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Influence of viscous heat dissipation on efficiency in high-speed high-performance liquid chromatography

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Abstract

The effect of viscous heat dissipation on efficiency in high-speed high-performance liquid chromatography was investigated for columns packed with non-porous 1.5 μm particles. When these columns are operated at high flow-rates and therefore high-pressure drops, viscous heat is generated leading to axial and longitudinal temperature gradients within the column which are translated into secondary gradients of the mobile phase viscosity, the flow velocity, the diffusion rate, and the retention factor. This resulted in additional band broadening which can destroy the separation quality as demonstrated by chromatograms and plate height curves. Thermostating of the eluent to a specific temperature below the column wall temperature compensated for this effect. The necessary degree of precooling decreased with increasing column temperature. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Temperature effects; Heat dissipation; Viscous heat dissipation; Eluent precooling; Efficiency; Steroids; Nitrobenzenes; Nitrotoluenes

1. Introduction

In a number of time-critical HPLC applications (e.g., process analyses, screening tests or multidimensional techniques) extremely high analysis speeds are mandatory. The theory of modern liquid chromatography (e.g. [1–9]) points out that high efficiency and a simultaneous reduction of analysis time is only attainable by radically decreasing the particle diameter. The decrease in minimum plate height and increase in optimum mobile phase velocity with decreasing particle diameter is illustrated by the plate height curves in Fig. 1. It is obvious that in case of a particle diameter of only 1.5 μm the optimum mobile phase velocity is much higher than that for larger particles. Even higher mobile phase velocities may be applied without a loss in efficiency

because the curve, which is dominated in this part by the resistance to mass transfer, runs nearly parallel to the x-axis. As already discussed in a previous paper [10] the influence of the particle diameter on the optimum mobile phase velocity and the analysis time is much larger than that of the presence or absence of pores. However, small non-porous silica (NPS) particles have some advantageous features [11,12] such as a high uniformity in size, a high mechanical and thermal stability, a low specific surface area (low carbon content if modified and hence organic modifier content in RP mode), and a high sample recovery, which is especially important for biological samples [13–17]. Apart from that such stationary phases offer an increased mass sensitivity and lower solvent consumption. Therefore, they are the ideal packing material for high-speed HPLC.

On the other hand, small particle diameters cause high-pressure drops even for short columns and the

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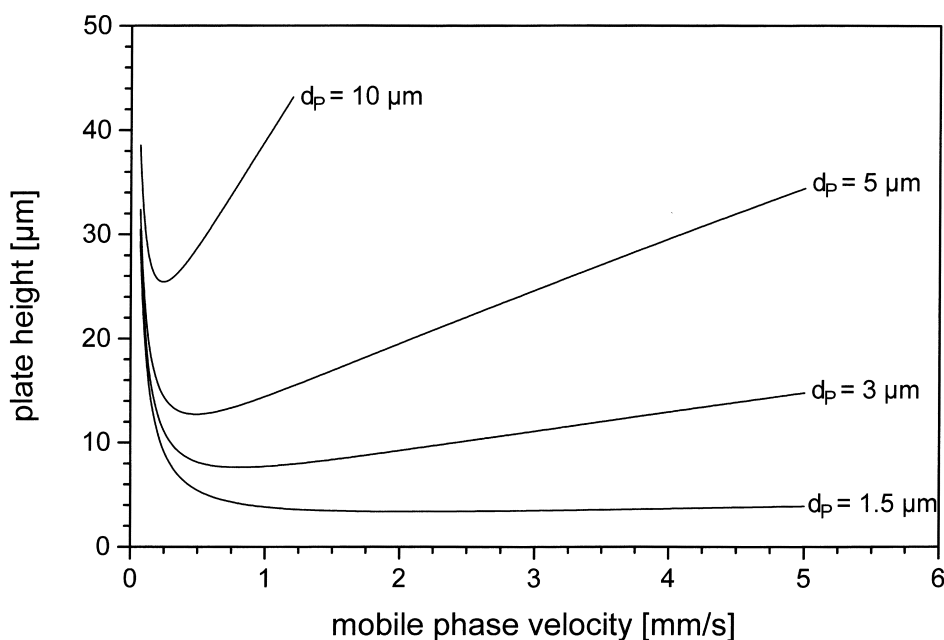


