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Temperature gradients in preparative high-performance liquid chromatography columns

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Abstract

One rarely investigated but important factor in the performance of HPLC columns is temperature. It influences the thermodynamic and hydrodynamic properties of the column. The subject of this work is the distribution of temperature within the column bed. Analytical columns operated at high flow-rates, and large diameter columns packed with small particles are subject to viscous heat dissipation which can seriously affect the quality of the separation. Since the thermal conductivity in a packed column is rather weak, axial and radial temperature profiles will appear in the chromatographic bed. The present paper reports temperature profiles measured in a preparative LC column of 60 mm diameter as a function of the packing material, the eluent flow-rate and the eluent composition. Van Deemter plots show the influence of temperature on the separation process. The influence of the operating parameters on the performance of the column is discussed.

Keywords: Preparative chromatography; Temperature gradients; Viscous heating; Temperature effects; Acetophenone; Diethyl phthalate

1. Introduction

Many thermal separation processes are governed by temperature, which is also true for chromatography.

A lot of work has been done to examine the influence of the temperature on the thermodynamics and the kinetics of the separation process in a chromatographic column [1–13]. The importance of the distribution coefficient and its dependency on the temperature in the chromatographic separation has been thoroughly investigated and is very well understood. Furthermore, many results about the temperature dependency of the plate height achieved in the column have been published [14–16].

These publications clearly show that the temperature of the column needs to be controlled for the sake of reproducibility and quality of the separation.

Thermostatting the chromatographic column does not guarantee a uniform temperature inside the column. Frictional effects also influence the temperature distribution in the chromatographic bed.

The pump of the HPLC system provides the necessary energy to overcome the back pressure of the column at the adjusted flow-rate. The fluid experiences the packing of the column as a homogeneous resistance. The energy provided is dissipated equally over the chromatographic bed (Fig. 1).

In the small interstices between the particles velocity gradients build up. Shear forces causing friction lead to a heat production in the chromatographic bed. Since the thermal conductivity in a column is rather weak only the wall region is cooled down to the wall temperature.

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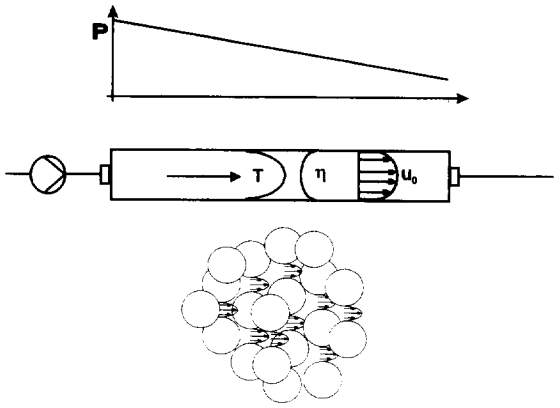

Fig. 1. Model of the dissipation of pump energy in the chromatographic bed. T = temperature, η = viscosity, u_0 = linear velocity.

In the column center the temperature rise causes a decrease of the mobile phase viscosity resulting in an increase of the fluid velocity.

Parabolic velocity profiles lead to band spreading which decreases the separation performance of the column. The effect of the viscous heat dissipation depends on the properties of the packing material and the eluent (thermal conductivity, heat capacity).

The phenomenon of viscous heat dissipation has been reported many years ago [17–19]. It has been mentioned briefly in some publications about viscosity and pressure drop as limiting factors in HPLC columns [20–23]. An extensive theoretical treatment can be found in Refs. [24–26]. Welsch et al. published some further results extending the considerations to preparative columns [27]. The authors stated in their work that a decrease of resolution is due to viscous heating in the column. They concluded that a compensation of the temperature increase inside the column is possible by cooling the eluent to a certain temperature below the column wall temperature. A recent publication presented experimental work and calculations on the temperature and its influence on the peak shape in chromatographic columns [28].

