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LONGITUDINAL TEMPERATURE GRADIENTS IN LIQUID CHROMATOGRAPHY WITH A BINARY MOBILE PHASE

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SUMMARY

The use of longitudinal temperature gradients in liquid chromatography with a binary mobile phase is described. The chromatographic column is heated gradually to a certain temperature and, in the heated section of the column, desorption of the more polar component of the mobile phase from the sorbent occurs and its concentration in the mobile phase increases. The method involves a combination of concentration and temperature gradients. The time of analysis is shortened owing to the use of the longitudinal temperature gradient in the system considered (sorbent, silica gel; mobile phase, 0.2% di-*n*-butyl ether in *n*-heptane) and the column efficiency is increased. The retention volumes of the components of the mixture being separated can be controlled by changing the ratio of the mobile phase flow-rate to the rate of movement of the temperature gradient.

INTRODUCTION

Temperature gradients, as used in gas chromatography, are only seldom used in liquid chromatography. The use of temperature programmes[1] for sorbent–liquid systems has been described, both the normal programme when the temperature increases with time and the inverse programme when the temperature decreases during the analysis. The normal temperature programme is chiefly used in single-component mobile phase systems[2,3], accelerating the analysis and improving the separation, although to a lesser extent than in gas chromatography. The inverse temperature programme is used in binary mobile phase systems[4,5], which include a highly polar substance at a low concentration (the so-called moderator). Scott and Lawrence[6] quoted a special case of temperature programming with a binary mobile phase.

The dynamic temperature gradient method[7,8] has been described for a liquid–sorbent system. The temperature gradient was created by a moving oven over the column[7] or by a heating spiral fixed to the column[8]. In both instances the temperature gradient moved along the column in the direction of flow of the single-component mobile phase, and the temperature in the gradient decreased in the same direction. The linear velocity of the mobile phase was chosen so as to be higher than that of the temperature gradient procedure at all times. The dynamic temperature gradient

permits regulation of the capacity ratio, increases the column peak capacity and enables the resolution of significant pairs of peaks to be controlled.

A longitudinal temperature gradient has been used for binary mobile phase–sorbent systems, the column being gradually heated to a certain temperature. In the heated section of the column desorption of the more polar component of the mobile phase (the moderator) from the sorbent occurs, which means that this method involves a combination of concentration and temperature gradients.

The principle of the method is shown in Fig. 1. The column is gradually heated to a certain temperature, $T_{max.}$, in the direction of flow of the mobile phase. Part of the moderator is gradually desorbed as the temperature increases and thus the concentration of the moderator in the mobile phase increases. In a particular sorbent–binary mobile phase system with a given composition of the mobile phase, c_0, the total amount of moderator desorbed from the column is proportional only to the increase in temperature. If the time of passage of the longitudinal gradient along the column (i.e., the time necessary to reach the increased temperature at the end of the column) is longer than the retention time of the moderator, t_M, at the original temperature, a concentration pulse of the moderator is created in the mobile phase, which moves along the column ahead of the temperature gradient. The course of the concentration pulse in the given system is dependent on the linear velocity of the mobile phase (u) and on the rate of movement of the temperature gradient (w). The increase in the concentration of the moderator in the mobile phase will appear in the column output at the retention time of the moderator. The initial concentration of moderator in the mobile phase, c_0, in the column output will be reached after heating the whole column at the temperature $T_{max.}$, but the sorbent coverage by the moderator is lower and is reduced by the amount eluted in the concentration pulse.
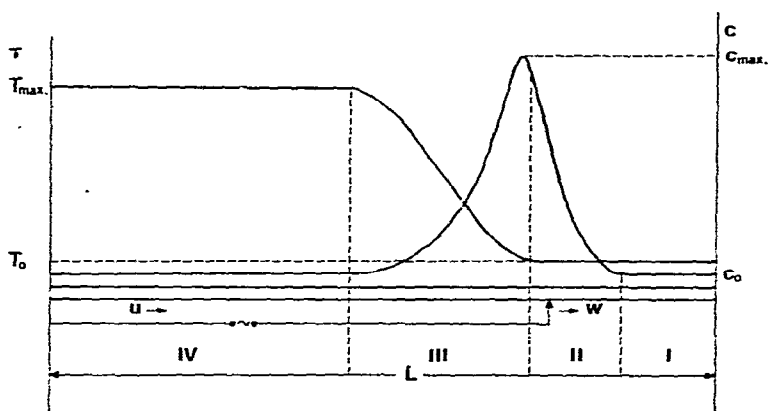

Fig. 1. Diagram of the longitudinal temperature gradient. Description in the text.

