluted in the gradient

$$n_G = 1 + (N^{\frac{1}{2}}/m) \ln (1 + l/L) \tag{7}$$

and n_I, the contribution for the components eluted at the isothermal regime before the gradient

$$n_I = 1 + (N^{\pm}/m) \ln (u/w) \tag{8}$$

The dynamic temperature gradient offers a number of advantages similar to those obtained by the programming of the mobile phase composition, *i.e.*, the possibility of controlling the capacity ratio of the components, reducing the time required for the analysis, making the adsorption isotherms linear, etc. It possesses several advantages over programming of the mobile phase composition. The composition of the mobile phase does not change during the experiment and therefore the detector response can always be used for quantitative purposes. After having used the temperature gradient, the sorption properties of the column are not changed and consequently a time-consuming column regeneration procedure is not necessary.

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