High speed LC with 0.5 mm i.d. columns: overcoming the challenges of frictional heating in UHPLC.

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Abstract

HPLC columns operating at high flow rate and high pressure can produce substantial heat, which in turn can produce a non-uniform temperature distribution within the column. In a traditional column oven, the column is semi-insulated. Although this will prevent a radial temperature profile that would otherwise reduce column efficiency, the actual column temperature, hence retention time, will be poorly controlled. Retention times depending on the details of column mounting and on operating conditions, greatly complicate method development and method transfer. Recent reports in the literature have confirmed this behavior in conventional bore columns and it has been suggested that the issue can be addressed by using smaller diameter columns.

Introduction

The advent of sub-2µm stationary phases heralds a new level of performance in HPLC, offering increases in plates and speed since both plates and optimum velocity scale inversely with bead diameter. But a decrease in bead diameter requires a noticeably higher operating pressure. The product of pressure and flowrate represents the power required to push liquid through the column. A substantial fraction of this power shows up as heat within the column that raises the temperature of the phases and thus alters retention factors. At the optimum velocity the heat deposited scales as d_c^2/d_b^4 where d_c and d_b are the column- and bead-diameters. Thus a 2-fold decrease in bead diameter results in a 16-fold in heat.

Theory

The issue of frictional heating and the resulting non-uniform temperature distribution within a column has been considered by Halasz *et al.* and Poppe *et al.*, and more recently studied by Colon *et al.* and de Villiers *et al.*. The temperature distribution within a column is quite complex owing to issues of temperature- and pressure-dependent transport properties, heat conduction in the column jacket and the vagaries of heat transfer through connections and mountings. However there are two simple cases that provide instruction:

The first case treats the column as insulated thus having negligible radial heat loss, the result being that there is little radial temperature variation but the temperature does increase from inlet to outlet. De Villiers *et al.* considered a 2.1 x 50mm Waters Acquity BEH C18, 1.7µm column operated with 30/70 acn/water at 1mL/min giving an inlet pressure of 819 bar with the column in an Acquity column holder (suspended in still air thus nominally insulated) at an oven temperature of 25°C. For these conditions they calculated a power of about 1.36W, a temperature rise of 23.6°C and they measured a rise of 13°C. The lower measured value can be attributed to order 30% of the power being reversibly recovered in decompression of the liquid and to some finite heat loss from the column to the oven. They observed that average retention factors decreased by upwards of 10% as column pressure was changed from 212 to 778 bar (flowrates from 0.3 to 1.1mL/min) and attributed this trend to the corresponding increase in temperature.

Under semi-insulted operating conditions thermal time response must also be considered. This time response is determined the total thermal mass of the column (body and phases) and by the total heat transfer rate (i.e. transfer from the column body and convention by the mobile phase). Under semi-insulated conditions this time constant can easily be many tens of minutes. Thus any change that alters the heat deposited in the column (e.g. a change in flowrate or in mobile phase composition) can require tens of minutes before the column temperature hence retention time stabilizes. Such a long time constant degrades productivity, can cause what may appear as erratic operation and certainly makes gradient operation difficult to interpret and near-impossible to reliably scale.

The second case treats the column jacket as held to a fixed temperature so there is little or no temperature variation along the column axis, but heat is still deposited within the column leading to higher temperatures on the column centerline that at the walls. This temperature variation can be relatively small but the resulting change in retained velocity can add substantial dispersion, through temperature dependent viscosity and retention factor. The Aris theory provides that the additional contribution to plate height due to such variation scales as $H_x \propto \beta^2 u^5 d_c^6 / d_b^4$. Here β is the relative slope sensitivity of retained velocity to temperature. This relation shows a strong dependence on operating parameters but a more severe dependence is revealed by taking velocity as some multiple of the optimum velocity (i.e. $u = a\, u_{opt}$) giving $H_x \propto \beta^2 a^5 d_c^6 / d_b^9$. This consideration is important since one purported advantage of using smaller beads is a smaller contribution from the C-term hence the ability to operated at higher than optimum velocity (i.e. $a > 1$) without a loss in plates.

De Villers *et al.* reported plate height measurements for a 2.1 x 100mm Acquity BEH C18, 1.7µm column using 30/70 acn/water operated in one case as mounted in an Acquity column holder (nominally insulated) and in a second case as suspended in a stirred water bath (nominally fixed wall temperature). Their results (taken from their figure 4) are shown in figure 1 where we also plot a curve fit to their 'insulated' data (fit of the form $H_1 = A + B/u + Cu$) and a fit for the second case of the form $H_2 = H_1 + Fu^5$. The quality of the later fit (done with one free parameter, F) lends some credence to the scaling suggested here. Poppe *et al.* performed similar experiments and we observe a like u^5 behavior in the high velocity branch of their HETP results.

