The future of HPLC and UPLC: are higher pressures and smaller particles opportune?

Ken Broeckhoven(1), **Konstantin Choikhet**(2), **Gerard Rozing**(2) **and Gert Desmet**(1)
(1) Vrije Universiteit Brussel, Department of Chemical Engineering, Brussels, Belgium *(Transport Modelling & Analytical Separation Science-group)*
(2) Agilent Technologies, Waldbronn, Germany
tel.: (+)32.(0)2.629.37.81, fax: (+)32.(0)2.629.32.48, e-mail: kbroeckh@vub.ac.be

I. ABSTRACT

In the current generation of HPLC columns and systems, the limit on the maximum operating pressure seems to be established around 1000 bar or 15000 psi. From an instrument manufacturer's point of view it is an interesting question whether this limit is worth expanding towards 1500 or 2000 bars. Similarly it is interesting to know whether a further reduction of particle size is desirable when the pressures needed to operate these at an optimal flow rate are beyond the possibilities of current systems. Both experimental [1], combined experimental-theoretical [2,3,4] and theoretical studies [5,6] have shown recently that the currently used systems, that can run at ultra-high pressure, operate near the limit where viscous heating in the column becomes detrimental for the total performance of the chromatographic system. For a better understanding of the effects originating from the use of these ultra-high pressures, computational fluid dynamics (CFD) was used to determine both the flow and temperature profile inside the packed bed and the column wall for the most common thermal boundary condition. A classical ultra-high pressure chromatographic column (2.1mm ID, 10cm, sub 2µm particles) was studied, including the complex column geometry, more specifically the large surface area of the column endfittings. In order to deepen the understanding of the complex set of interacting physicochemical effects leading to a deterioration of the column performance, the (temperature dependent) retention was studied in detail. Physico-chemical properties of commonly used chromatographic solvent mixture were taken from experimental results, as well as the influence of temperature on retention.

II. Aim and Introduction

- Investigating how significant the transfer of heat of the column to the environment in still air conditions and the backflow of heat through the column wall can effect the chromatographic performance of columns operated at the maximal operating pressure currently available and beyond. This is done for both retained and non-retained conditions, including the influence of temperature on the retention coefficient.
- Determining the influence of the value of the retention enthalpy on the performance deterioration.
- Examining the decrease in retention due to viscous heating.

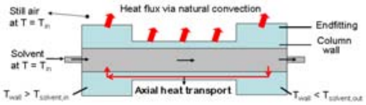


Figure 1. Schematic model of the chromatographic column (including endfittings) and the applied thermal boundary conditions.

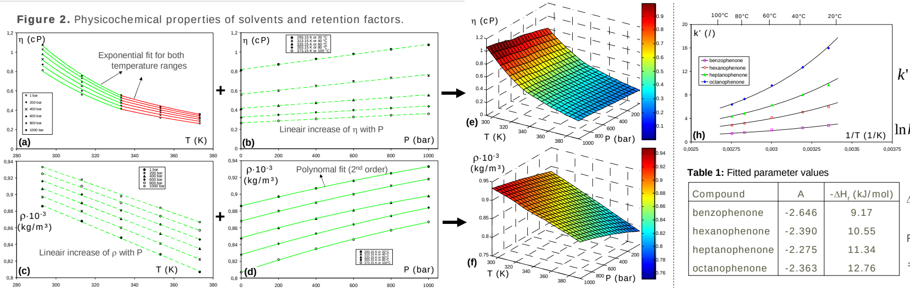
III. Method:

a. Experimental results for physico-chemical properties and temperature dependence of retention coefficients:

Experimental measurements [7] were performed to determine the density (ρ) and viscosity (η) of common chromatographic solvents and solvent mixtures (water-methanol = H_2O-MeOH and water-acetonitrile = H_2O-ACN) as a function of the temperature and pressure. The temperature range measured was from 20°C to 100°C and the pressure range from 1 bar (ambient pressure) up till 1000 bar. The solvent mixture considered in this study was 60-40 (vol%) ACN-H_2O. The viscosity as function of temperature was readily fitted by an exponential function in a temperature interval of about 40°C (fig. 2a) and depended linearly on the pressure (fig. 2b). The density depends linearly on the temperature (fig. 2c) and can be fitted with 2nd order polynomials as a function of pressure (fig. 2d) . These dependencies allow an interpolation to cover the range of temperatures and pressures in the simulations up till 1000 bar and 100°c (fig. 2e and 2f) and extrapolation to higher pressures.

