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INFLUENCE OF THERMAL CONDITIONS ON THE EFFICIENCY OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS

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SUMMARY

The effect of thermal conditions on efficiency in high-performance liquid chromatography is investigated with special reference to the role of the viscous heat dissipation. In systems thermostatted by a water-bath or jacket it turns out to be advantageous to pre-cool the mobile phase when high flow-rates and small particle sizes are used. This is explained by a compensation of band broadening in the first and second part of the column. The wall region of the band moves faster in the first part, but it moves slower in the second part and it is eluted together with the solute from the core region.

Our results indicate that a similar compensation effect occurs when the column is operated in still air. This is due to back-flow of heat through the stainless-steel column wall.

For large diameter columns (larger than 3 mm), packed with small particles, a linear temperature profile imposed on the column wall may diminish the detrimental effects of viscous heat dissipation. Results obtained with such a system are promising.

INTRODUCTION

In a previous paper[1] the viscous heat dissipation[2-4] in liquid chromatographic columns was discussed from the point of view of the heat balance equation, and the predicted temperature increase for columns thermostatted by a liquid bath or jacket was verified experimentally. It was shown that under conditions commonly occurring in practical high-performance liquid chromatographic (HPLC) work (particle size 5 μm, pressure drop a few hundred bar, 4.6 mm I.D.) the inner core of the packed bed is warmer than the wall region by 0.5 to several degrees centigrade, depending on the mobile phase used. In that paper, it was argued, that such a radial temperature profile is bound to cause a non-uniformity in the migration velocity of the solute by two mechanisms.

First, there is the dependence of viscosity on temperature, which is of the order of 2% per degree centrigrade. In the column axis the mobile phase velocity will be higher because the same pressure gradient prevails over the whole cross-section.

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Secondly, the capacity factors of retained compounds depend sufficiently on the temperature to cause an additional and often greater non-uniformity of migration.

The corresponding peak broadening is difficult to describe. Radial re-equilibration, by diffusion and streamline splitting, interacts with the velocity profile, much as in dispersion in open tubes[5]. However, the situation is more complicated, because the profiles are not always parabolic and because only slight equilibration occurs. In fact, in contemporary columns, with lengths of between 6 and 25 cm, inner diameters of 4–5 mm and particle sizes of 3–10 μm, the radial mass transfer is very slow and in many cases negligible[6]. In that case, the column acts as a set of independent parallel ones, each of which corresponds to a certain radial position, and of which the effluents are mixed in the outlet tube. Each of these columns produces an elution curve with a given retention time and peak width, and the observed peak is the summation of these. It follows that under such conditions the rate theory of band broadening does not apply, as the variance of the elution curve will not be proportional to the column length.

The magnitude of this additional band-spreading effect may, depending on the conditions, be comparable with or even larger than the known phenomena described by the usual plate height equations, such as axial diffusion, convective mixing and resistance to mass transfer. It was noted earlier[1] that the trend towards smaller particles and higher mobile phase velocities, aiming at higher speed of separation, can be predicted to promote increased thermal non-uniformity.

The purpose of this work is twofold. We want to obtain direct experimental evidence on the magnitude of the effect, and we also intend to gain insight into the relationship between the thermal conditions and the observed efficiencies. In this way, the very good performance observed by various workers[7–10] with 3-μm packings, which is unexpected on the basis of our earlier estimations[1] of thermal effects, can be explained. Also, this insight will allow guidelines to be derived for future work on high-speed, high-efficiency LC separations.

The next section is devoted to a theoretical derivation of the radial temperature profiles onder conditions of wall thermostatting, with the mobile phase feed at a different temperatures. This derivation is needed for the interpretation of some experimental results to be presented.

TEMPERATURE PROFILES

For the case in which the column wall is kept at a temperature T_e and the mobile phase reservoir is thermostatted at the same value, we have derived earlier[1]:

$$T(r,z) = \triangle \left\{ 1 - \rho^2 - \sum_{s=1}^{s=\infty} \frac{8\, J_0\,(\lambda_s \rho)}{\lambda_s^3\, J_1\,(\lambda_s)}\, e^{-\xi \lambda_s^2} \right\} + T_e \qquad (1)$$

in which $T(r,z)$ is the temperature at longitudinal position z ($z = 0$ at column entrance) and radial position r; $\triangle$ equals $v_s \dfrac{dp}{dz} R^2/4\, \bar{\lambda}_{\rm rad}$, where v_s is the superficial velocity, $\dfrac{dp}{dz}$ is the pressure gradient, R is the internal radius of the column, $\bar{\lambda}_{\rm rad}$ the average effective heat conductivity in the radial direction; and ρ equals r/R; J_0 and J_1 are the Bessel functions of the first kind and zeroth and first order, respectively; λ_s are the roots of $J_0(x)$, ξ equals $z\,\bar{\lambda}_{\mathrm{rad}}/v_s\,d_m c_m\,R^2$ where $d_m c_m$ is the heat capacity per volume unit of the mobile phase.

Conditions under which eqn. 1 can be assumed to be valid are mentioned in ref. 1.

When the mobile phase feed is at a different temperature, the deviation at the column entrance from the wall temperature T_e is conveniently expressed as a multiple of $\triangle$, which is the axial temperature increase that would eventually be reached if the column would be lengthened indefinitely. The entrance temperature is thus $T_e + a\triangle$.

