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Enhancement of the separation efficiency through temperature control in preparative high-performance liquid chromatography columns

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Abstract

The flow regime in analytical and preparative HPLC columns is most commonly assumed to have the shape of an ideal plug. This is a helpful simplification for the mathematical treatment of thermodynamics and kinetics of the separation process, but does not necessarily depict the realistic conditions in the separation column. In reality there are different factors causing a deviation from the plug flow pattern in a chromatographic column. The homogeneity of the chromatographic bed determines its permeability. The temperature in the column has an impact on the local viscosity of the eluent. The permeability of the chromatographic bed and the viscosity influence the local velocity of the eluent. The distribution system of the column inlet affects the shape of the eluting band profile. The problem of an even distribution of the injected mixture over the entire diameter of the column increases with increasing column dimensions. The present paper reports some results about the influence of the eluent temperature on the flow regime and the shape of the eluting peaks. It reports clearly how to control the shape of band profiles and how to compensate for a distributional lack by means of the eluent temperature. Pictures taken from eluting dye bands resulting from different chromatographic conditions serve as a clear basis for the effect of the discussed observations. © 1998 Elsevier Science B.V.

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1. Introduction

A preceding publication [1] reported frictional effects in a preparative HPLC column leading to an increase in the temperature in the center of a thermostated column. The viscosity of the eluent decreases with increasing temperature, resulting in an increase in the velocity in the center of the column. Different eluent velocities in a chromatographic column lead to a band distortion which has a detrimental effect on the separation performance.

Experiments showed [1] that compensating for the temperature increase in the center of the column, by cooling the eluent to a certain temperature below the column wall temperature, improved the separation performance significantly. Surprisingly the optimal subcooling temperature T_{opt} is higher than the temperature increase due to friction in the center of the column. Accordingly there must be another reason besides viscous heat dissipation for a non symmetrical band spreading phenomenon inside the column.

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The shape of the eluting band can be influenced by the homogeneity of the packed bed. Guiochon et al. [2,3] impressively demonstrated the influence of the homogeneity of the chromatographic bed on the local velocity of the eluent. Kaminski [4] reported the difference of the bed structure comparing dry packed and wet packed columns.

Another source of peak asymmetry is the design of the distribution system in the inlet and outlet of the column. The distribution of the eluent stream in the inlet and outlet of the column causes the parabolic shape of the eluting band. The problem of distributing the eluent stream over the whole diameter of the column is more pronounced with increasing column diameter. Cooling the eluent to a temperature below the column wall temperature results in a smaller linear velocity in the center of the column compared to the region close to the wall.

By finding the right subcooling temperature, it seems to be possible to compensate for both viscous heating and the distributional lack.

The subject of this paper is a closer examination of the influence of the eluent sub-cooling on the shape of the eluting band and the resulting separation performance in a chromatographic column.

2. Experimental

2.1. Set-up

All experiments were carried out using a dynamic axial compression column LC-60 (Prochrom, Champigneulles, France). A membrane pump (Lewa Herbert Ott, Leonberg, Germany, type EKM-2) which provides a flow-rate up to 250 ml/min and a UV detector (Knauer, 14163 Berlin, Germany) were used. The chromatograms were recorded with a chart recorder (Kipp and Zonen, Delft, The Netherlands, type BD111). 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). The temperature of the column wall was set by means of a water-jacket thermostated by another temperature bath to $T=$ 296.3 K for all experiments.

Data acquisition was done with a digital multimeter (Prema Präzisionselektronik, Mainz, Germany, type 6001). Data about the detector signal, the temperature of the column wall and the temperature of the eluent entering the column, were collected and stored in the computer for later evaluation.

2.2. Chemicals

The eluent used was acetonitrile of standard plant purity. The sample injected was a mixture of indophenol blue, Sudan Red G and 4-dimethylaminoazobenzene dissolved in acetonitrile (0.15%). The injected volume was 1 ml. The column was repeatedly packed with spherical silica $d_P=10$ μm, 100 Å (AKZO Nobel, Bohus, Sweden, type KR100-10-C8). Ethanol was used as the slurry solvent.

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) and (2)).

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

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

Between runs, the system was allowed to reach thermal equilibrium for about 1 h.