Fig. 1. Calculated plate height vs. mobile phase velocity curves for different particle diameters, d_p , on the basis of the Knox equation [7,10] $H = [A\nu^{1/3} + B/\nu + C\nu \cdot d_p]$; the reduced velocity $\nu = ud_p/D_m$. Parameters: $A=1$, $B=2$, $C=0.1$, $D_m=1 \cdot 10^{-9} \text{ m}^2/\text{s}$.

peak volumes are extremely small. Conventional HPLC equipment cannot be used without problems to operate such columns [10,18–20] because the extra column variances are too large. Thus, the injection and detection volumes as well as the lengths and internal diameters of the connecting tubes must be reduced. Also, the time constant of the detector and the data acquisition rate have to be adapted appropriately. A response time lower than 100 ms and a data acquisition rate greater than 25 Hz [20] are strongly recommended.

In practice a further decrease of the particle size seems to be limited only by the tolerable pressure drop. Fig. 2 shows the dependence of the pressure drop, Δp , and the retention time, t_R , on the particle diameter, d_p , calculated for a mobile phase consisting of acetonitrile and water (40:60) pumped with a flow-rate, F , of 1.0 ml/min for a component with a retention factor of $k=10$ on 4 mm I.D. analytical columns having 6000 plates. It becomes clear that analysis times in the sub-minute range have to be paid for by pressure drops of several hundred bars. Pressures of 300–400 bar are not unusual at optimum mobile phase velocities using commercially

available columns packed with 1.5 μm NPS particles [10–12,20,21].

Apart from the high-pressure drop there is an additional problem when using micro-particle packed columns in high-speed HPLC. High-pressure drops at high flow-rates are linked to the generation of heat by friction within the column (viscous heat generation). The consequence of this heat by friction is both a radial and a longitudinal temperature gradient in the column. The gradients are due to the poor heat conductivities of the mobile phase as well as the stationary phase (silica packing material). This phenomenon and the negative effects of inner column temperature gradients on column performance have already been discussed by Halász et al. [22] and Horváth and Lin [8] and were extensively treated theoretically and experimentally by Abbott et al. [23], and Poppe, Kraak and Huber [24,25]. The loss of efficiency and peak resolution caused by viscous heat dissipation may be considerable already for commonly used analytical and semipreparative columns packed with 5 μm particles [26].

In this work, the effects of viscous heat dissipation on column efficiency in high-speed HPLC using