The expression for the temperature distribution is then:

$$T(r,z) = \triangle\left\{1 - \rho^2 + \sum_{s=1}^{s=\infty}\left(\frac{-8}{\lambda_s^3} + \frac{2a}{\lambda_s}\right)\frac{J_0(\lambda_s \rho)}{J_1(\lambda_s)}\cdot e^{-\lambda_s^2 \xi}\right\} + T_e \tag{2}$$

Although eqn. 2 is also generally useful and can serve (with $\triangle = 0$, $a\triangle$ finite), *e.g.*, for the calculation of thermostatting in the column of an insufficiently pre-thermostatted mobile phase (*cf.* ref. 11), we need it here primarily for the cases where the viscous heat is also important and where the mobile phase feed is at a lower temperature. The calculations according to eqn. 2 can be carried out with reasonable effort on a programmable hand-calculator. Resulting profiles in dimensionless representation for some cases of importance are shown in Fig. 1.

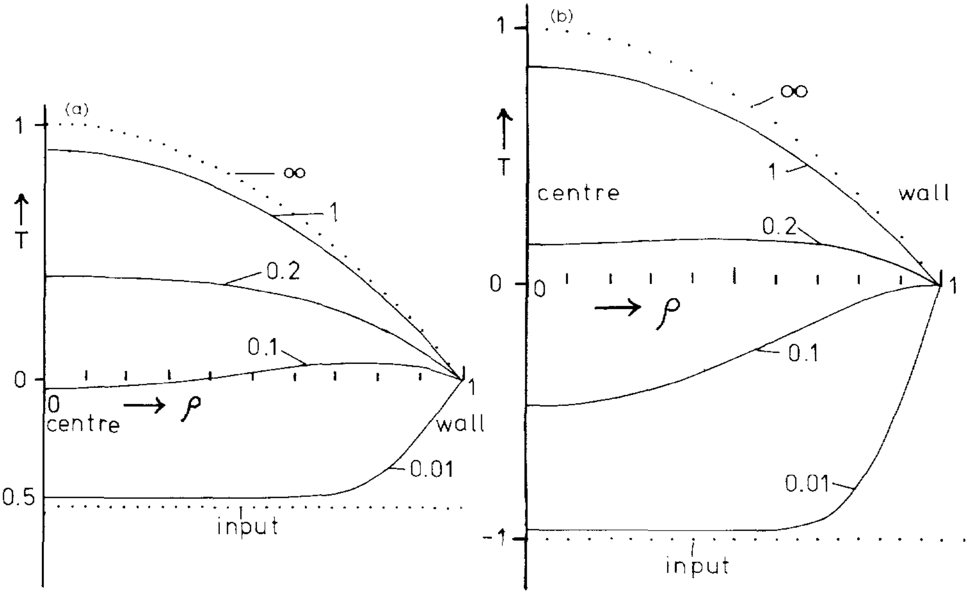

Fig. 1. Radial temperature profiles in wall-thermostatted columns, with the mobile phase feed cooler than the wall, such that (a) $a = -0.5$ and (b) $a = -1$ (see text). For translation of dimensionless temperature increase $\Delta T/\triangle$ and dimensionless length ξ into absolute values with particle diameter $d_p = 5\ \mu$m, see ref. 1. Numbers on the curves indicate the longitudinal position to which the profiles apply.

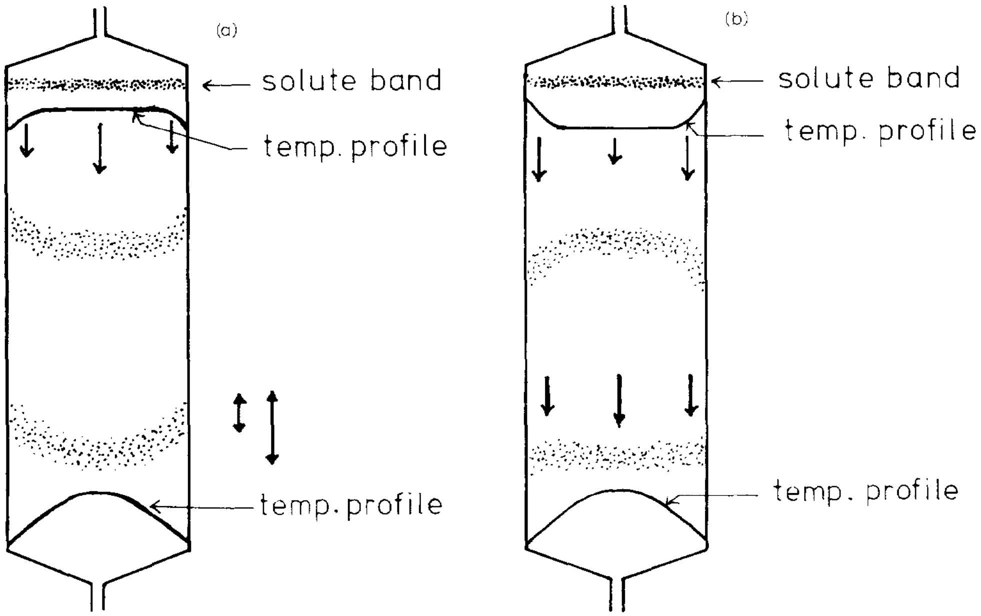

Fig. 2. Schematic view of the transport of a solute band through the column, as influenced by the radial temperature profile under wall-thermostatted conditions (a) with the mobile phase at the same temperature and (b) with the mobile phase cooler.

As can be seen the history of a solute eluted from a column of sufficient length involves two stages. In the first stage ($0 < \xi < 0.1$ for the case $a = -0.5$) the axis of the column is cooler and the solute moves slower in the axis than it does in the wall region. In the second part of the column ($\xi > 0.1$ for the case $a = -0.5$) just the opposite occurs, as the viscous heat dissipation more than outweighs the entrance effect and a higher temperature prevails in the axis.