2.3.1. Visual proof of the band profiles

The column we had in use was packed with a bed length of 210 mm (internal diameter: 60 mm). After equilibrating the column with the eluent at a flow-rate of 92 ml/min, the first injection was done. Subsequently the pump was run for 2 min and stopped for the next injection. This procedure was repeated four times and the samples were eluted until the first injection started to leave the column. After that, the packing was pressed out into a special tube that consisted of two half shells. Within this tube, a metal sheet was installed which cut the packing into two parts, like a guillotine. After taking the tube apart, a picture of the half cut was taken for documentation.

3. Results and discussion

The chromatographic separation of acetophenone and diethylphthalate improves significantly when the eluent stream is cooled down to a specific temperature below the column wall temperature [1]. In the present paper, a mixture of three dyes (yellow, red, blue) was used as the injected sample to investigate the effect of the sub-cooling on the flow regime in a chromatographic column.

Fig. 1 shows the efficiency HETP of the column for the red dye peak for different eluent sub-cooling temperatures ΔT. The red dye elutes after yellow and before blue in the chosen chromatographic system. The efficiency of the separation increases with decreasing eluent temperature to a certain subcooling temperature. For a further decrease of the eluent temperature, the efficiency decreases again. The optimal sub-cooling temperature resulting in a HETP of 2.5×10^{-5} m is $\Delta T=3.7$°C for the given chromatographic system.

The three dyes elute in close order. From the resulting chromatogram it is hardly possible to calculate the resolution of the separation. To demonstrate the improvement of the separation for the optimal subcooling temperature, the resulting chromatograms were plotted in one figure (Fig. 2). The shape of the chromatogram for a subcooling of $\Delta T=3.8$°C, which is very close to the optimum, depicts the best separation of the three adjacent peaks. The peaks show a straight front and tail. Working with a subcooling smaller or higher than the optimum produces distorted peaks.

To get a better understanding of the influence of the eluent temperature on the separation process, one needs to take a closer look at the flow regime inside the column during the separation process.

Injecting a mixture of three dyes four times with different eluent temperatures before unpacking the column demonstrates the influence of the subcooling on the shape of the eluting band profile. Fig. 3 shows three photographs of the extruded cake cut in the center of the chromatographic bed. The flow direction of the mobile phase was from bottom to top.

Fig. 3a shows the case when the eluent is thermostated to the same temperature as the column wall. The mixture of colours enters the column packing with a parabolic band shape. The center of

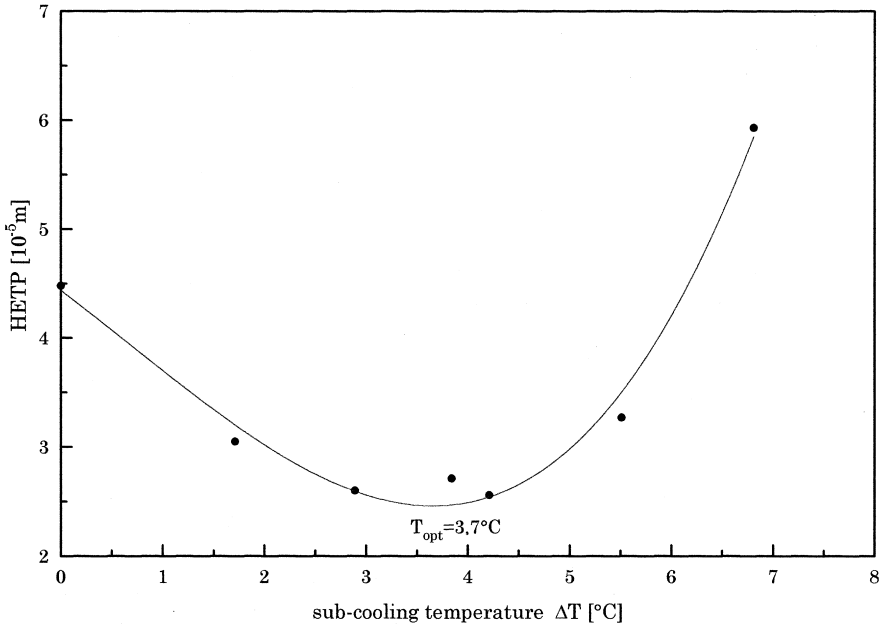

Fig. 1. Influence of the eluent sub-cooling temperature on the HETP of Sudan Red. Eluent: acetonitrile, flow-rate 92 ml/min.