The subject of this paper is a further discussion of the effect of viscous heating in chromatographic columns and a more detailed look on the influence of the eluent temperature on the performance of the separation.

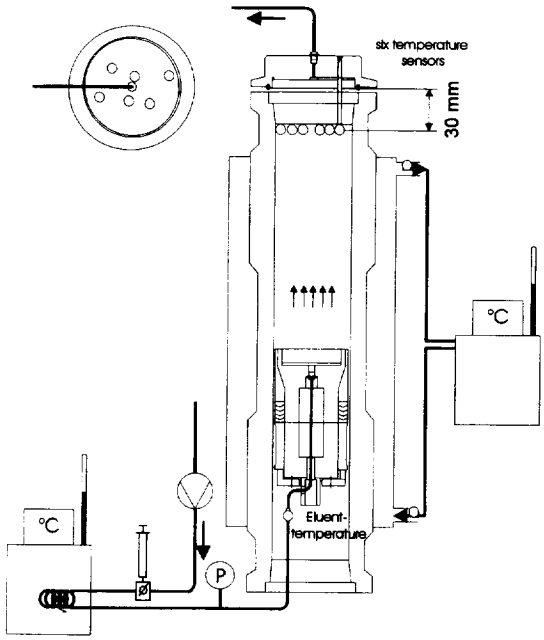

Fig. 2. Experimental set-up.

2. Experimental

2.1. Set-up

The experimental set-up can be seen in Fig. 2. A membrane pump (Lewa Herbert Ott, 7250 Leonberg, Germany, type EKM-2) which provides a flow-rate up to 250 ml/min and a UV detector (Knauer, Berlin, Germany) were used. The chromatograms were recorded with a chart recorder (Kipp and Zonen, Delft, Netherlands, type BD111). The pressure was measured by means of a pressure transducer (Wika Tronic Line, Klingenberg, Germany, type 891.20.501). The samples were injected with a syringe. The eluent temperature was set by immersing a coil made of 1.5 m stainless-steel tube (2.7 mm I.D.) in a temperature bath (Lauda, Königshofen, Germany, type RM 6). As a column we used a dynamic axial compression column LC-60 (Prochrom, Champigneulles, France). This column can be packed with all available packing media. The bed length is determined by the amount of material packed. It is possible to set the temperature of the

column wall by means of a water-jacket thermostated by a temperature bath (Lauda, type RM 6).

The head of the column was equipped with six temperature sensors (PT-100, Type FW51, Sensycon Hartmann and Braun, Hanau, Germany) to measure the temperature at different radial positions about 30 mm away from the outlet frit. The sensors were held in the upright position and separated from the chromatographic bed with six sockets soldered into the top flange of the column. Data acquisition was done with a digital multimeter (Prema Präzisionselektronik, Mainz, Germany, type 6001). Data about temperature distribution over the column, column inlet pressure, detector signal and eluent flow-rate were collected and stored into the computer for later evaluation. With this set-up all experiments, analytical and preparative scale were carried out.

2.2. Chemicals

All solvents used were of standard plant purity. The samples injected were of analytical grade. Two types of packing media were used: (1) coarse silica d_P=35–70 μm (Amicon, Witten, Germany, type matrex granular silica 633), (2) spherical silica d_P= 10 μm (Eka Nobel, Bohus, Sweden, type KR100-10SIL).

2.3. Procedures

For the determination of plate height (HETP) and resolution (R_S), peak width at half of the maximum height was used (Eqs. (1,2)). Unresolved substances were injected separately. Between each measurement the system was allowed to reach thermal equilibrium for about 1 h.

$$N = 5.54\left(\frac{t_R}{W_{0.5}}\right)^2, \quad \text{HETP} = \frac{L}{N} \tag{1}$$

$$R_S = 1.18\left(\frac{t_{R,2} - t_{R,1}}{W_{0.5II} + W_{0.5I}}\right) \tag{2}$$

3. Results and discussion

The effect of the viscous heat dissipation depends on the amount of energy dissipated in the chromatographic bed, the thermal conductivity and heat capacity of the eluent and packing material and the diameter of the column.