On cooling the column is regenerated. The mobile phase moderator is adsorbed on the sorbent until equilibrium is reached at a temperature T_0. During regeneration, mobile phase with a decreased concentration of moderator leaves the column. The column regeneration time is dependent on the rate of cooling of the column, when the mobile phase composition, maximal temperature, $T_{max.}$ and the linear velocity of the mobil phase, u, are constant.

The components of a mixture being separated in a gradient of this type can thus be divided into the following four groups (see Fig. 1).

Component group I

These are components eluted in the isothermal isocratic regime. They are components of the analysed mixture with a retention time shorter than that of the moderator, t_M.

Component group II

These are components eluted in the isothermal regime in the concentration gradient. This interval is given by the time period (t_M, t_1), where t_1 represents the time at which the temperature at the end of the column begins to increase. The retention time of components eluted in this interval is dependent on the concentration course in the gradient; if c_0, u and T_{max}. are constant it depends on the rate of movement of the temperature gradient along the column.

Component group III

These are components eluted in the temperature and concentration gradient within the time period (t_1, t_2), where t_2 represents the time during which equilibrum is reached in the entire column at temperature T_{max}., and the mobile phase leaving the column contains the initial moderator concentration, c_0. During this interval, the components of the mixture are eluted in the temperature gradient with increasing temperature and in the concentration gradient with decreasing moderator concentration.

Component group IV

These are components eluted in the isocratic regime at the temperature T_{max}.. In this instance the initial moderator concentration is in the mobile phase, and the sorbent coverage by the moderator is lower than that at the initial temperature, T_0. The change in the retention volume of the solute related to the isothermal regime at temperature T_0 will depend on the particular sorbent–moderator–solute system. Within a certain temperature interval the retention volume of the solute can decrease, increase, or remain almost constant with increasing temperature[4,5,9]. Under the conditions used in this work, using a moderator of low polarity (di-*n*-butyl ether), we can expect a decrease in the retention volume of the solutes with increasing temperature.

It thus appears that the concentration course of the moderator in the concentration gradient, the temperature course in the temperature gradient and the separation of the mixture being analysed, for a particular system of sorbent and binary mobile phase with given composition, are dependent on the temperature, T_{max}., to which the column is heated, and on the ratio of the flow-rate of the mobile phase to the rate of movement of the temperature gradient.

EXPERIMENTAL

Materials

Sorbent. Silica gel CH (Lachema, Brno, Czechoslovakia) with the specific surface area of 395 m²/g and grain size 50–63 μm was used as a sorbent.

Mobile phase. The binary mobile phase consisted of *n*-heptane plus 0.2% of di-*n*-butyl ether. The *n*-heptane (analytical grade, Lachema) was purified prior to use to remove aromatic hydrocarbons and water[10]. The di-*n*-butyl ether (pure- grade; Reachim, Moscow, U.S.S.R.) was purified by vacuum distillation and dried over the molecular sieve 4A.

Solutes. The solutes were benzene of UV spectroscopy grade and *m*-dinitro-benzene of analytical grade (Lachema), and pure-grade azobenzene and benzaldehyde (Reachim).

Description of the Instrument

A modified dynamic temperature gradient measuring instrument[8] was used for the longitudinal gradient. The mobile phase is led from the container by a diaphragm pump (Type DMP 1515; Orlita, Giessen, G.F.R.) and enters the column, via a pressure impact damper accessory according to Locke[11]. A UV analyser operating at 254 nm (Development Workshop of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia) was used for detection. A differential refractometer (Knauer, Eppelheim, G.F.R.) was used for monitoring the changes in moderator concentration in the mobile phase.