Band deformations, as depicted in Fig. 2b, can be expected. These should be compared with those in Fig. 2a, applying to the case where the mobile phase is thermostatted at the same temperature and the column axis is always warmer. For the case of a cooler feed temperature the slow radial mass transfer allows a compensation of the band deformations in the two parts of the column (see Fig. 2b). With suitable adjustment of the feed temperature thermal non-uniformity might be corrected for to a large extent, and better efficiencies can indeed be observed under such conditions[2].

EXPERIMENTAL

Equipment

The liquid chromatograph consisted of a syringe pump (LC 8500, Varian, Walnut Creek, CA, U.S.A.). A valve injector (7010, Rheodyne, Berkeley, CA, U.S.A.), equipped with a custom-made bypass system, and a UV absorption detector (PM2D, Zeiss, Oberkochen, F.R.G.).

Thermostatting of the column (if applied) was carried out by means of a water-jacket system (custom) and a circulating liquid thermostat (Type FE, Haake, Berlin, F.R.G.). Prethermostatting of the mobile phase was accomplished by insertion of a 30-cm coiled stainless steel tube, immersed in the water reservoir of a second liquid thermostat (Type 423, Haake), between the pump and the injector[10]. The housing of the latter was likewise immersed.

Chemicals

All solvents and solutes were of analytical grade.

Columns

Column type T was 15 cm × 4.6 mm I.D. It was first packed with 5-μm octyl-modified silica (Hypersil, Shandon, Runcorn, U.K.) and used with 31% aqueous ethanol as the mobile phase, and later packed with octadecyl-modified silica (Shandon) and used with 40% aqueous ethanol.

Column type D was 8 cm × 6.2 mm I.D., obtained from DuPont de Nemours, Experimental Station, Wilmington, DE, U.S.A., by courtesy of Dr. J. J. DiStefano and were delivered packed with 3-μm octadecyl-modified silica (Zorbax ODS). First experiments were carried out with these packings; at a later stage the columns were repacked by us with the same material.

Procedures

Peak widths were measured at 0.6 and 0.1 maximum heights, and from these values plate numbers and plate heights were calculated from the known equations ($H_{0.6}$ and $H_{0.1}$, respectively). Care was taken to allow the system to reach thermal equilibrium: usually 5 min was sufficient.

Column wall temperatures were measured by pressing one wire of a thermocouple pair onto the column wall at the desired position by means of a plastic-covered wooden clothes-peg. Another wire of the same material was pressed in the same way against the first nut of the column hardware at the column top; this junction served as a reference. In preliminary calibration experiments, carried out by means of the two liquid thermostats, it was established that such temperature readings, obtained with the aid of a microvoltrecorder (BD 5, Kipp en Zonen, Delft, The Netherlands) were sufficiently precise.

RESULTS AND DISCUSSION

Liquid thermostatting

In the first series of experiments column T was used with a water jacket at 30°C and fed with mobile phase at various temperatures. This was done with flow-rates of 1, 2 and 3 ml/min. The mobile phase was 31% aqueous ethanol, chosen because of its high viscosity, which can be expected to result in relatively large thermal effects.

The results for the plate height $H_{0.6}$ are given in Figs. 3a–c for the three solutes uracil, nitrobenzene and dinitrobiphenyl at the three flow-rates as a function of the temperature of the mobile phase feed, T_m.

These figures show that for low flow-rates (1 ml/min) there is no influence of the mobile phase temperature; the thermostatting in the column is effective. This is in agreement with theoretical prediction. The relaxation length, l_1, over which the temperature deviation from the ultimate profile declines with approximately a factor e (2.718), as introduced in ref. 1, can be calculated from Table I and eqn. 12 of ref. 1 to be 6 mm.