The dissipated energy is proportional to the pressure drop over the length of the column. The pressure drop depends on the physical properties of the eluent and the packing material. Fig. 3 shows two temperature distributions measured with the same eluent at identical flow-rate with different particle shape and diameter.

The coarse matrex material forms a bed with high intra-particle pore volume resulting in a comparatively wide cross-section of the column accessible for the eluent. The resulting pressure drop is low and so the increase of the temperature in the center of the column is small (lower points). A spherical shaped material with small particles forms a more dense bed structure which causes a higher pressure drop resulting in a stronger temperature increase (upper points).

Fig. 4 shows the influence of the properties of the eluent (thermal conductivity, heat capacity and viscosity). These measurements have been done at the same flow-rate on spherical material and with different eluent compositions. It is very obvious that the properties of the liquid have a great influence on the temperature profile in the column. The increase of the temperature in the center of the column is stronger for a liquid with a high viscosity, small thermal conductivity and small heat capacity.

Poppe et al. [25] suggested a mathematical model for the calculation of the temperature profile in a chromatographic column (Eq. (3)). Solving this differential heat balance equation by means of a numerical method gives us the possibility to model the measured profiles. ρ is the density of the liquid. For c the effective value of the heat capacity has to be taken. $\overline{\lambda_{ax}}$ and $\overline{\lambda_{rad}}$ mean the effective thermal conductivity in axial and radial directions and are taken equal for simplification. u_0 is the mean linear velocity of the mobile phase. dp/dz is the pressure drop in the column. All data used for the calculations are summarized in Table 1 and assumed to be independent of the temperature and the position in the column. For further details see [25].

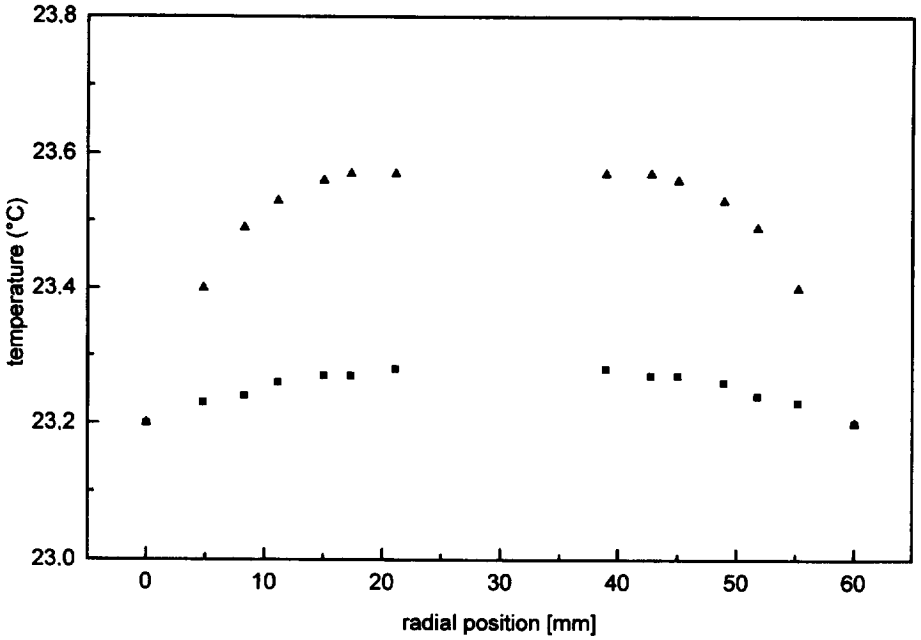

Fig. 3. Influence of the shape of the packing material. Eluent hexane–ethyl acetate (70:30), flow-rate 6.2 cm/min, ▲ spherical silica 10 μm, ■ irregular silica 30–70 μm.