A stainless-steel analytical column (65 cm long, O.D. 4 mm, I.D. 2 mm) was heated by a resistance wire (constantan, diameter 0.3 mm) coiled round it. An adjustable contact moves along the column, connected to one lead of the regulation transformer, the other lead being connected to the beginning of the resistance wire. As the contact moves along the column a constant current is maintained and the column is gradually heated to the desired temperature. The linear velocity of the contact along the column can be varied over the range from about 30 to 150 mm/min. The temperature was measured at the end of the column by a copper–constantan thermocouple.

Measurement

Longitudinal temperature gradient experiments were carried out in the silica gel-*n*-heptane + 0.2% di-*n*-butyl ether system, the mixture being separated containing benzene, azobenzene, nitrobenzene, benzaldehyde and *m*-dinitrobenzene. A constant flow-rate of mobile phase and a constant maximal temperature of the column were maintained, the rate of movement of the temperature gradient was changed, and the influence of these changes on the course of the concentration gradient, on the temperature course in the temperature gradient and on the separation of the mixture being analysed was examined.

RESULTS AND DISCUSSION

The influence of the rate of movement of the temperature gradient, w, in the range 0.070–0.200 cm/sec on the shape of the concentration pulse and on the temperature course in the gradient with u constant at *ca.* 0.7 cm/sec and $T_{max.} \approx 67°$ was observed.

Fig. 2 shows the results of the measurements for the minimal and maximal rates of movement of the gradient used. In addition to the concentration pulse course, the temperature course at the end of the column is also shown. With increasing w, both the concentration and the temperature gradient have a steeper course and a

greater increase in the concentration of the moderator occurs in the concentration gradient (for $w = 0.070$ cm/sec, $\Delta c_{max.} = 0.11\%$, v/v; for $w = 0.200$ cm/sec, $\Delta c_{max.} = 0.41\%$, v/v).

On cooling the column, the di-n-butyl ether in the mobile phase is adsorbed on the silica gel. The column regeneration time was about 20 min under the conditions used.
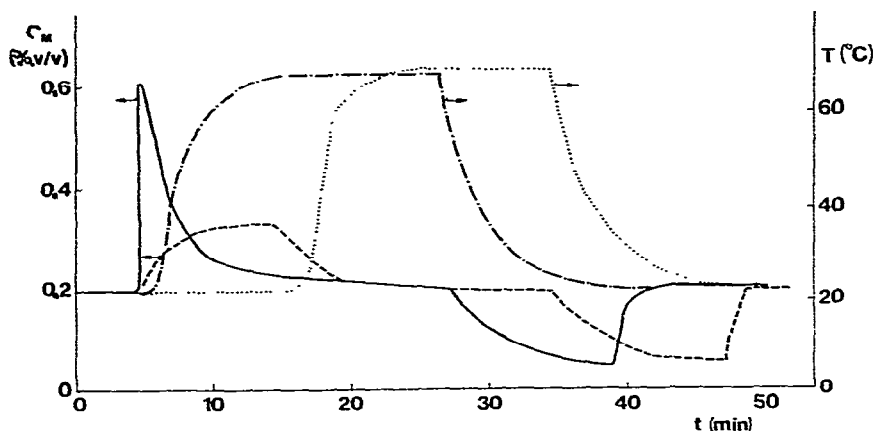