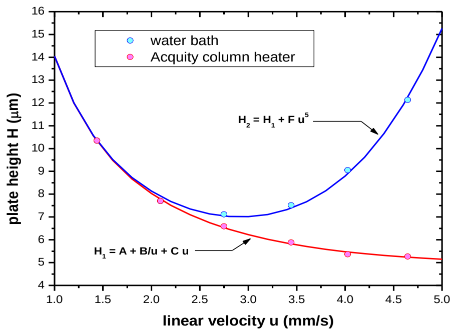

Figure 1. HETP vs. velocity for 2.1mm bore insulated and iso-thermal columns.

Operation of a sub-2mm packing in a conventional bore column then appears as a balancing act between using a semi-insulated column to achieve good plate height performance but at the cost of systematic error in retention times and selectivity since column temperature is essentially uncontrolled. Versus using a column held at fixed temperature to achieve stable retention times but at the cost of a severe degradation in plates. Colon *et al.* have suggested that a better balance is achieved by using a 1mm column. In figure 2 we re-plot the curve-fits from figure 1, H_1 and H_2 labeled 2.1mm insulated and 2.1mm iso-thermal, respectively. Using the derived relationship we rescale the iso-thermal curve to also plot curves for 1.0 and 0.5mm columns. It is apparent that use of a 1mm column provides improvement, but near-ideal performance requires the use of a 0.5mm column.

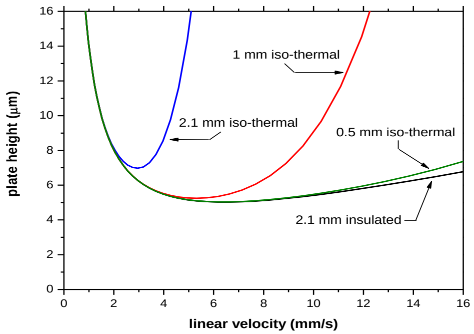

Figure 2. HETP vs. velocity for an insulated and iso-thermal columns of various diameters.

To test the prediction a 0.5 x 100mm Eksigent-format column with a sub-2µm packing (Restek Pinnacle DB C18 1.9µm) was mounted in an Eksigent ExpressLC-Ultra . This instrument includes mobile phase preheating and column oven/mountings designed to provide sufficient heat transfer to regulate column temperature. The instrumented provides column inlet pressures to 10,000 psi, data rates to 100Hz and is designed with suitable extra-column variance to provide column-limited performance with 0.5mm columns. Figure 3 shows plate height data for acenaphthene with 60/40 acn/water as the mobile phase. Data are shown for three column temperatures. Also plotted are fits to the van Deemter equation. At higher linear velocity there is no indication of a power-law in velocity (i.e. u^5) showing that the effect of frictional heating can be made negligible by using a sufficiently small bore column. The minimum reduced plate heights achieved (values of about 2.3) attest to the ability to operate under column-limited conditions. It is important to note that under the high heat transfer conditions used, the column thermal time constant is reduced to tens of seconds. At the same time the column is held at a well-regulated temperature providing reproducible and stable retention times.

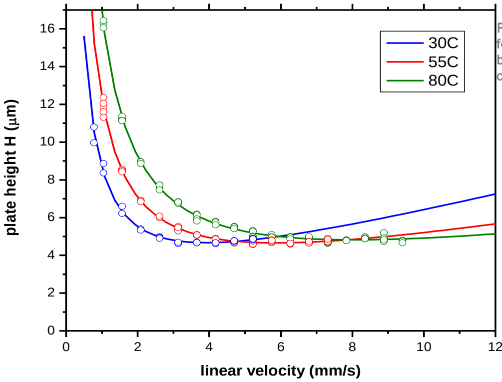

Figure 1. HETP vs. velocity for an iso-thermal 0.5mm bore column and for three column temperatures

Conclusions

Recent reports in the literature and engineering computations show that operation of sub-2µm media in conventional bore columns under semi-insulated conditions can provide the expected improvements in plates. But then the column temperature is poorly controlled and retention time reproducibility must suffer. As noted by de Villiers *et al.*, the resulting variably in retention times will complicate method transfer and method development. Further the long thermal time constant incurred with this configuration can degrade productivity and compromise gradient operation. The issues of retention time reproducibility and thermal time response are corrected by increased heat transfer to hold the column at fixed temperature. But in a conventional bore column this results in a severe degradation in plates.

We have demonstrated that good performance is found using 0.5mm columns with sub-2µm beads operated with suitable heat transfer and in a well-designed instrument. It is then possible to achieve in combination: as-promised plate heights well into the high-velocity branch of the van Deemter curve, stable and reproducible retention time and hence selectivity, and sufficiently fast column thermal response to make predictable fast gradient operation practical.

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