Experimental measurements of the retention coefficient of a series of alkylphenones at different mobile phase temperatures (20-100°C) allows the determination of the retention coefficient for all possible temperatures (fig. 2h).

Figure 2. Physicochemical properties of solvents and retention factors.



Van't Hoff's equation

$$k'(T) = A^* \cdot e^{-\frac{\Delta H_r}{R \cdot T}}$$

$$\downarrow$$

$$\ln k'(T) = A - \frac{\Delta H_r}{R \cdot T}$$

Table 1: Fitted parameter values

Compound	A	$-\Delta H_r$ (kJ/mol)
benzophenone	-2.646	9.17
hexanophenone	-2.390	10.55
heptanophenone	-2.275	11.34
octanophenone	-2.363	12.76

ΔH_r: retention enthalpy

R: molar gas constant = 8.31 J/(mol·K)

b. Numerical simulations

A commercial flow solver (Fluent®) was used to determine both the temperature and velocity field in the column geometry studied (2.1 mm I.D. column, $K_{v0} = 6 \cdot 10^{-15}$ m², 10 cm long). The mobile phase chosen was 60-40 (vol%) acetonitrile water with solvent inlet and still air temperature 20°C. For the thermal boundary conditions, natural convection was assumed on the column wall surface, using correlations for heat transfer coefficients found in literature [8]. The heat generation in the column was corrected for the heat of decompression.

velocity (u_0) found in numerical simulations can be converted into retained species velocity (u_r) using:

$$u_r = \frac{u_0}{1 + k'(T)} \quad with \quad \ln k'(T) = A - \frac{\Delta H_r}{R \cdot T} \qquad (1)$$

Retained species velocity field in column is used in a in house written numerical code (Fortran®) to determine the peak band broadening. The convection diffusion equation (Eq. 2) is solved implicitly in the space domain (with 2nd order accurate (or higher) finite differences) and explicitly in the time domain (with a 4th order Runge-Kutta scheme).

$$\frac{\partial C}{\partial t} = D_{rad} \cdot \left(\frac{\partial^2 C}{\partial y^2} + \frac{1}{y} \frac{\partial C}{\partial y} \right) + D_{ax} \cdot \frac{\partial^2 C}{\partial x^2} - u \cdot \frac{\partial C}{\partial x} \qquad (2)$$

IV. Results

Due to the backflow of heat in the column wall, the radial temperature gradients at the front of the column are inverse as opposed to those near the back. The dependency of the solvent viscosity with temperature results in the same behaviour for the radial velocity profiles. As a result, the solute peak experiences very strong dispersion near column inlet and outlet which, in part, compensate each other (sign of the velocity gradient in front and back are opposed). Nevertheless, a measurable influence on the column performance due to the heat transfer (especially from the bulky endfittings) to the surroundings can observed even at 1000 bar. The column performance deteriorates quickly if the backpressure is further increased. All these effects are even more pronounced for retained compounds and increase with increasing retention enthalpy of the component. The effective retention coefficient of the components also is decreased due to viscous heating in the column.

a. Temperature and velocity profiles in column

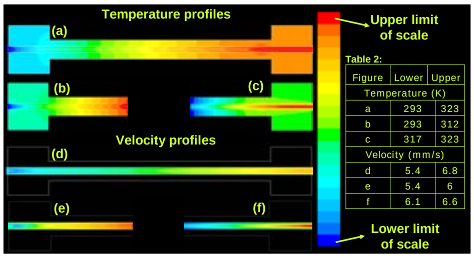


Table 2:

Figure	Lower	Upper
Temperature (K)		
a	293	323
b	293	312
c	317	323
Velocity (mm/s)		
d	5.4	6.8
e	5.4	6
f	6.1	6.6

