Giving Up Constant Flow in Modern Liquid Chromatography - Expanding the Horizon

Monika M. Dittmann[1], Konstantin Choikhet[1], Klaus Witt[1], Gerard Rozing[1], Matthias Verstraeten[2], Ken Broeckhoven[2], Gert Desmet[2]
(1) Agilent Technologies GmbH, Germany Waldbronn, (2) Vrije Universiteit Brussel, Department of Chemical Engineering, Brussels, Belgium

HPLC 2010
Poster P-2408-T

Agilent Technologies

Introduction

In HPLC a mixture of analytes is eluted from a chromatographic column after a certain volume of eluent is passed through the column. The required elution volume depends on the column dimensions and affinity of analytes to the stationary phase. Typically a chromatogram is acquired at constant flow rate and recorded as trace of detector signal vs. time. In the early years of automation of HPLC generating constant flow and a constant paper feed was easier than providing precise real-time value for passed eluent volume and referencing a detector signal to it.

Thus representation *versus* time combined with the assumption of constant eluent flow rate turned identical to signal representation *versus* run volume. However,

"Physically correct is chromatogram evaluation vs. run volume rather than vs. run time."

The use of time-based constant-flow chromatography also generates a number of issues for example:

- Flow rate limitation by maximum viscosity in gradients
- Necessity to account for pressure head space
- Column temperature variations in constant flow gradients

Special routines were created that allow to:

- recalculate programmed gradient timetables so that they can be executed with varying flow rate keeping gradient shape over volume unchanged.
- run analysis in variable flow mode
- acquire run volume data over time
- transform the detector data (chromatograms) recorded vs. time into chromatograms vs. volume

In this poster the authors would like to focus on the aspects of varying the flow rate in the course of a gradient run to operate at constant pressure during the execution of the gradient. When the eluent is changed from aqueous to organic in a gradient run the viscosity changes according to the solvent properties. The flow rate is limited by the pressure drop at maximum viscosity. In the variable flow mode the pressure is kept constant and the flow rate is increased as the eluent viscosity decreases. This would typically result in a decrease of analysis time.

Separation performance of gradients run in **constant flow mode (cst. F)** were compared to those run in **constant pressure mode (cst. P)** where the gradient slope in volumetric units was kept the same. For the cst. F mode runs the flow rate was varied in the range of 0.1 and 1.2 ml/min depending on column length. The cst. P gradients were run at pressures corresponding to the pressures at maximum viscosity of the constant flow gradients. Peak capacities were calculated and compared for the two modes. Gradient kinetic plots were constructed (Figures 6 and 8) and compared for the two operation modes (for the theory of gradient kinetic plots see posters P-2827-W and P-1303-W)

Experimental

All experiments were performed on an Agilent 1290 Infinity System where the pump was modified to allow operation in variable flow mode. Special experimental firmware has been designed to allow the pump to run gradient programs vs. run volume (actually delivered volume since run start) and to provide a real-time output for the run volume over time:

$$V_{run} = f(t)$$

The columns used were Zorbax Eclipse Plus RRHD, 1.8 µm, 2.1x50, 100 and 150 mm. HPLC grade acetonitrile and methanol were purchased from Merck Darmstadt, water was purified using a Millipore water purification system. Sample A was a mixture of Uracil (1), Benzene (2), Toluene (3), Ethylbenzene (4), Propylbenzene (5), Mesitylene (6) and Pyrene (7) in 66/33 vol% MeOH/H_2O, Sample B was the Agilent RRLC-checkout sample (mixture of alkylphenones, part no 5188-6529).

For the cst. P experiments the cst. P methods were set to the same pressure as the maximum pressure observed during the cst. F runs (see Figure 1). While the viscosity change in the course of the gradient resulted in non-constant flow rate, the gradient slope in volumetric units (V_B/V_D) was kept the same in cst. P and cst. F mode by the special firmware of the pump.

The data for both run modes were acquired vs. real time and later converted to be displayed vs. virtual time or run volume using the run volume vs. real time dependency data, supplied by the special firmware routines of the pump. Based on these data other signals (e.g. Absorbance) vs. time $A_r = s(t)$ were transferred to the volume domain and further processed as volume-dependent variables:

$$V_{run} = f(t) \Rightarrow t = f^{-1}(V_{run});\ A_r = s(f^{-1}(V_{run}))$$

Results and Discussion

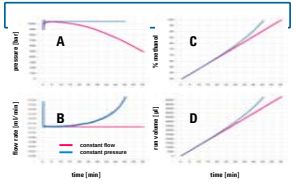

Figure 1: Curves for pressure drop (A), flow rate (B), % organic modifier (C) and run volume (D) over time for a linear gradient. In the cst. F mode % organic and run volume vary linearly over time, while they show a non-linear behaviour according to the change in flow rate in the cst. P mode.