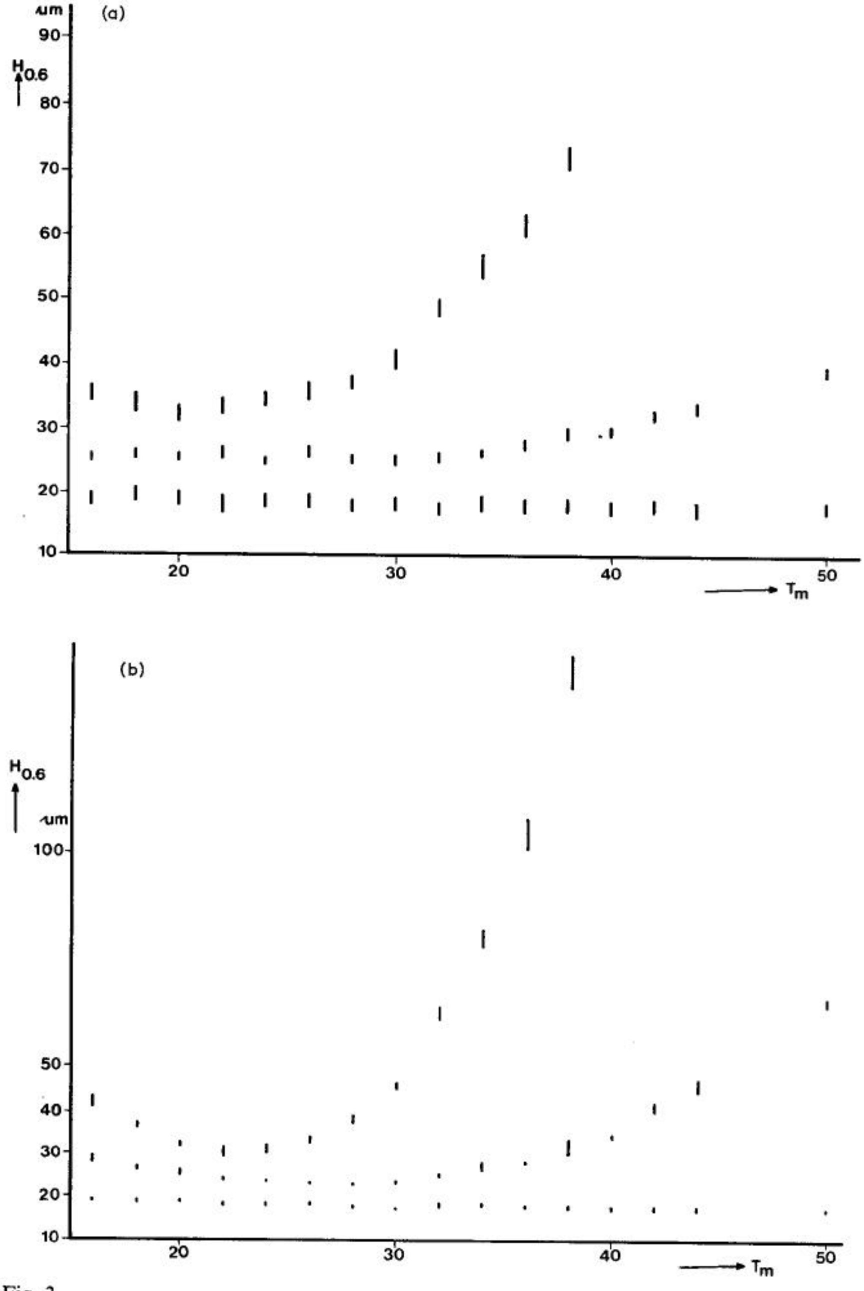

Fig. 3.

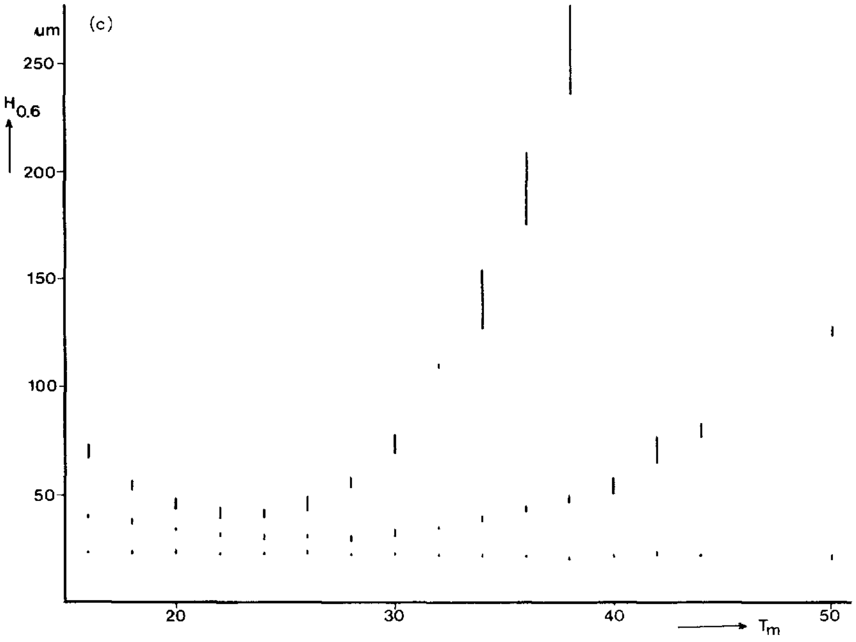

Fig. 3. Dependence of column efficiency, observed via $H_{0.6}$, on the mobile phase feed temperature, T_m. Column water-jacket at 30°C. Column type T. Flow-rates 1, 2 and 3 ml/min for lower, middle and upper plots, respectively. Pressure drops, 100, 200 and 300 bar, respectively. Solutes: (a) uracil (unretarded); (b) nitrobenzene (capacity factor, $k' = 3.7$); (c) dinitrobiphenyl ($k' = 11$). Mobile phase, 31% aqueous ethanol. Bars indicate the ranges of the results obtained.

The plots for the flow-rate of 3 ml/min show a more-or-less quadratic dependence of $H_{0.6}$ on T_m. The relaxation length l_1 is proportional to the flow-rate and thus it is *ca.* 18 mm here. This means that a significant part of the column is influenced by the value of T_m. The final temperature rise in the column axis at the end of the column with respect to the wall is virtually equal to the asymptotic value, Δ, and can be calculated according to eqn. 1 to be 1.7°C.

The most important feature of the 3 ml/min plots in these figures is the fact that the minimum is well below 30°C, the column wall temperature. Higher efficiencies are found by using a mobile phase that is slightly cooler than the column jacket. This indicates that the compensation effect, as illustrated in Fig. 2b, indeed occurs.