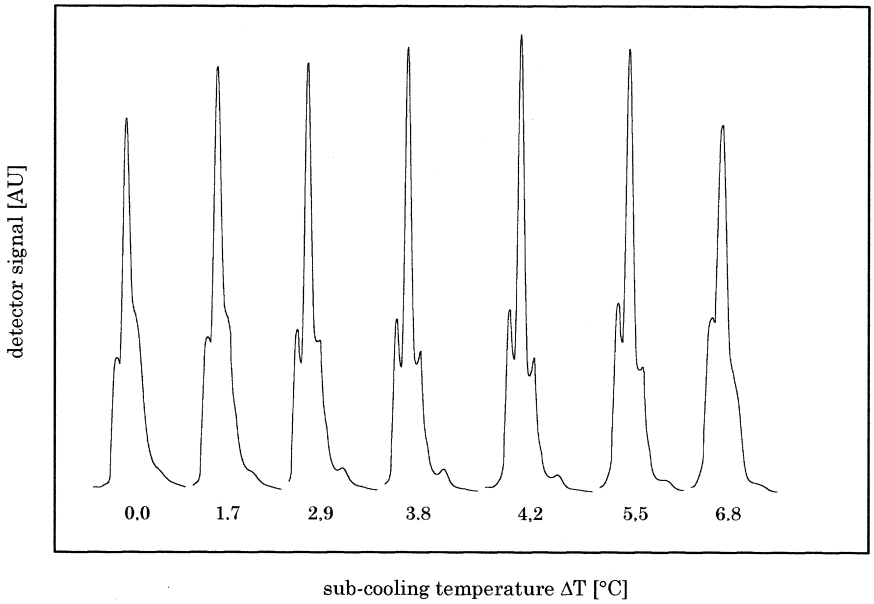

Fig. 2. Comparison of different chromatograms resulting from eluent sub-cooling.

the band shows a small peak, probably due to the fact that the eluent stream enters the column through one central inlet.

The shape of the eluting band is similar to the one reported by Kaminsky [4] for wet packed preparative columns. As a result of the slurry packing method, the density of the chromatographic bed close to the wall is higher than the density of the rest of the bed [3]. A higher density of the bed reduces the flow velocity of the eluent close to the wall. The phenomenon is called the wall effect. Dye molecules in the external region, close to the column wall, propagate a little bit slower than molecules in the rest of the diameter. Comparing the second and fourth injection shows that the influence of the wall effect on the shape of the profile is small compared to the effect of the inlet design.

The frictional heating measured in a previous publication [1] causes fluid in the core region of the column to travel faster than the fluid closer to the wall. Comparing the four profiles in Fig. 3a shows a small increase of the flow velocity at the center of the column with increasing axial position. As a consequence the profile of the first injection at the exit of the packing is more distorted than the fourth injection just entering the column.

It should be emphasized that the strongest contribution to the deformation of the eluting band is caused by the design of the inlet frit. Molecules travelling in the center of column will elute in the front part of the peak. Molecules in the annular region around the center elute a little bit later and shape the center of the peak while those lagging behind, eluting from the region close to the wall, form the rear part of the resulting peak. The major part of the substance elutes in the region close to the wall. As a consequence a distorted band profile like in Fig. 3a contributes to a significant tailing of the peak.

Travelling through the column, the dyes separate with no major change of the band shape. Leaving the column, the eluting band will experience a distortion in the outlet frit, the same as it did in the inlet of the column. As a consequence, the components of a mixture eluting in close order will not be separated sufficiently.

Fig. 3b shows the case when the eluent is thermostated to the optimal temperature below the