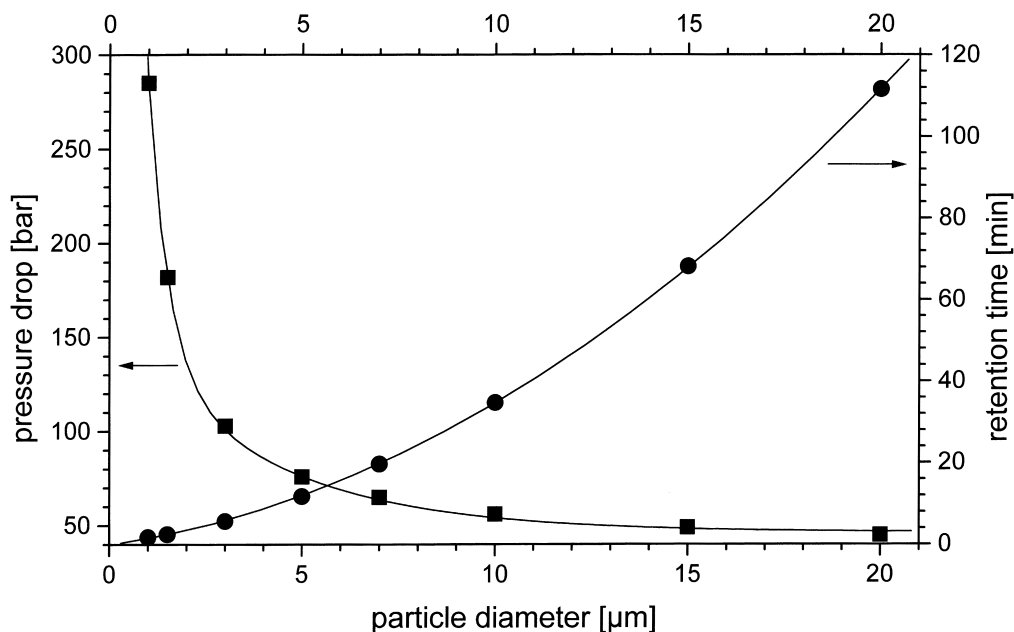


Fig. 2. Dependence of retention time and pressure drop on particle diameter. Equations [10] and parameters used for the calculation: $t_R = (1 + k) \cdot (Nhd_p^2 / \nu D_m)$ and $\Delta p = (D_m \nu \epsilon h N \eta / d_p^2)$. Parameters: A, B, C, and D_m as in Fig. 1; $k=10$, $h=2.4$, $N=6000$, $u=1.66$ mm/s, $\epsilon=0.8$, $\eta=1$ cP.

short columns packed with non-porous 1.5 μm particles are investigated and the application of temperature gradients between the mobile phase and the column (wall) to counterbalance the deleterious effect of the viscous heat generation are reported.

2. Experimental

2.1. Chemicals

Acetonitrile (HPLC Grade) was obtained from Rathburn (Walkerburn, UK). All solutes were purchased from Sigma (Deisenhofen, Germany). Bidistilled water was delivered by Bender und Hobein (Ulm, Germany).

Eluents were prepared by mixing of acetonitrile and water at different ratios.

2.2. Columns

Commercially available NPS columns (stainless

steel tube material) from different manufacturers were used:

(A) ChromSpher UOP 1.5 C₁₈ Column, 30×4.6 mm (Chrompack, Middelburg, Netherlands);

(B) MICRA NPS 1.5 C₁₈ Column, 33×4.6 mm (Micra Scientific, Northbrook, IL, USA, provided by Bischoff, Leonberg, Germany);

(C) Kovasil MS 1.5 C₁₄ Column, 33×4.6 mm (CU Chemie Uetikon, Uetikon, Switzerland).

2.3. Instrumentation and chromatography

Two HPLC pumps Model 422 (Kontron, Neufahrn, Germany) and an UV detector Model 535 (Kontron) were used. Injections were done employing an electrically driven injection valve Model C4-1004.5EH4, with a 0.5 μl internal sample loop (VICI/Valco Europe, Schenk, Switzerland). The eluent was thermostatted by pumping it through stainless-steel tubing (50 cm×0.5 mm I.D.) [23] placed inside a liquid thermostat type B (Lauda, Königshofen, Germany). The main problem of thermostating the eluent is the great contact surface, and

the heat conductivity and heat capacity of the injection valve. For this reason the eluent temperature is always close to room temperature if the valve is not thermostatted additionally. To investigate conditions with eluent temperatures above and below ambient temperature, the injection valve was provided with a water jacket system which is connected to the eluent thermostat. This system allows a precise adjustment of the eluent temperature in a temperature range of 5°C to 80°C at flow-rates of up to 4 ml/min. The columns were thermostatted by a separate liquid thermostat (Lauda) via a custom-made water-jacket system. Stainless steel tubing (0.125 mm I.D.) used to connect the injection valve and the column was as short as possible (10 cm). The actual entrance temperatures of the eluent into the packing bed ($T_{E \text{ inlet}}$) were measured with a thermo sensor Model 925 (Testo, Lenzkirch, Germany) behind the inlet frit (for the position see Fig. 3) of an unpacked column (type B). The temperatures of the eluent at the column outlet ($T_{E \text{ outlet}}$) were measured directly after the outlet frit (Fig. 3).

The mobile phase was degassed by applying vacuum in an ultrasonic bath before use, and during work with an inline degasser Model Degasys DG-1210 (Besta-Technik, Wilhelmsfeld, Germany).

Data acquisition and evaluation was performed with the Chromeleon software (Gynkotek HPLC, Germering, Germany).

The entire HPLC system was allowed to reach thermal equilibrium before measurements were

started. For that purpose, a period of 5 min was sufficient as the temperature measurements behind the inlet and the outlet frit showed.