The minimum in the plate height is found at *ca.* 21–25°C. This large deviation

TABLE I

THERMAL PROPERTIES OF THE MOBILE PHASES USED (APPROXIMATE VALUES)

	$d_m c_m$ (10^6 Jm^{-3} $^0C^{-1}$)	λ (Wm^{-1} $^0C^{-1}$)
31% (v/v) Aqueous ethanol	3.8	0.47
40% (v/v) Aqueous ethanol	3.3	0.41
50% (v/v) Aqueous acetonitrile	3.0	0.4

from 30°C (compared with the value of 1.7°C predicted for △) may seem surprising at first glance. However, it should be noted that the feed temperature and, thus, its radial profile is operative only in the few first centimeters of the column. The band distortion due to the viscous heat profile is therefore operative throughout the larger part of the column. In order to compensate for this in the first few centimetres an intensive thermal gradient must be imposed on this part.

Similar experiments were carried out with the type D columns, for two mobile phases, 40% aqueous ethanol and 50% aqueous acetonitrile. The results for the second, low-viscosity phase are shown in Figs. 4a and 4b for flow-rates of 3 and 6 ml/min, respectively.

The calculated values for △ and l_1 for the 3 ml/min experiment are 3.5°C and 20 mm, respectively. Fig. 4a shows a minimum at *ca.* 25°C for the two retarded

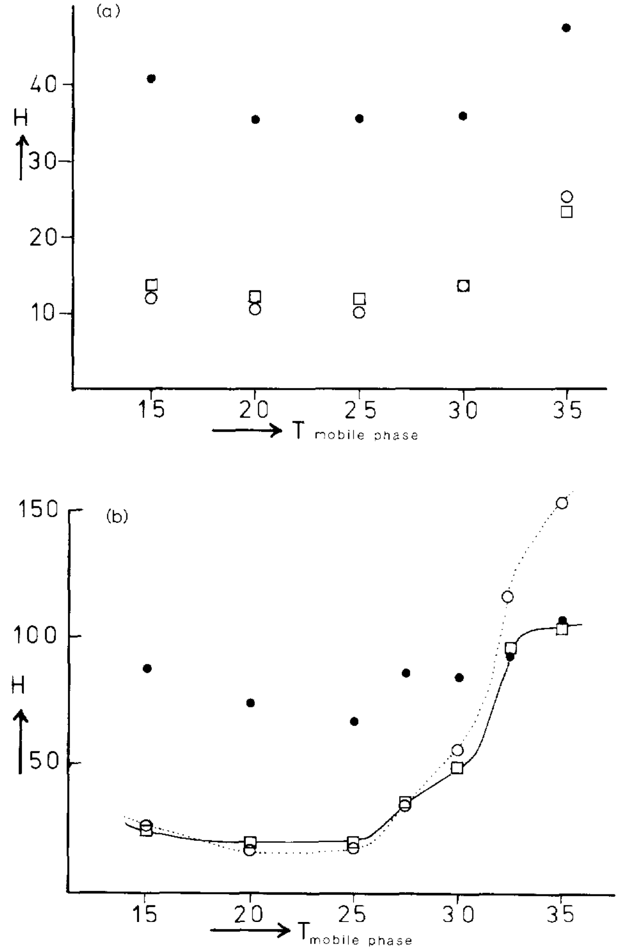

Fig. 4. Dependence of the column efficiency, observed via $H_{0.6}$, on the mobile phase feed temperature, T_m. Column water-jacket at 30°C. Column type D. Flow-rates (a) 3 and (b) 6 ml/min of 50% aq. (v/v) acetonitrile. Pressure drops (a) 150 and (b) 285 bar. Solutes: uracil (●, unretarded); nitrobenzene (○, k' = 2.2); dinitrobiphenyl (□, k' = 4.1).

solutes. The measurements for the unretarded component are too much impaired by the external peak broadening effects. These were practically inavoidable, because of the long connecting lines necessary in the thermostatting experiments.

The minimum at 25°C indicates that the temperature difference imposed on the mobile phase must be larger than $\triangle$ under these conditions. The explanation, taking into account the small l_1 value, is the same as that given above for the type T column experiment.

A much lower minimum is found in Fig. 4b. For these conditions values of 14°C and 40 mm for $\triangle$ and l_1 can be calculated. The position of this minimum is not readily discernible, but it probably lies at *ca.* 20°C. The compensation of the band distortion in the last part of the column is therefore accomplished in this case by cooling the mobile phase by an amount about equal to $\triangle$. In view of the fact that l_1 is equal to half the column length, this is understandable.

Fig. 5 shows an example of the peak shapes obtained during these experiments. These shapes could be obtained reproducibly by manipulation of the two temperatures. The maximum peak height obtained in the example shown for 20°C corresponds to the minimum plate height obtained in Fig. 5b. The trend towards peak-doubling for the two extreme temperatures, 15°C and 35°C, is noteworthy, but as yet unexplained. Computer simulation studies on the shape of elution curves under such conditions are underway.

In the experiments carried out with aqueous ethanol, pressure limitation (*ca.* 600 bar) did not allow flow-rates higher than 3 ml/min. At this flow-rate the phenomena were comparable with those obtained at 6 ml/min with aqueous acetonitrile. Also, at 2 ml/min, significant effects due to viscous heating were found.

Air thermostatting

The experiments described above are at first sight in disagreement with successful operation (reduced plate heights 2–3) of 3-μm columns at high velocities found by several workers[7–10]. With the exception of Cooke *et al.*[9], they did not take the unusual precaution of thermostatting the mobile phase at lower temperatures. It was therefore decided to study the operation of columns further in still air and in a circulating air bath.