Fig. 2. Course of the concentration pulse of the moderator in the mobile phase and of the temperature at the end of the column for two rates of movement of the temperature gradient. ———, c_M for $w = 0.200$ cm/sec; — — —, c_M for $w = 0.070$ cm/sec; —·—·—·, T for $w = 0.200$ cm/sec; ······ T for $w = 0.070$ cm/sec.
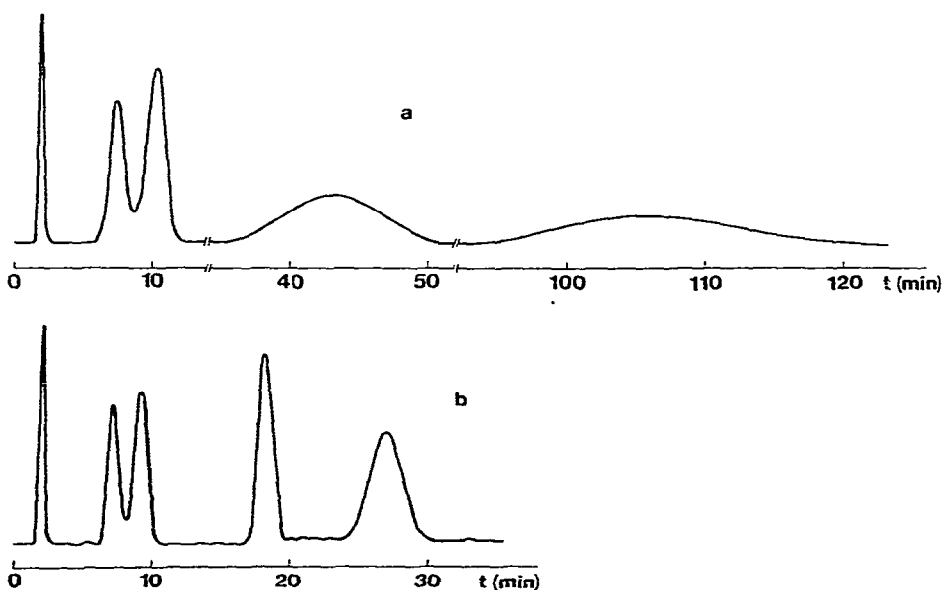

Fig. 3. Chromatograms of a mixture of benzene, azobenzene, nitrobenzene, benzaldehyde and m-dinitrobenzene. Column length, 65 cm; I.D., 2 mm; packing, silica gel CH, 50–63 μm; mobile phase, 0.2% di-n-butyl ether in n-heptane; $u = 0.72$ cm/sec. (a) Isothermal experiment; (b) experiment with the longitudinal temperature gradient, $w = 0.070$ cm/sec.

The application of a longitudinal temperature gradient for separating a mixture containing benzene, azobenzene, nitrobenzene, benzaldehyde, and *m*-dinitrobenzene is shown in Fig. 3. For comparison the separation in the isothermal regime is also shown.

Because the rate of movement of the temperature gradient along the column influences the course of the concentration and temperature gradients, it also influences the retention volume of the solutes eluted in groups II–IV. In the isothermal regime and in the concentration gradient (group II) azobenzene is eluted from the mixture being separated. The dependence of the adjusted retention volume of azobenzene on the rate of movement of the temperature gradient can be understood from Table I, which shows the concentrations of di-*n*-butyl ether in the mobile phase, c_M, at the time when azobenzene leaves the column. The minimal value, V_R, is reached at the maximal temperature of gradient movement, and the adjusted retention volume was 1.59 times lower than that in the isothermal experiment. The column efficiency increased with increasing rate of movement of the temperature gradient. Compared with the isothermal regime, the height equivalent to a theoretical plate (H) was 21 times lower at the maximum.

TABLE I

DEPENDENCE OF THE ADJUSTED RETENTION VOLUME, V_R, OF AZOBENZENE ON THE RATE OF MOVEMENT OF THE TEMPERATURE GRADIENT

V_R (ml)	w (cm/sec)	c_M (%, v/v)	H (mm)
6.25	0.070	0.27	1.078
5.93	0.097	0.33	0.724
5.66	0.102	0.34	0.598
5.07	0.132	0.43	0.488
4.64	0.142	0.44	0.218
4.22	0.175	0.50	0.362
4.27	0.180	0.49	0.247

Nitrobenzene is eluted in the concentration (group II) and the temperature (group III) gradients. The results of the measurements are shown in Fig. 4. At lower values of *w*, when nitrobenzene is eluted in group II, the corrected retention volume is decreased with increasing *w* (open circles). At higher values of *w*, nitrobenzene is eluted in group III (closed circles). As the rate of movement of the temperature gradient increases, the temperature at which nitrobenzene is eluted from the column also increased but at the same time the moderator concentration in the mobile phase decreases. The course of the dependence of V_R on *w* represents a sum of both of these influences. Compared with the isothermal experiment, the corrected retention volume in this section was 1.79 times lower at the maximum.