The hold-up time was determined using thiourea which was added to the standard solutions. To investigate the influence of eluent temperatures on plate numbers and separation quality and to find out the optimum eluent temperature for a preset column temperature, data at different flow-rates (inlet pressures) were measured and Knox curves were plotted. Possible compressibility effects of the mobile phase were neglected.

3. Results and discussion

Radial temperature gradients within a chromatographic column—no matter how they have emerged e.g. by large temperature differences between column (wall) and feeding eluent [23] and/or the generation/dissipation of viscous heat within the column [22,24–26]—result in radial differences of the mobile phase viscosity, the mobile phase velocity, the diffusion coefficient, and the retention (k -value) which in turn affect the peak width, the peak shape and hence the separation performance [23,25–27]. The partially considerable contribution of this secondary gradients caused by viscous heat dissipation to overall band broadening can be counterbalanced by thermostating the eluent below the column wall temperature [26–28]: If the mobile

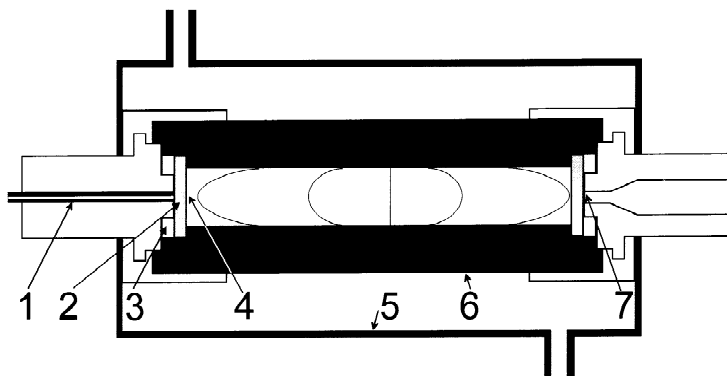


Fig. 3. Schematic of the column showing the location of temperature measurement points and the qualitative radial temperature profiles formed at optimum compensation temperatures of the eluent. 1 = inlet capillary, 2 = inlet frit, 3 = seal, 4 = temperature measurement point 1 ($T_{E \text{ inlet}}$), 5 = water jacket cabinet, 6 = column tubing (stainless steel), 7 = temperature measurement point 2 ($T_{E \text{ outlet}}$).

phase enters the column with a temperature lower than that of the column wall, an inverse temperature profile is formed in the first part of the column, opposite to the profile in the second part, which arises from viscous heat (the possible temperature profiles are qualitatively outlined in the schematic of Fig. 3). At a certain optimum temperature difference between the column (wall) and the feeding mobile phase the gradients are just offset. Correspondingly, the secondary gradients are offset and the additional band broadening by viscous heat dissipation is compensated.

We investigated these effects for NPS columns of different manufacturers packed with 1.5 μm non-porous particles (see experimental). The influence of viscous heat dissipation and the compensating effect of eluent precooling on efficiency is illustrated by the chromatograms in Figs. 4 and 5. The chromatograms show that the peaks are broadened and deformed, even shoulders or double peaks appear if the temperature of the eluent is either higher or lower than the optimum necessary for compensation of the

temperature gradients caused by viscous heat dissipation. The occurrence of shoulders and double peaks in preparative columns has been attributed to radial temperature gradients by Dapremont et al. [27], especially in connection with inhomogeneities of the packing bed [28–31], which may be true of NPS columns too.

The plot of plate numbers for two test analytes in dependence on the adjusted mobile phase temperature shown in Fig. 6 demonstrates the effects of viscous heat dissipation and its compensation by precooling of the eluent very impressively. At a flow-rate of 0.5 ml/min the optimum linear mobile phase velocity for maximum plate number (plate height curve optimum) is not yet reached (see Fig. 1). The plate numbers are both relatively low and independent of the eluent temperature. The generation of viscous heat at a pressure drop of only 100 bar seems to be too low to cause additional band broadening. No increase of the outlet temperature of the eluent (see $T_{E \text{ outlet}}$ in Table 1) is measured. However, at a flow-rate of 1.48 ml/min corre-