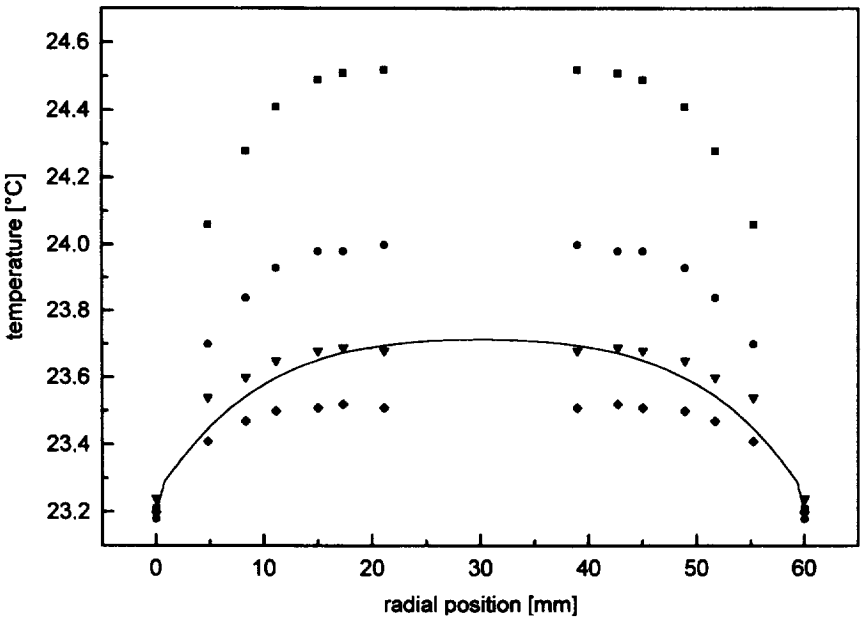

Fig. 4. Influence of the properties of the eluent. Flow-rate 8.8 cm/min, spherical silica 10 μm, ■ cyclohexane, ● toluene–ethyl acetate (90:10), ▼ hexane–ethyl acetate (70:30), ♦ hexane–methyl *tert.*-butyl ether (MTBE) (70:30), ——— calculated profile for hexane–ethyl acetate.

Table 1
Data used for the calculations

c (kJ/kg K)	ρ (kg/m^3)	$\overline{\lambda_{ax}}$ (W/mK)	$\overline{\lambda_{rad}}$ (W/mK)	u_0 (m/s)	dp/dz (bar)
2.0	700	0.38	0.38	0.00295	34.4

$$\rho c\frac{dT}{dt}=\overline{\lambda_{ax}}\frac{d^2T}{dz^2}+\overline{\lambda_{rad}}\left(\frac{d^2T}{dr^2}+\frac{1}{r}\frac{dT}{dr}\right)-u_0\rho c\frac{dT}{dz}+u_0\frac{dp}{dz} \quad (3)$$

It should be possible to compensate for the generation of heat in the center of the column by cooling the eluent to a certain temperature below the temperature of the column wall.

Fig. 5 shows clearly how the performance of the separation of two analytical peaks increases significantly with decreasing eluent temperature. It is obvious that there is an optimum sub-cooling temperature of $T_{opt}=4.59°C$ where the resolution reaches a maximum for the separation of acetophenone and diethyl phthalate in the applied column. A further decrease of the eluent temperature reduces the resolution again. The symbols above the HETP curve refer to Fig. 6.

The temperature profiles in the column used for the separation experiments are reported in Fig. 6. In the case of the eluent having the same temperature like the column wall, the temperature profile is slightly convex due to the heat generation in the column (full circles). The compensation of the generated heat should lead to a maximum of the separation performance (plus signs). Surprisingly, the best separation is reached at a temperature far below that compensation.