Figure 3. Temperature and velocity profiles (u_0) inside the column operated at 2000 bar. The same color scale is used for both quantities, with the ranges given in the Table 2.

b. Radial velocity gradients and resulting band broadening

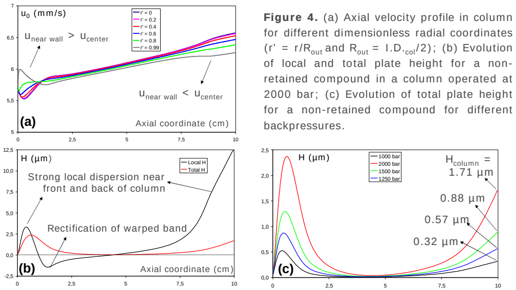


Figure 4. (a) Axial velocity profile in column for different dimensionless radial coordinates ($r' = r/R_{out}$ and $R_{out} = I.D._{col}/2$); (b) Evolution of local and total plate height for a non-retained compound in a column operated at 2000 bar; (c) Evolution of total plate height for a non-retained compound for different backpressures.

C. Effects of viscous heating for retained compounds

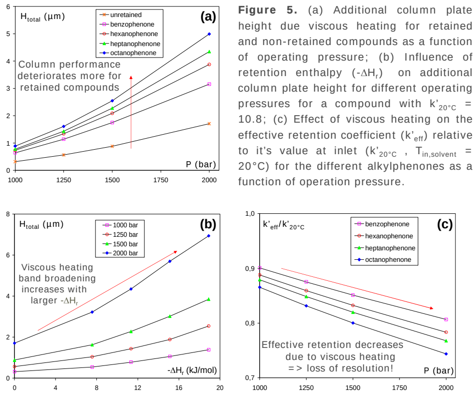


Figure 5. (a) Additional column plate height due viscous heating for retained and non-retained compounds as a function of operating pressure; (b) Influence of retention enthalpy ($-\Delta H_r$) on additional column plate height for different operating pressures for a compound with $k'_{20°C}$ = 10.8; (c) Effect of viscous heating on the effective retention coefficient (k'_{eff}) relative to it's value at inlet ($k'_{20°C}$, $T_{in,solvent}$ = 20°C) for the different alkylphenones as a function of operation pressure.

V. Conclusions

- The heat transport in the column wall has to be taken into account when studying the effect of viscous heating in columns operated at ultra-high pressures.
- Even for the currently available operating pressures and for columns operated in still air conditions, a measurable additional band broadening can occur due to viscous heating.
- Further increase of the operating pressure beyond 1000 bar will quickly results in deterioration of the performance of columns operated at such high pressures.
- The effects of viscous heating are even more pronounced for retained compounds and increase with increasing component retention enthalpy.
- Further reduction of the particle size would require such high pressures to operate at the optimal flow rate that the gain in performance would be strongly reduced due to the viscous heating effect.
- Loss of retention in the columns operated at ultra-high pressures due to viscous heating can cause a loss in separation resolution.

[1] de Villiers, H. Lauer, R. Szucs, S. Goodall, P. Sandra, J. Chromatogr. A 1113 (2006) 84-91
[2] F. Gritti, G. Guiochon, J. Chromatogr. A 1138 (2007) 141-157
[3] F. Gritti, G. Guiochon, J. Chromatogr. A 1166 (2007) 47-60
[4] K. Kaczmarski, F. Gritti, G. Guiochon, J. Chromatogr. A (2007), doi:10.1016/j.chroma.2007.11.009
[5] G. Desmet, J. Chromatogr. A 1116 (2006) 89–96
[6] K. Broeckhoven, J. Billen, G. Desmet, K. Choikhet, G. Rozing, presented at the 31st International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2007), Ghent, 17–21 June 2007, oral presentation L22.01.
[7] Billen, J., Broeckhoven, K., Liekens, A., Choicket, K., Rozing, G., Desmet, G., Influence of pressure and temperature on the physicochemical properties of mobile-phase mixtures commonly used in high performance liquid chromatography, in preparation
[8] Çengel, Y.A., Heat transfer – A Practical Approach, 2nd ed., McGraw Hill: New York, 2003.