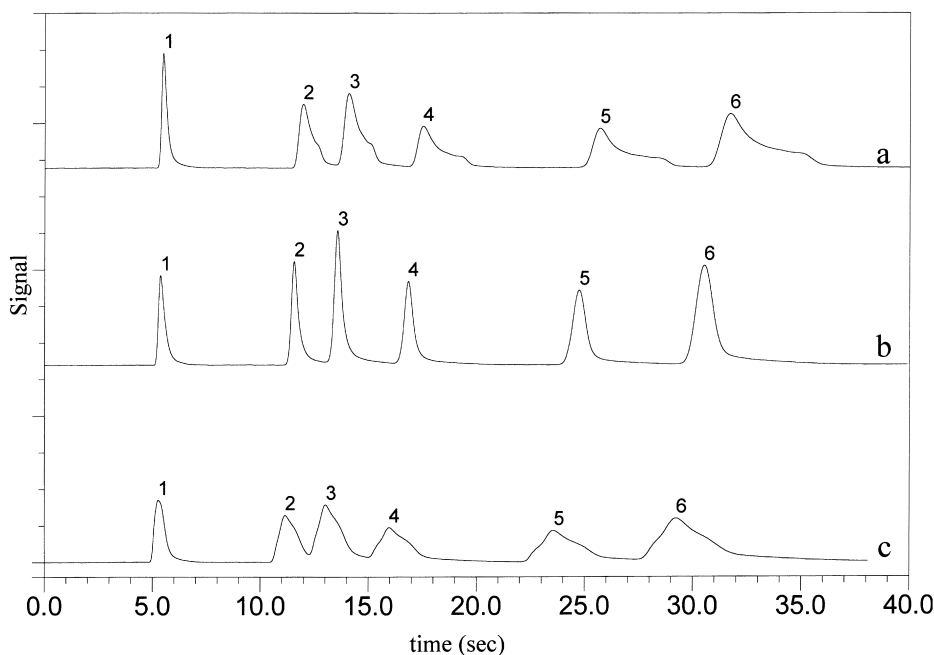


Fig. 4. Separation of 5 nitroaromatic compounds at different eluent temperatures. Column: C; column temperature 40°C; trace a: eluent temperature 10°C, pressure drop 542 bar; trace b: eluent temperature 25°C, pressure drop 532 bar; trace c: eluent temperature 40°C, pressure drop 515 bar; eluent: acetonitrile–water (10:90, v/v); flow rate: 2.4 ml/min; injection volume: 0.5 μl ; peak assignment: 1=thiourea; 2=1,3,5-trinitrobenzene, 3=1,3-dinitrobenzene, 4=1,2-dinitrobenzene, 5=2,4,6-trinitrotoluene, 6=3-nitrotoluene.