The result of that experiment shows that there must be another reason besides viscous heat dissipation for a non-symmetrical band spreading phenomenon inside the column.

In general there is no difference between the separation of two substances in an analytical and preparative column.

A preparative column has a larger diameter than the analytical although their lengths are similar. That means that the plug of substance coming to the entrance of the column must be distributed onto a

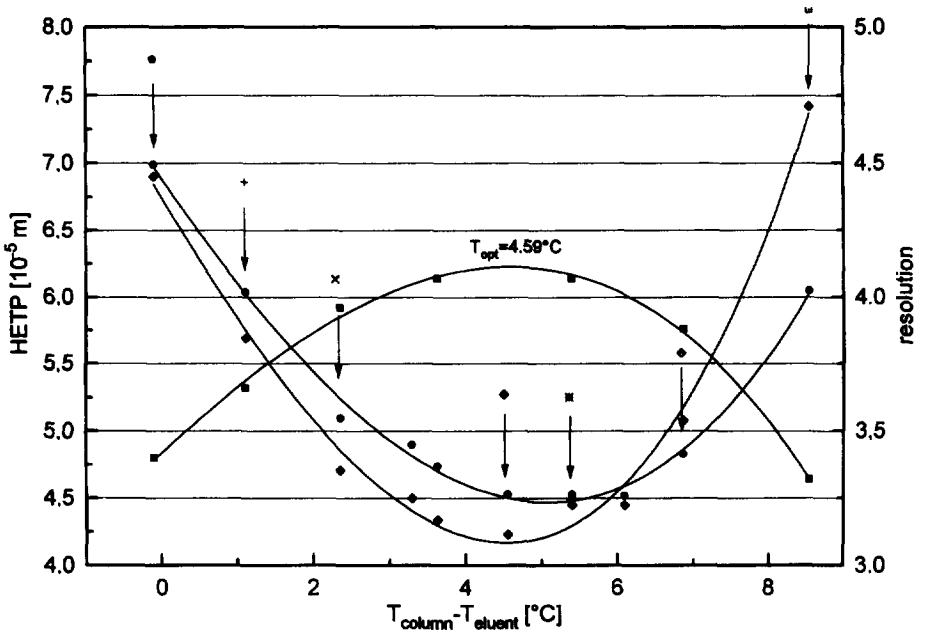

Fig. 5. Influence of the eluent temperature on the HETP (left scale) and resolution (right scale) of acetophenone and diethyl phthalate. Eluent hexane–MTBE (86:14), flow-rate 4.0 cm/min, ♦ acetophenone, ● diethyl phthalate, ■ resolution.

