How Scalable are the Dispersion Processes in Real Columns Packed with Solid Core Material?

Tony Edge,[1] Luisa Pereira,[1] and Frank Steiner[2]
[1]Thermo Fisher Scientific, Runcorn, UK
[2]Thermo Fisher Scientific, Germering, Germany

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Overview

Purpose: To determine the scalability of the performance improvements seen with solid core particles.

Methods: Two different particle sizes will be investigated by comparing efficiency and impedance determinations. An investigation into pressure induced temperature effects is also performed.

Results: The data demonstrates that the expected benefits in reducing particle size can be realized, however the use of low dispersion, ultra high pressure solvent delivery systems is critical. The data also shows the effect of different modes on heating on the chromatographic performance.

Introduction

There has been much interest recently in the applications of solid-core silica based substrates for improved chromatographic efficiency. The improvement in efficiency comes from the greater uniformity in the particle substrate. This allows for better packing efficiencies, the 'a' term in the van Deemter equation, a reduction in the void volume of the column, which lowers the longitudinal diffusion, and also the reduction in mass transfer effects due to the reduced pore depth offered by the solid-core material.

The use of smaller particles creates higher pressure drops across the columns which will result in increased temperature gradients. Pressure induced temperature gradients can have an impact on the performance of the separation, and understanding how to optimise the heating of the column, whether that be by performing the separation in a nominally isothermal or an adiabatic mode, will allow better design of column ovens.

Methods

Columns: Thermo Scientific™ Accucore™ XL C18 4 µm, 150 x 4.6 mm; Accucore C18 2.6 µm, 100 x 2.1 mm

Efficiency and pressure testing:

Mobile phase: water / ACN (50:50)

Flow rates: 0.4 to 2.0 mL/min (every 0.1 mL/min) for the 4 µm, 150 x 4.6 mm column; 0.05 to 0.8 mL/min (every 0.05 mL/min) for the 2.6 µm, 100 x 2.1 mm column

Temperature: 30 °C

Detection: UV at 254 nm (0.1s rise time, 20 Hz)

Injection volume: 1 µL

Test probe: o-Xylene (t_0 marker – theophylline

FIGURE 1. Vanquish UHPLC system which was used to perform the evaluation.

Results

van Deemter plots

It can be seen from the reconstructed van Deemter plots, Figure 2, that reducing the particle size results in a reduction in the plate height minimum. The Accucore XL C18 4 µm has a minimum plate height of 6.2 µm, compared to that obtained with the Accucore C18 2.6 µm, which has a minimum of 3.4 µm. It can also be clearly seen that there is an increase in the linear velocity associated with the minimum plate height as the particle size is reduced. Thus, with the Accucore XL C18 4 µm the minimum plate height is obtained at a linear velocity of 2.2 mm/s, compared with the higher linear velocity of the Accucore C18 2.6 µm, 4.8 mm/s, required to obtain the minimum plate height.

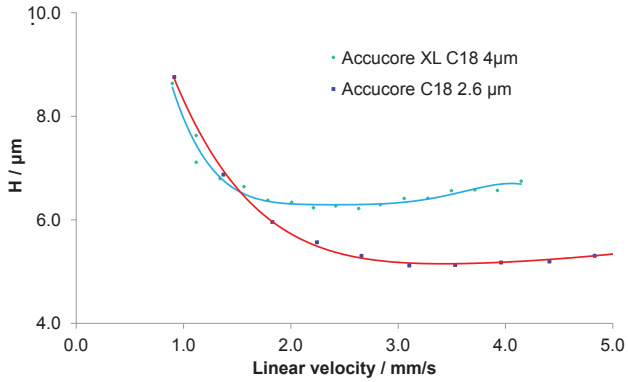

FIGURE 2. Comparison of van Deemter plots obtained for the Accucore XL C18 4 µm, and Accucore C18 2.6 µm.