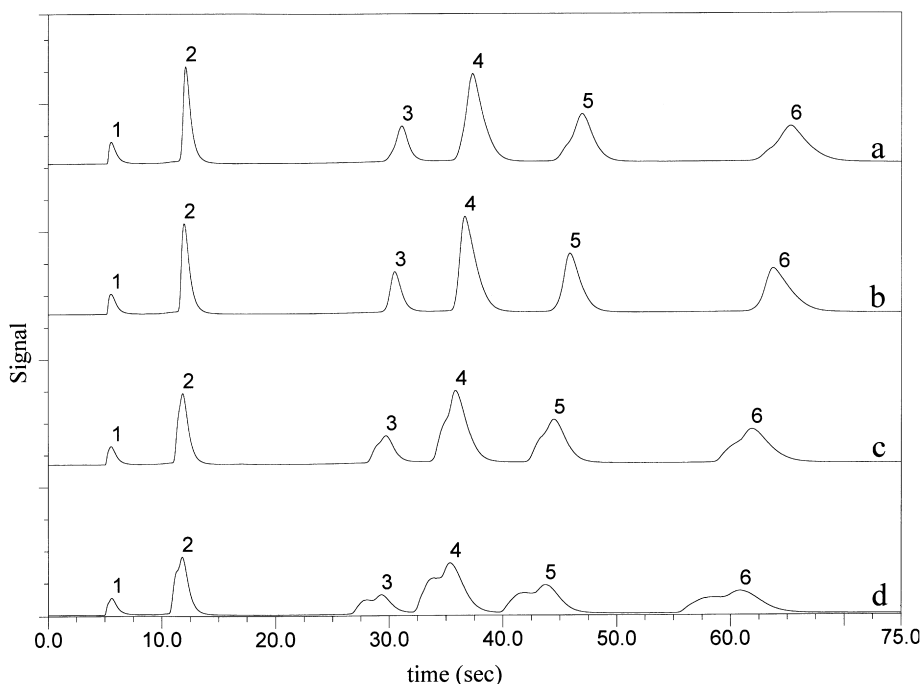


Fig. 5. Separation of 5 hormones at different eluent temperatures. Column: A; column temperature: 30°C; trace a: eluent temperature 5°C, pressure drop 508 bar; trace b: eluent temperature 10°C, pressure drop 503 bar; trace c: eluent temperature 22°C, pressure drop 490 bar; trace d: eluent temperature 30°C, pressure drop 480 bar; eluent: acetonitrile–water (20:80, v/v); flow rate: 2.0 ml/min; injection volume: 0.5 μ l; peak assignment: 1=thiourea, 2=hydrocortisone, 3=nortestosterone, 4=dehydro-17 α -methyltestosterone, 5=testosterone, 6=17 α -methyltestosterone.

sponding to an inlet pressure of 301 bar the dependence of the plate numbers on the eluent temperature becomes very pronounced: A distinct maximum in plate numbers appears at an adjusted eluent temperature of 22–25°C corresponding to an entrance temperature of the eluent into the packing bed of 36.6°C (Table 1). In order to verify that the observed dependence of column efficiency is based on effects of the secondary gradients mentioned above and not on changes in the retention behaviour of the solutes, the selectivity of the peak pair 2-nitrotoluene ($k=4.98$) and 2,4,6-trinitrotoluene ($k=3.56$) was determined for a column wall temperature of 40°C in dependence on the eluent temperature. The measured selectivity value of 1.40 was not influenced by the eluent temperature within the experimental accuracy of the measurement. This underlines that the compensation of the efficiency loss caused by viscous heat dissipation is the only reason for the observed maximum in Fig. 6.

To investigate the negative effects of viscous heat dissipation for a wide range of flow-rates and to determine the optimum eluent temperature to be adjusted for different column temperatures in more detail, experiments to establish plate height curves were performed and the experimental curves were compared to the theoretical curve for a non-porous 1.5 μ m packing material (Fig. 1).

Fig. 7 shows three exemplary Knox plots. The only curve which actually follows the theoretical curve shape is based on experimental values obtained with an adjusted eluent temperature of 23°C at a column wall temperature of 40°C. Both, eluent precooling below this optimum temperature ($T_E=10^\circ\text{C}$, $T_C=40^\circ\text{C}$) and identical temperatures of eluent and column ($T_E=T_C=40^\circ\text{C}$) result in an increasing slope of the resistance to mass transfer dominated part of the curves. Such a radial thermal gradient contribution to plate height which can be explained both by local flow velocity differences and retention