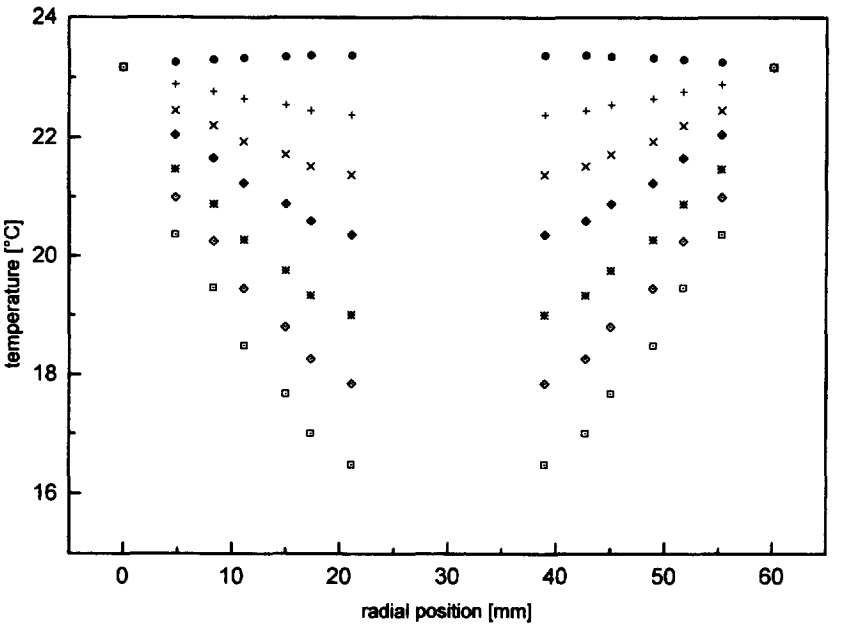

Fig. 6. Temperature profiles in the column with subcooling of the eluent. Eluent hexane–MTBE (86:14), flow-rate 4.0 cm/min. Sub-cooling: ● 0°C; + 1.13°C; × 2.39°C; ♦ 3.65°C; * 5.35°C; ◇ 6.85°C; □ 8.5°C.

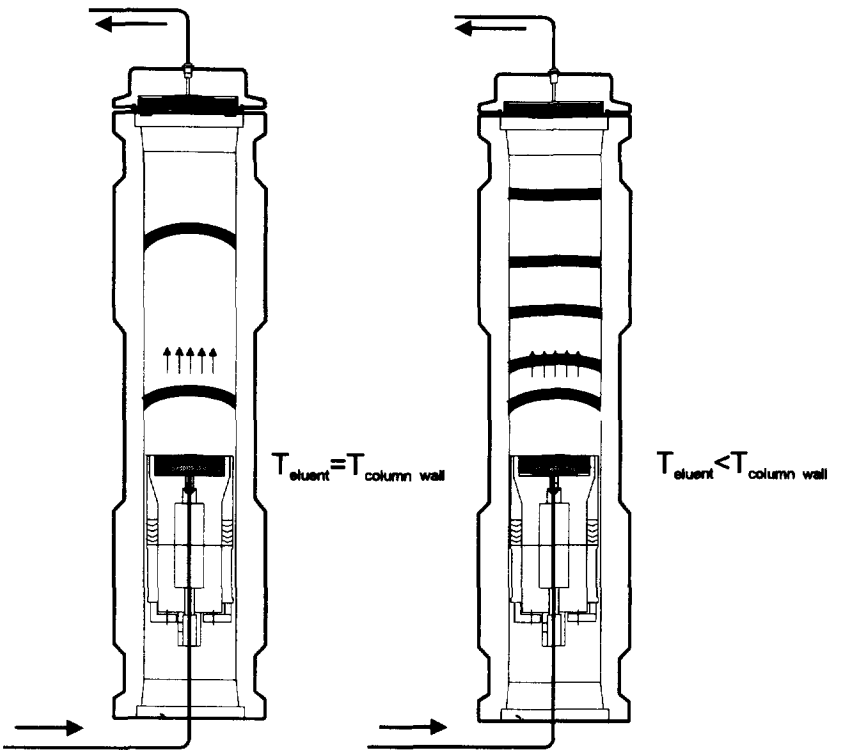

Fig. 7. Influence of eluent temperature on band broadening in a well thermostated column.