Although in still air a considerable extent of heat transport to the surroundings may remain, it may be useful to treat such a condition to a first approximation like that of a thermally insulated ("adiabatic") columns. As noted before[1,3], the viscous

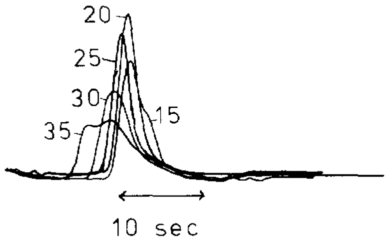

Fig. 5. Peak shapes observed for various mobile-phase feed temperatures. Conditions as in Fig. 4. Solute, dinitrobiphenyl. Overlapping recordings were started at the same position at the same moment after injection. Peak shapes for nitrobenzene observed under the same conditions have roughly the same appearance.

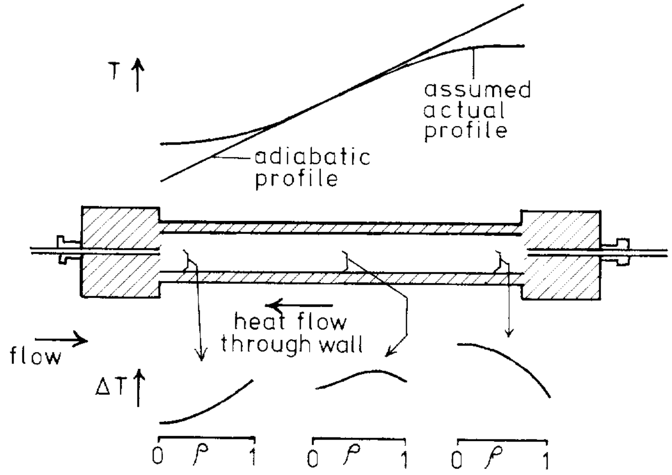

Fig. 6. Thermal conditions in an thermally insulated column with a stainless steel wall of reasonable thickness.

heating then tends to establish the truly adiabatic situation, a linear longitudinal gradient, with zero radial gradients. However, the stainless-steel wall conducts heat from the warm end of the column to the entrance. The result is that the longitudinal gradient is diminished in magnitude and that radial gradients of opposite signs develop in the beginning and end portions of the column. The situation is illustrated in Fig. 6, but only qualitatively because we did not succeed in deriving mathematical expressions for the temperature profiles in these, more complicated, cases.

For the transport of solutes, such a situation might provide the same compensation of band distortion in two column portions, as illustrated in Fig. 2b, and this might explain the good efficiencies found by other workers under less well-defined thermal conditions with 3-μm particles.

Fig. 7a–c gives evidence of the validity of this description. The efficiencies found for the still-air operation are always the highest (open symbols). At the bottom of the plots the gradient of the surface temperatures of the column wall is given. It

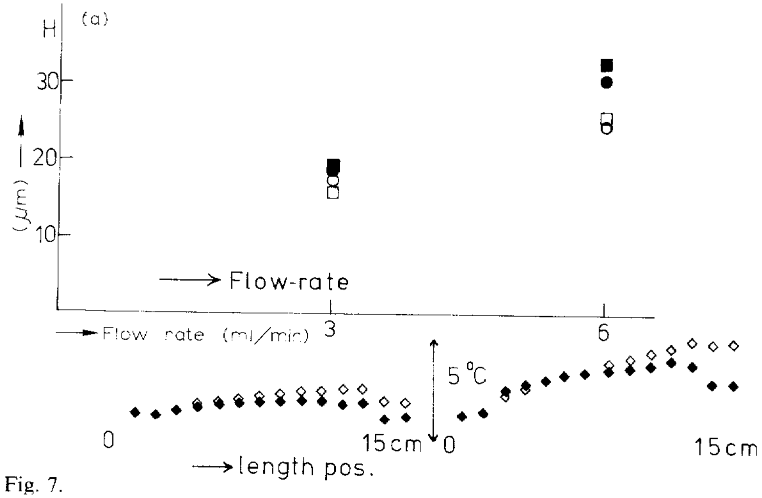

Fig. 7.