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Impedance Plots

The van Deemeter plot allows for the visualisation of the performance of the column, in terms of plate height, compared to the linear velocity. It does not however account for:

- analysis time
- pressure restrictions
- or different morphologies of the packing materials

Kinetic plots are an alternative method of plotting the same data (HETP and linear velocity values) which takes into account the permeability of the columns, and allows for a broader methodology for comparing different columns. One such kinetic plot was devised by John Knox, [1] and is referred to as impedance, E.

The mathematics underlying the kinetic plot method is very simple and is based on three 'classical' chromatographic equations, given below:

$$t_0 = \frac{L}{\mu} \qquad L = NH \qquad \mu = \frac{\Delta P K_V}{\eta L}$$

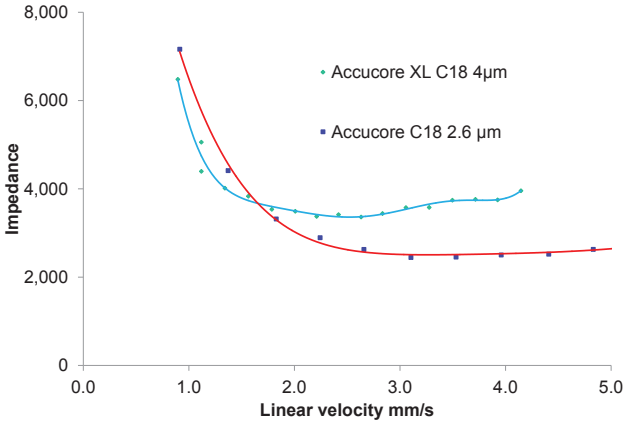

FIGURE 3. Comparison of Impedance plots obtained for the Accucore XL C18 4 µm and Accucore C18 2.6 µm.

It can be seen from Figure 3 that when comparing the impedance of the two forms of the columns that a different profile is obtained. Theory suggests that the impedance is the same for the same particle morphology, assuming that the packing is consistent. In the data presented it is clear that the Accucore C18 2.6 µm material has the lowest impedance at the higher linear velocities compared to the Accucore XL 4 µm. However, these particles do have a different morphology as the ratio of the solid core to the particle diameter is different. This value is termed as, ρ, with the value for Accucore 2.6 µm being larger, 0.78 compared to 0.72 for the larger particle. The reduction in porosity means that there is an improvement in efficiency.

Modeling of experimental data

Models have been developed which will allow for the variation of the particle morphology. Using a model developed by Desmet [2] it is possible to demonstrate that changing the relative amount of porous layer relative to the solid core will alter the impedance plot. The equations used are described in detail elsewhere, with the important parameter, ρ, being the amount of solid core relative to the whole particle diameter.

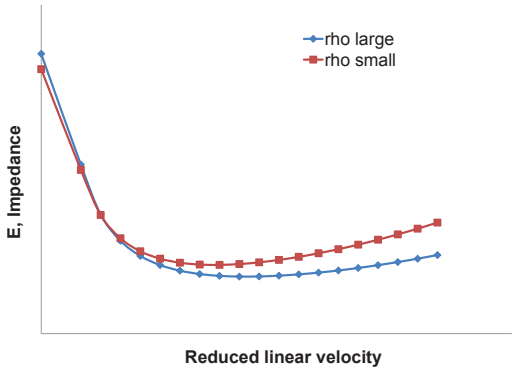

FIGURE 4. Modeling work demonstrating the effect of altering the shell thickness has on the impedance plot

Effect of Temperature

With the reduction in the particle there is an associated increase in the backpressure, which also results in an increased in the temperature gradient along the column and also potentially radially across the column. As the backpressure along the column increases above 600 bar, the temperature effects become more noticeable and it is important to ensure that these are minimized by using the correct design of heating. Different scenarios can exist depending on the nature of the heating, whether isothermal or adiabatic heating is employed. Schematics representations of the observations are shown in Figure 5. These highlight the issues associated with running at elevated pressures above 600 bar. Under adiabatic temperatures there are no radial thermal gradients, which means that the peak shape will in general be better. It should also be noted that pre-heating the solvent will in general result in better efficiencies (sharper peaks).