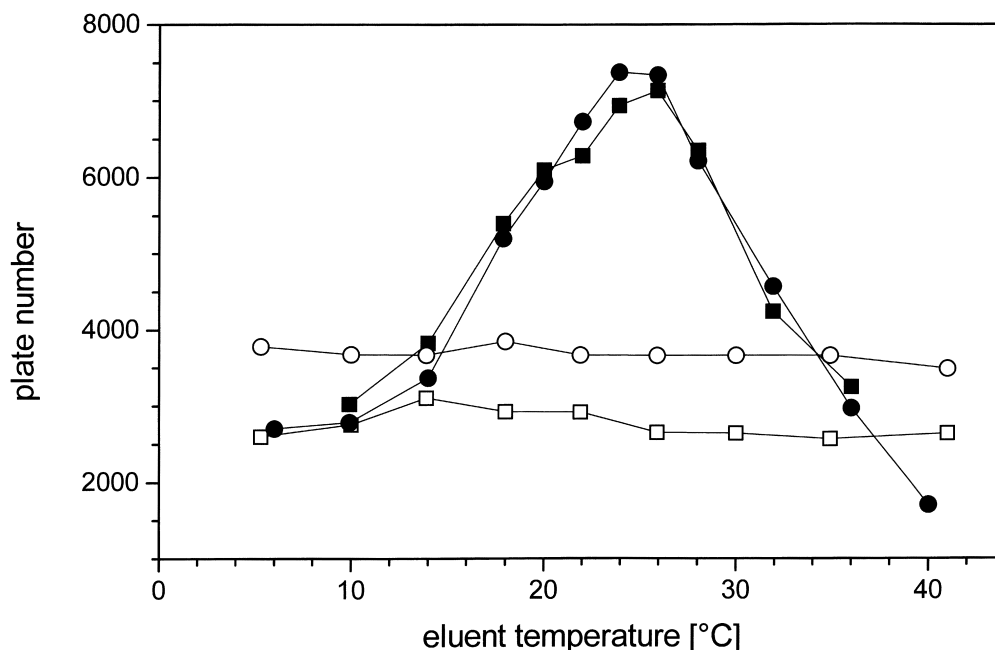


Fig. 6. Dependence of plate numbers on eluent temperature. Column: B; column temperature 40°C; eluent: acetonitrile–water (10:90, v/v); flow rate: 0.5 ml/min, pressure drop 100 bar (open symbols); flow rate: 1.5 ml/min, pressure drop 301 bar (full symbols); analytes: ○ = 2,4,6-trinitrotoluene; □ = 2-nitrotoluene.

factor differences has been discussed in several papers [23,25,26]. Viscous heat compensation in conjunction with efficient column thermostating seems therefore mandatory in fast HPLC analysis

Table 1

Relations between mobile phase flow, F , pressure drop, Δp , and measured temperature increase of the mobile phase after passing the inlet frit ($T_{E \text{ inlet}}$) (temperature measurement point 1 of Fig. 3) and at column outlet ($T_{E \text{ outlet}}$) (temperature measurement point 2 of Fig. 3). The eluent temperature, T_E , was adjusted to 22°C and the column wall temperature, T_C , to 40°C. Column: B; eluent: acetonitrile–water (23:77; v/v). (Note that the eluent temperature at column outlet, $T_{E \text{ outlet}}$, represents a mean value. The temperature rise at column axis due to viscous heat generation is much higher)

F (ml/min)	u (mm/s)	Δp (bar)	$T_{E \text{ inlet}}$ (°C)	$T_{E \text{ outlet}}$ (°C)
0.024	0.07	6	39.5	39.9
0.50	1.38	105	38.4	39.9
1.00	2.78	208	37.2	40.2
1.48	4.10	316	36.6	40.5
1.96	5.43	409	35.9	41.0
2.78	7.69	573	34.3	41.7

using high mobile phase velocities which are inevitably connected to high-pressure drops.

During the measurements it turned out that the necessary eluent temperature to be adjusted for maximum plate numbers at a fixed column (wall) temperature is dependent on the latter but nearly independent on the flow-rate and the pressure drop. The answer to this seeming contradiction is given by the measured actual entrance temperatures of the eluent ($T_{E \text{ inlet}}$) in comparison to the adjusted temperatures of the eluent and the valve and the column wall temperature ($T_C=40^\circ\text{C}$). The values, listed in Table 1, show that from the initially adjusted temperature difference of 18°C only little remains at the packing bed inlet due to the contact of the eluent with the column head and the stainless steel inlet frit. At low flow-rates and hence pressure drops the eluent temperature is unimportant for the column performance because the eluent is warmed up anyway to column temperature. At higher flow-rates, the contact time decreases and larger temperature differences are to be left. Thus, the real entrance temperature of the eluent (necessary for the compensation of