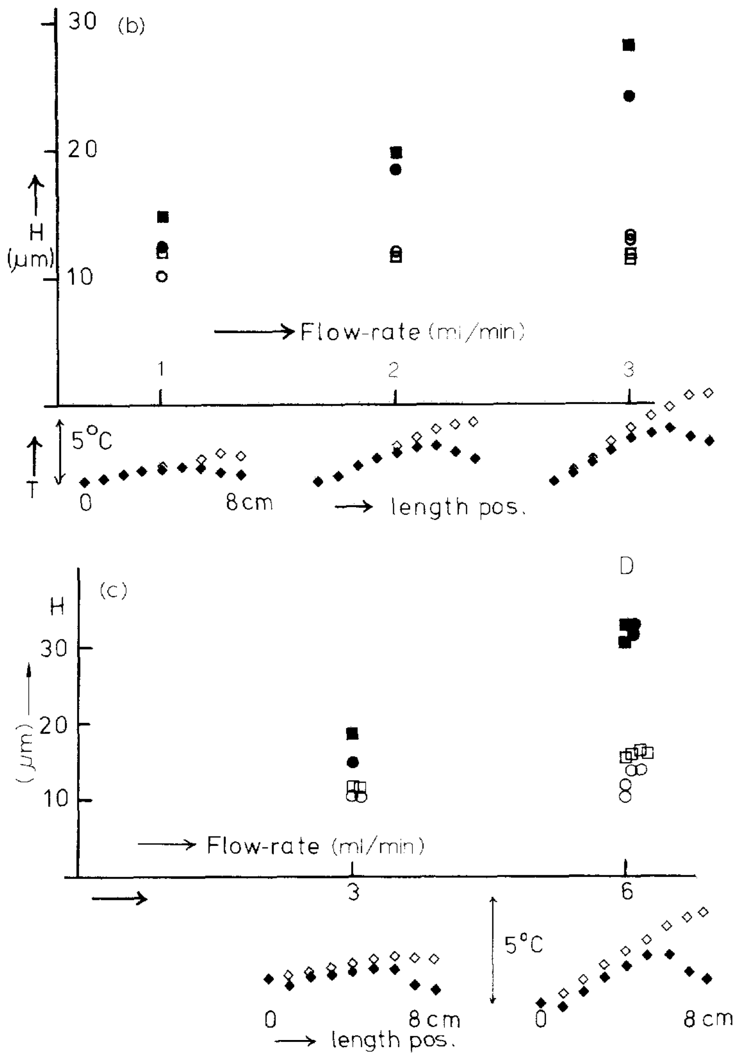

Fig. 7. Dependence of the efficiency, observed under air-thermostatting conditions, via $H_{0.6}$, on the mobile phase flow-rate and the air velocity. Columns: (a) type T; (b) and (c) type D. Mobile phases: (a) and (c) acetonitrile; (b) 40% aqueous ethanol. Symbols for solutes as in Fig. 4. Open symbols, measurements in still air; closed symbols, measurements in an air flow perpendicular to the column axis with a velocity of *ca.* 2 m/sec. Bottom plots represent the surface temperature of the chromatographic tubes at 1-cm intervals, the two extreme points on both sides being located on the terminator nuts. Capacity factors for nitrobenzene and dinitrobiphenyl, respectively, in aqueous acetonitrile were 2.1 and 4.0, and in aqueous ethanol were 3.6 and 6.3.

can be seen that these gradients are approximately linear for the still-air case. However, the calculated adiabatic temperature increases, $\Delta p/d_m c_m$ according to Halász *et al.*[3], equal to 9, 15 and 14°C, respectively, for the highest flow-rates in Figs. 7a, b and c, are considerably higher than the observed ones, the differences between entrance and exit terminator nuts. This indicates that considerable back-flow of heat occurs, with concurrent radial profiles and compensation of band deformation.

By application of an air flow perpendicular to the column this condition is disturbed. The linear temperature profile is distorted (closed symbols in the bottom plots of Fig. 7), and radial profiles of the normal sign predominate. Compensation no longer takes place, and a significant to large effect on the plates heights is observed in Fig. 7.

Linear wall profile experiments

From the point of view of efficiency, the truly adiabatic condition, with no radial profiles and a linear longitudinal profile, is obviously the most favourable. With stainless steel of reasonable thickness as the wall material this is impossible to accomplish. However, it appears possible to mimic this condition by imposing a

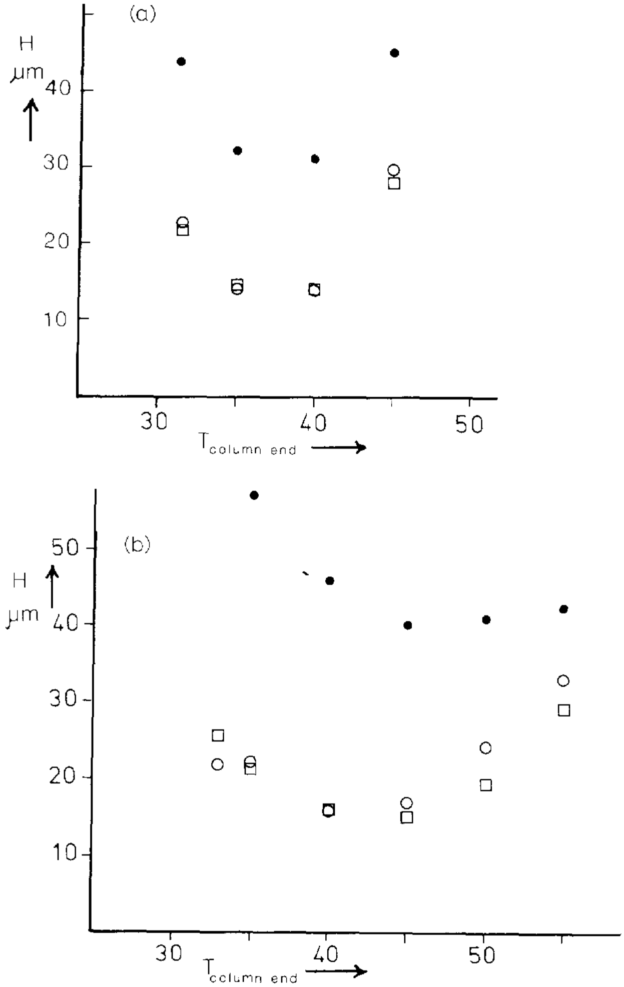

Fig. 8. Results of the linear wall profile experiment. Efficiency, observed via $H_{0.6}$, as a function of the temperature of the outlet terminator. For thermal conditions see text. Column type D. Mobile phase, 40% aqueous ethanol at flow-rates of (a) 2 and (b) 3 ml/min. For solutes, symbols and capacity factors see Fig. 7b.

linear temperature gradient onto the wall artificially. The temperature difference between the end and the beginning should be equal to the adiabatic increase according to Halász *et al.*[3]. There would then be no temperature difference between the wall and the packing at any longitudinal position and, thus, radial, heat transport with concurrent profiles would be absent.