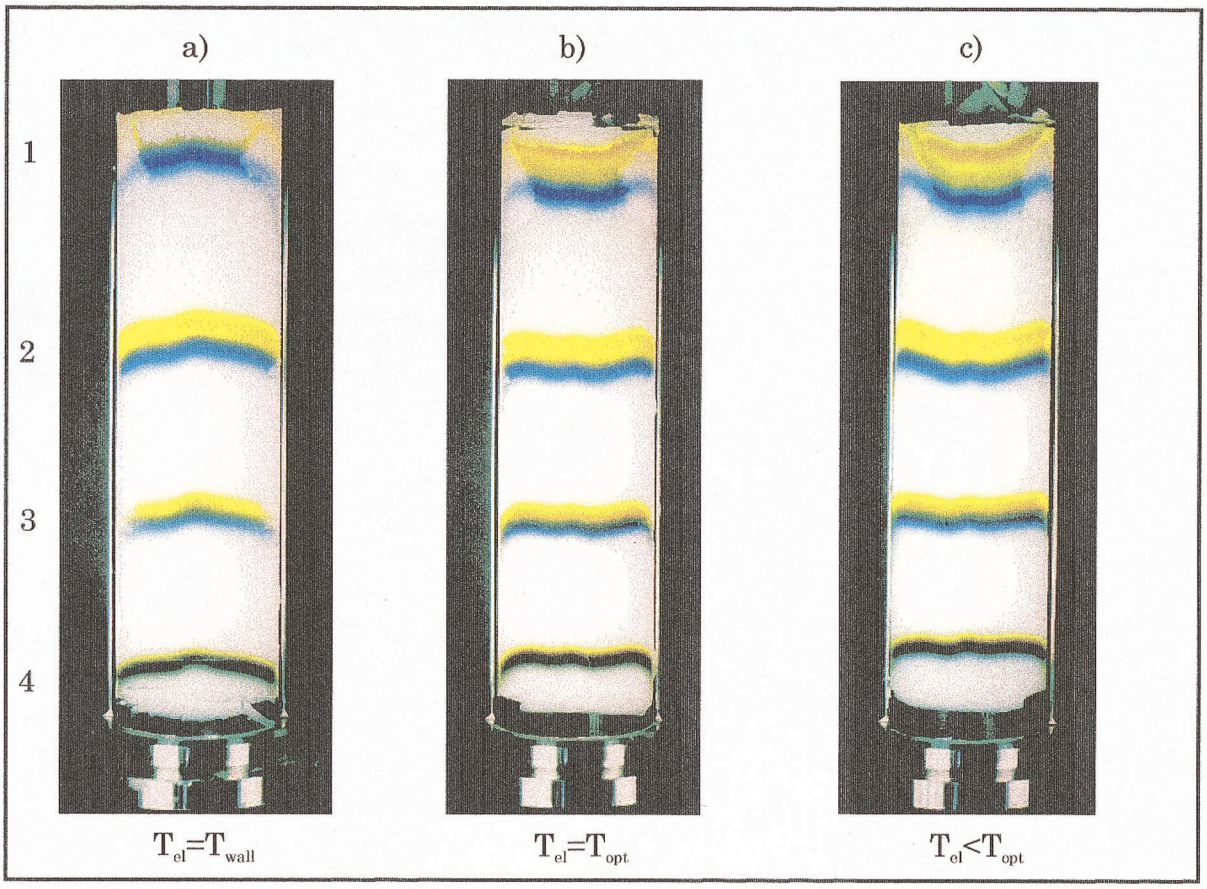

Fig. 3. Photographs of a mixture of three colours eluting from a C8-RP material with different eluent temperatures, (a) $T_{el}=T_{wall}$, (b) $T_{el}=T_{opt}$, (c) $T_{el}<T_{opt}$.

column wall temperature, determined in the experiment described in Fig. 1. The parabolic band entering the column is flattened and eventually turned over on its way through the column which just compensates for the band distortion in the outlet frit. Cooling the eluent stream to the optimal temperature decreases the band width of the eluting peak significantly. Close to the wall, the band migrates slower due to the wall effect.

Fig. 3c shows the case when the eluent is thermostated to a temperature far below the optimal temperature. The point of inflection of the band is closer to the inlet frit and the band turns over, strongly deteriorating the separation improvement.

4. Conclusions

Different factors contribute to an unfavourable distortion of the eluting bands in preparative HPLC columns.

Non symmetric band spreading is caused by frictional heating, inhomogeneities of the column packing and a lack in the distribution system in the inlet and outlet of the column. The contribution of the frictional effects and packing inhomogeneities to the detrimental band spreading is comparatively small compared to the distributional lack.

For a given separation, it is possible to optimize the efficiency of the separation by cooling the eluent

to a certain temperature below the column wall temperature.

References

[1] A. Brandt, G. Mann, W. Arlt, J. Chromatogr. A 769 (1997) 109.
[2] T. Farkas, M.J. Sepaniak, G. Guiochon, J. Chromatogr. A 740 (1996) 169.
[3] T. Yung, G. Guiochon, J. Chromatogr. A 760 (1997) 17.
[4] M. Kaminski, Isolation and Purification 2 (1994) 1.