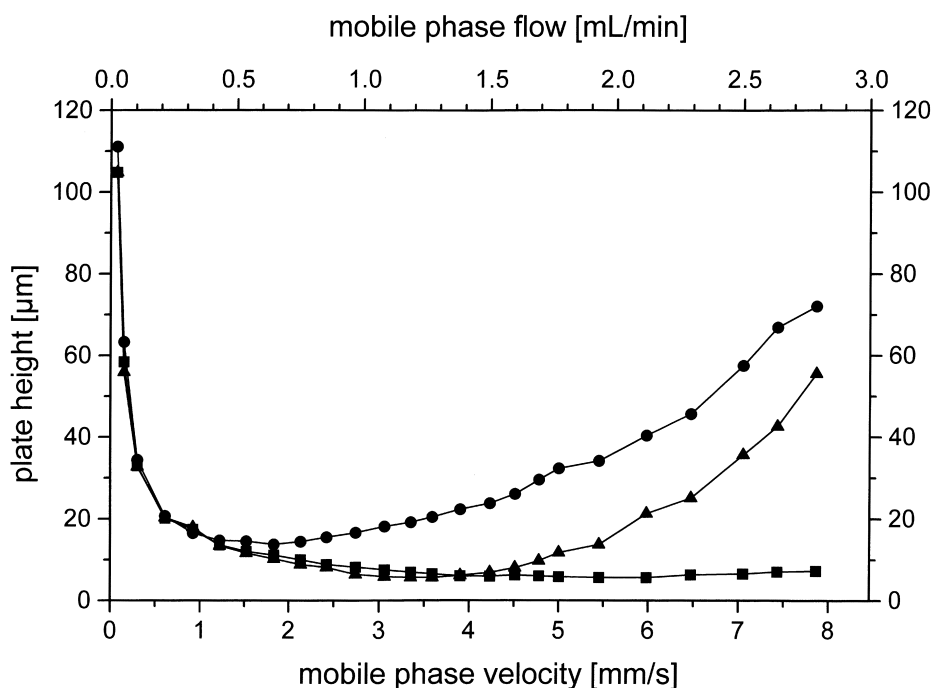


Fig. 7. Knox plots for butyl phenyl ketone at different eluent temperatures; column: A; Column temperature 40°C; eluent: acetonitrile–water (23:77, v/v); ●=eluent temperature 40°C ($k=5.16$); ■=eluent temperature 20°C ($k=6.13$); ▲=eluent temperature 10°C ($k=5.91$).

plate height contributions due to radial thermal gradients) is “automatically” adopted by the flow-rate which is responsible for the pressure drop and the magnitude of the generated viscous heat. This fact proved to be a big advantage for the practical realisation of eluent precooling because the optimum eluent and valve temperature has to be determined experimentally only once for a given column type, column temperature, and mobile phase composition and can be kept constant for any flow-rate.

The optimum precooling temperature of the eluent, T_E , approximates to the column wall temperature, T_C , with increasing column wall temperatures. At a column wall temperature e.g. of 30°C the optimum “compensation” temperature of the eluent was 7°C whereas this optimum temperature is 52°C at a column wall temperature of 60°C. This can be explained by the decreasing viscosity of the eluent and hence the diminished pressure drop which in turn results in the generation of less viscous heat. In addition, a temperature increase at higher temperatures changes the viscosity to a lesser extent than at low temperatures. A slightly improved diffusionaly

relax of the flow and retention inhomogeneities due to an increased diffusion coefficient at higher temperatures may also play a role [23,32]. Thus, the secondary gradients within the column are smaller at higher column temperatures and so are its negative effects on efficiency. This leads to the conclusion that an effective compensation of viscous heat is especially important at low column temperatures and/or when using viscous mobile phases.

4. Conclusions

The use of small non-porous particles and high mobile phase velocities in high-speed HPLC requires high-pressure drops which in turn lead to the generation of viscous heat within the column. The dissipation of viscous heat to the thermostatted column wall produces temperature gradients within the column which diminish the achievable efficiency. These negative effects which may be considerable especially at low column temperatures could be counterbalanced by a defined precooling of the mobile phase.

The optimum temperature difference between the mobile phase and the column (wall) necessary to compensate the viscous heat dissipation effects becomes smaller with increasing column temperature. Both, control of the column wall temperature and of the mobile phase temperature is an important prerequisite for good column performance and reproducibility in high-speed HPLC using columns packed with small non-porous particles.

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