Some experiments along these lines were carried out with the type D column. The entrance union was immersed in the water bath of the mobile-phase thermostat. A heating wire was wound around the end union. The temperature of the latter could be measured by means of a small thermistor (*ca.* 0.5 mm diameter), glued into a 0.7-mm hole of 3-mm depth, drilled into the nut. The column tube and most of the terminator sections were wrapped in a thermally insulating material (glass fibre). By adjustment of the voltage on the heating coil of the end terminator the temperature of the latter could be controlled.

Results for the 40% aqueous ethanol mobile phase are given in Figs. 8a and 8b for flow-rates of 2 and 3 ml/min, respectively. Again the results for the unretarded solute are unreliable because of external effects. The graphs for the retarded solutes show minima at *ca.* 37 and 42°C, respectively; *i.e.* 7 and 12°C higher than the mobile phase feed temperatue.

These differences should coincide with the adiabatic increases. Under the conditions used these are equal to 5 and 9°C, respectively. In view of the approximations involved (constant values of heat capacity and viscosity, no pressure drop in terminators, etc.) the agreement can be considered to be quite satisfactory.

Unfortunately, the column used for this experiment, having been in use for a considerable time under severe thermal and flow-rate conditions, no longer had optimal efficiency. Therefore, the anticipated improvement in efficiency in comparison to air-bath operation could not be demonstrated.

Throughout this work, measurements of plate heights on the basis of 0.1 maximum height were also carried out. The results did not deviate substantially from those with 0.6 maximum height.

CONCLUSION

The purpose of this work was to elucidate the role of thermal effects as a limitation to the increase of speed and efficiency in HPLC. Although this has been accomplished in our view, it should be admitted that the gain in speed and efficiency arrived at during this work, in comparison to state-of-the-art performance reported by other workers, is marginal or absent.

To a large extent, this has been due to experimental difficulties. Most of the work has been carried out with columns (both types) the packing structure of which was no longer optimal, with the result that reduced plate heights even under the most favourable thermal conditions are larger than 3. The columns were deteriorating during the preliminary stages of this work, when rather drastic conditions were applied. As a result, we were unable to show that special measures with respect to thermal conditions can lead to better performance. Comparison should be made, of course, with the straightforward use of such columns, well packed and with a well-chosen mobile phase under reasonable flow-rates. Under such conditions others found long-lasting, good efficiencies (reduced plate height, h = 2–3) for the 6.2[7] as well as for the 4.6-mm columns[10].

However, this state of affairs in our view does not impair the validity of our findings. The thermal effects, as an additional cause of band broadening, are observable to an extent that supersedes the normal broadening mechanisms. If it is significant in the damaged columns we used, it will certainly be so for good columns.

Although we sometimes chose conditions in which the thermal effects are magnified (large diameters, high viscosities), it can be deduced that under less drastic conditions the effect with 3-μm particles is also significant. This will hold even more so for future attempts to increase speed and efficiency by reduction of particle size and increase of flow-rate, as long as normal column diameters are used.

Conditions of compensation, which we believe play a role in most succesful columns at high flow-rates with 3-μm particles, or the linear wall profile conditions operation as proposed in this paper, constitute a reasonable option from the efficiency point of view. While it is accepted that the separation process takes place in a non-isothermal system, the drawbacks should be noted. Many phase systems do not withstand temperature gradients of the magnitude encountered in this work. Also, the reproducibility and the ease of interpretation of retention data, and even the reproducibility of the separation *per se* is at stake, even for less sensitive phase systems. The optimum compromise will depend greatly on the phase system and sample at hand.

A final remark should be made about the usefulness of thermostatting the column and the mobile phase at the same temperature, as is often done. Our results indicate that this has a negative effect on efficiency when high flow-rates and small particles are used. On the other hand, the reproducibility of separations and retention data will be improved compared with the condition where no thermostatting is applied. However, the accuracy of the retention data is still doubtful, because the inner part of the column bed is warmer to an extent that is usually unknown.

All this is also dependent on the effectiveness of the thermostatting of the column wall. Our results indicate that air thermostatting does not even keep the wall of the column at the right temperature (*cf.* Fig. 7) when viscous dissipation is large. The thermal resistance between column and air appears to be too large. Accordingly, the negative effect of air thermostatting on efficiency may not be as great as that of water thermostatting in some cases.

ACKNOWLEDGEMENTS

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REFERENCES

1 H. Poppe, J. C. Kraak, J. F. K. Huber and J. H. M. van den Berg, *Chromatographia*, 14 (1981) 515.
2 H. Poppe, *183rd ACS National Meeting, Las Vegas, Nevada, March 28 April 2 1982*, Abstracts No. Anyl 71.
3 I. Halász, R. Endele and J. Asshauer, *J. Chromatogr.*, 112 (1975) 37.
4 Cs. Horváth and H.-J. Lin, *J. Chromatogr.*, 149 (1978) 43.
5 R. Aris, *Proc. Roy. Soc., London Ser. A*, 235 (1956) 67.
6 J. H. Knox, G. R. Laird and P. A. Raven, *J. Chromatogr.*, 122 (1976) 129.
7 R. W. Stout, J. J. DiStefano and L. R. Snyder, *J. Chromatogr.*, 261 (1983) 189.
8 I. Halász, H. Schmidt and P. Vogtel, *J. Chromatogr.*, 126 (1976) 19.
9 N. H. Cooke, B. G. Archer, K. Olsen and A. Berick, *Anal. Chem.*, 54 (1982) 2277.
10 M. W. Dong and J. L. DiCesare, *J. Chromatogr. Sci.*, 20 (1982) 369.
11 S. Abbott, P. Achener, R. Simpson and F. Klink, *J. Chromatogr.*, 218 (1981) 123.