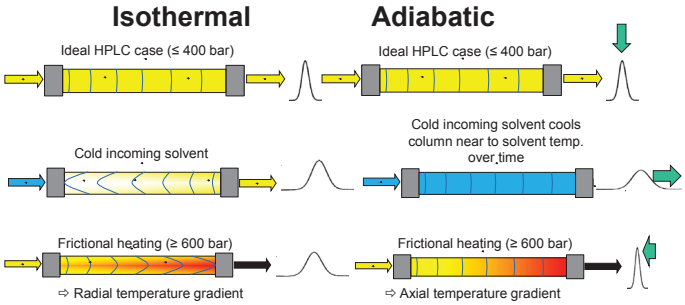

FIGURE 5. Schematic representation of the different forms of heating at different operating pressures and the effect that it can have on the peak shape.

The schematics also demonstrate how the different forms of heating can affect the peak shape, with the most notable being the situation where adiabatic heating occurs above 600 bar. In this situation, a longitudinal thermal gradient is established which results in a degree of peak focussing, meaning that it would be feasible to have sharper peaks than theory predicts, assuming that there are no thermal effects.

To verify that the proposed benefits associated with adiabatic heating exist a series of experiments were performed which investigated the use of nominally isothermal and also adiabatic heating. This was achieved by having using a forced air oven to simulate the isothermal behaviour and also a still air oven to simulate the effects of an adiabatic environment.

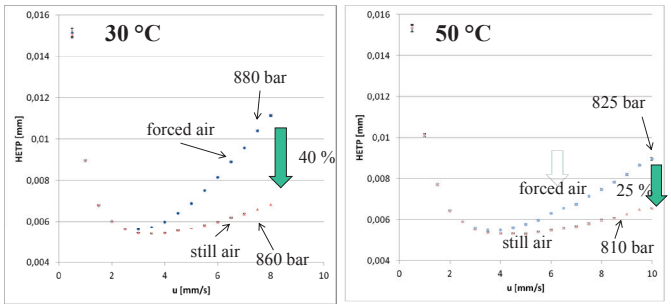

FIGURE 6. Comparison between adiabatic heating and isothermal heating on a 100mm column at two different temperatures.

Figure 6 depicts four sets of data which investigates the effects of the heating of the column at two different nominal temperatures, 30 °C and 50 °C. It can be seen at the lower temperature and at a low flow rate, the HETP for both the forced air and the still air are comparable. As the flow increases, and the back pressure across the column increases resulting in an increase in the temperature gradient across the column, the difference between the two modes of heating becomes greater. It can be seen at the highest linear velocity of 8 mm/s the still air heating has a 40% reduction in the HETP. Increasing the temperature to 50 °C and a similar observation can be made. At the lower linear mobile phase velocities the efficiencies of the two experimental arrangements are comparable. Increasing the linear velocity beyond the optimal efficiency level results in a disparity arising between the two column heating modes. It can be seen that running with the still air heating, which is an adiabatic approach to heating, that the performance of the chromatographic system is 25% better in terms of efficiency than that obtained with the isothermal or forced air approach to heating.

Conclusion

Two columns were evaluated under a variety of conditions:

- The greatest efficiency was obtained using the smaller particle size.
- The Accucore 2.6 µm C18 gave the lowest impedance.
- This was due to a different morphology, the porous layer had a reduced thickness.
- A model was developed which showed the same qualitative rend.
- The use of adiabatic (still air) heating gives lower HETP than isothermal heating (forced air).
- Scalability in dispersion processes is possible, however the particle morphology and also the effects of pressure induced heating do have to be considered.

References

1. P.A. Bristow, J.H. Knox, Chromatographia 1977, 10, 280.
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3. F. Gritti, T. Farkas, J. Heng and G. Guiochon, J. Chromatogr. A, vol. 1218, no. 45, pp. 8209-8221, 2011.

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