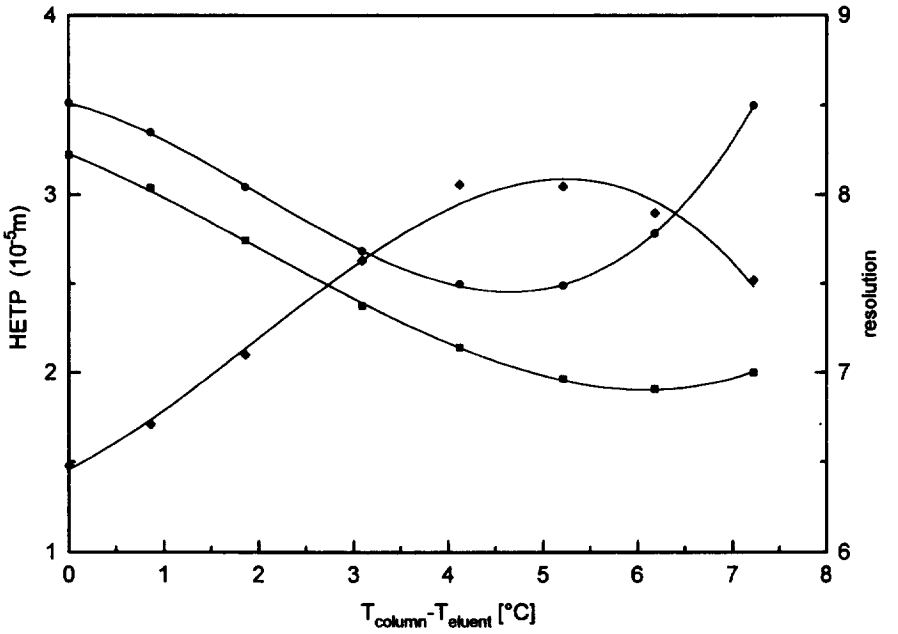

Fig. 8. Influence of the eluent temperature on the HETP (left scale) and resolution (right scale) of toluene and diethyl phthalate. Eluent hexane–MTBE (86:14), flow-rate 4.0 cm/min, ■ toluene, ● diethyl phthalate, ♦ resolution.

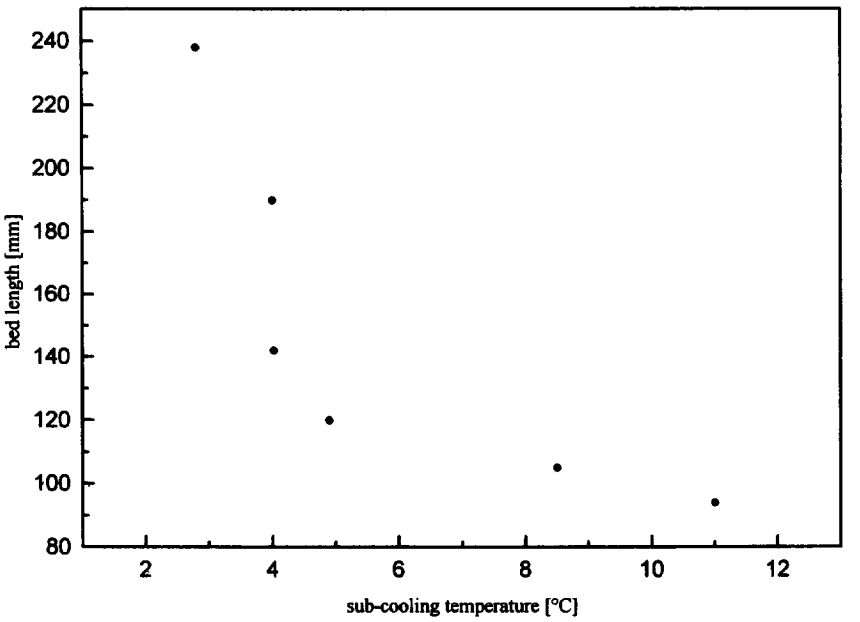

Fig. 9. Influence of the bed length on the optimal sub-cooling temperature. Eluent hexane–MTBE (86:14), flow-rate 4.0 cm/min.

much bigger area before travelling through the column (Fig. 7).

Considering this, it is very likely that a fluid portion travelling at the center of the entrance will pass the inlet frit earlier than another one having to reach a point far away from the center before entering the inlet frit.

Basically we encounter a distribution problem in both the inlet and outlet frit. The delay of the fluid portion having to reach the region near the column wall causes a parabolic band profile starting to travel through the column.

Every distortion of the flat eluting band means a decrease of the separation performance. The parabolic profile created in the inlet frit will encounter the same distortion at the outlet frit.