In the isocratic regime, benzaldehyde and *m*-dinitrobenzene are eluted at an increased column temperature, as shown in Table II. In addition to the adjusted retention volume, V_R, and the rate of movement of the temperature gradient, *w*, the temperature, T_R, at which the solute is eluted from the column, and the height equivalent to a theoretical plate (H) are also given.

Benzaldehyde is eluted from the column at lower rates of movement of the temperature gradient (*w* < 0.14 cm/sec) before reaching the maximal temperature,
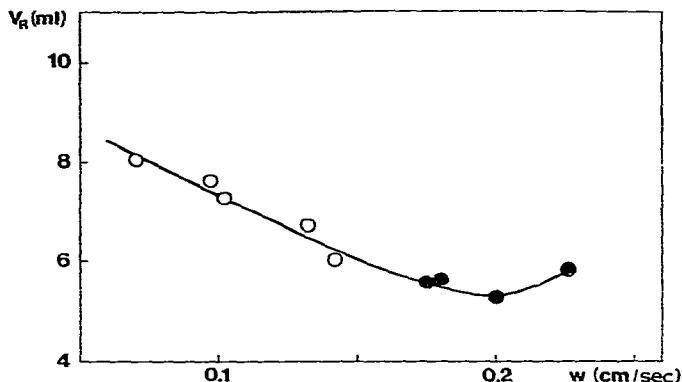
Fig. 4. Dependence of the adjusted retention volume of nitrobenzene on the rate of movement of the temperature gradient. ◯, Nitrobenzene eluted in group II; ●, nitrobenzene eluted in group III.

TABLE II

DEPENDENCE OF THE ADJUSTED RETENTION VOLUME, V_R, AND RETENTION TEMPERATURE, T_R, ON THE RATE OF MOVEMENT OF THE TEMPERATURE· GRADIENT FOR BENZALDEHYDE AND m-DINITROBENZENE

Benzaldehyde				m-Dinitrobenzene			
V_R (ml)	w (cm/sec)	T_R (°C)	H (mm)	V_R (ml)	w (cm/sec)	T_R (°C)	H (mm)
18.03	0.070	50	0.147	27.37	0.070	68	1.552
16.07	0.097	63	0.430	29.78	0.102	66.5	1.865
15.96	0.102	64	0.659	25.15	0.142	69	1.708
13.76	0.142	66.5	0.754	105.36	0	22	1.867
13.53	0.175	67	0.919				
47.93	0	22	1.870				

although the moderator concentration in the mobile phase is less than 0.21 %(v/v) (an exception is the lowest value of $w = 0.070$ cm/sec, when $c_M = 0.23\%$, v/v). The adjusted retention volume is a function of temperature and decreases as the temperature increases. Together with the increasing rate of movement of the temperature gradient, the adjusted retention volume at the maximum was decreased 3.54 times lower than that in the isothermal experiment. The height equivalent to a theoretical plate increases with increasing w but in all instances it has a lower value than that in the isothermal experiment (compared with the isothermal experiment, H is 12.7 lower at the maximum). The increase in H with increasing w can be explained by the fact that at higher rates benzaldehyde is eluted in the temperature gradient section shortly before reaching $T_{max.}$, with a slow temperature increase, so that the rear part of the curve is eluted at.a temperature that is only slightly higher than that of the front part.

m-Dinitrobenzene is eluted from the column after reaching the maximal temperature in the column, the adjusted retention volume being independent of the rate of movement of the temperature gradient. The differences in the retention temperatures and the V_R values are due to the differences in the maximal column temperature

reached. The adjusted retention volume is 4.19 times lower than that in the isothermal experiment. The value of H is also independent of w and is approximately constant.

CONCLUSION

Longitudinal temperature gradients permit the application of dynamic gradients in liquid chromatography with binary mobile phase systems. It shortens the time of the analysis and increases the column efficiency. The change in the ratio of the flow-rate of the mobile phase to the rate of movement of the temperature gradient, u/w, permits the retention volumes of the components of a mixture being separated to be controlled.

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