With cooling the eluent to a certain temperature below the column wall temperature, the center of the eluent stream will travel slower than the wall region, since the wall region is immediate subject to heating from the outside (right side Fig. 7). With a lower migration velocity and temperature in the center it is possible to compensate for both, viscous heating and distribution deficiency.

There are two ways to prove that the distribution deficiency in the head and the bottom is the governing bandspreading effect in a preparative column.

Experimental results (Fig. 8) show that even for an unretained component (here toluene on np-material) there is an optimal eluent temperature below the column wall temperature. The substance does not interact with the stationary phase. So, no influence of the sub-cooling on the adsorption process has to be considered. Obviously the effect is not governed by thermodynamics.

If the lower eluent temperature holds back the current in the core region eluent cooling should be smaller for long columns. The eluent in the wall region does not travel much faster than eluent in the center but has enough way to equalize the band profiles. In a short column the wall region must travel much faster i.e., needs a bigger temperature difference to compensate for the distorted elution band.

Another proof of our observation is the fact that longer columns need smaller sub-cooling (Fig. 9).

Having found the optimum temperature difference for the separation of two substances with analytical

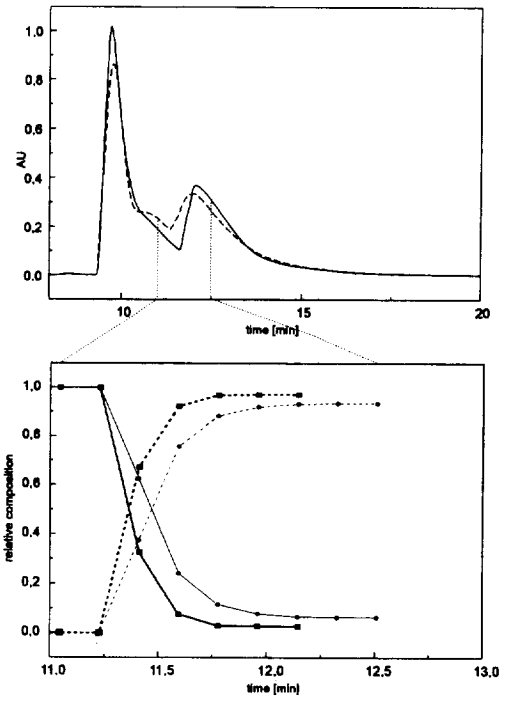

Fig. 10. (Top) Preparative separation of acetophenone and diethyl phthalate. Eluent hexane–MTBE (86:14), flow-rate 4.0 cm/min. Detector signal: - - - no temperature control, ——— with temperature control. (Bottom) Preparative separation of acetophenone and diethyl phthalate. Eluent hexane–MTBE (86:14), flow-rate 4.0 cm/min. Relative composition: no temperature control: ——●— acetophenone, – · – diethyl phthalate, with temperature control: —■— acetophenone, – ■ – diethyl phthalate.

concentration in the 60 mm column and having analysed the mechanism of improvement, we now applied the results to a separation in the overloaded mode.

The shape of the detector signal and the analysis of the mixing zone of the two substances show clearly that the separation could be remarkably improved (Fig. 10).

Since the distribution problem in the inlet and outlet frit becomes more pronounced with increasing column diameter we expect a stronger effect of sub-cooling in production scale columns.

4. Conclusions

The generation of heat inside a chromatographic

column depends on the shape of the packing material, the energy dissipated in the bed, thermal conductivity and heat capacity of the eluent and packing material.

Temperature profiles depending on these factors could be measured and calculated. Results also show that there is an optimal eluent sub-cooling temperature. The subcooling of the eluent is much bigger than the temperature increase due to viscous heating inside the column. In preparative columns a considerable bandspreading effect is the distribution in inlet and outlet frit. With sub-cooling the eluent it is possible to compensate both, viscous heating and distribution problems in the column. The temperature control is considered an interesting tool for the preparative separation of substances.

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