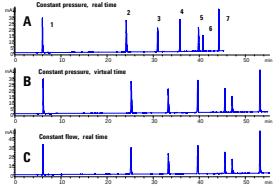

Figure 2: Gradient separations in cst. F and cst. P mode, coupled columns , L = 35 cm, flow rate = 0,124 ml/min, V_B/V_D = 0.1095 . Absorbance signal plotted vs. real time (A and C) and virtual time (B),

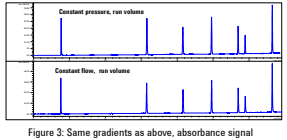

Figure 3: Same gradients as above, absorbance signal plotted vs. run volume

As shown in Figure 2 the elution time of the peaks in cst. P mode (A) vary from that in cst. F mode (C). This is a result of the non-linear behaviour of % organic modifier and run volume vs. time corresponding to the non-linear change in flow rate in the cst. P mode (Figure 1).

Using the run volume data, the cst. P absorbance signal can be represented in the virtual time domain, i.e. the elution time that would result at a given constant flow rate (B). Another possible representation of the data is in the volume domain, where the absorbance signal is plotted vs. run volume (Figure 3). As can be seen from figures 2 and 3, the virtual retention times and retention volumes for all peaks are the same for both elution modes. Thus the selectivity in cst. P mode is not different from that in cst. F mode.

This is, however, not true for the efficiency or peak width as the variation of flow rate in cst. P mode results in a different change of plate height compared to the cst. F case (Figure 4). During a gradient run the minimum of the H-u curve is shifted to different linear velocity values according to the change in eluent viscosity and the associated change in diffusion coefficient. The effect on peak width is different for gradients run in cst. P mode vs. cst. F mode.

$$u_{red} \propto \frac{1}{D_m} \qquad D_m \propto \frac{1}{\eta} \qquad u_{red} \propto \eta \propto \Delta P$$

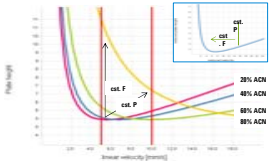

Figure 4: Change of plate height minimum with eluent composition. For gradients run in cf mode the reduced velocity changes according to the change in viscosity (diffusio), in cp mode the reduced velocity remains constant.

Results and Discussion

Construction of gradient kinetic plots [1] (see Poster P-1303-W)

$$\lambda = \frac{\Delta P_{max}}{\Delta P} \qquad t_{r,KPL} = \lambda \cdot t_{r,exp}$$

$$n_{p,KPL} = 1 + \sqrt{\lambda}(n_{p,exp} - 1) \qquad n_{p,exp} = 1 + \frac{t_{r,last} - t_{r,0}}{\frac{1}{n}\sum_{i}^{n} 4\sigma}$$

To compare peak widths in the two run modes, the 4-sigma values calculated from the signals in the virtual time domain were plotted vs. virtual retention time (figures 5 and 7). It can be observed that for the early eluting the compounds (propiophenone, benzene) no significant difference in sigma is observed. For the compound eluting towards the end of the gradient (octanophenone, pyrene) a significant difference in sigma, in particular in the B-term regime can be observed.

This magnitude of this gain depends on the gradient range, type of organic eluent and linear velocity.

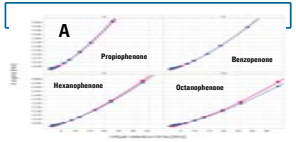

Figure 5: Plot of 4 sigma vs virtual retention time. Gradients from 0.1 to 1.2 ml/min, 10 – 90% Acetonitrile, Gradient slope V_B/V_D = 0.06 on a Zorbax Eclips Plus RRHD 2.1 x 50 mm

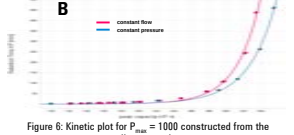

Figure 6: Kinetic plot for P_{max} = 1000 constructed from the gradient runs above.

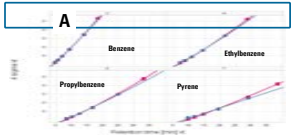

Figure 7: Plot of 4 sigma vs virtual retention time. Gradients from 0.1 to 0.3 ml/min, 50 – 100% Methanol, gradient slope V_B/V_D =0.1095 on a Zorbax Eclips Plus RRHD 2.1 x 150 mm

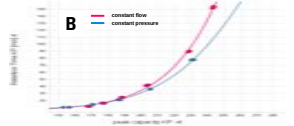

Figure 8: Kinetic plot for P_{max} = 1000 constructed from the gradient runs above.

Figures 6 and 8 show gradient kinetic plots [1] constructed from the experimental 4-sigma values in the virtual time domain and the experimental retention time data in the real time domain. In the C-term regime of the kinetic plots both modes perform very similar, in the B-term regime however a clear advantage of the cst. P mode can be observed.

Conclusions

It has been demonstrated that constant flow rate is not an absolute requirement in gradient (or isocratic) HPLC as long as real-time monitoring of run volume and control of gradient slope in the volume domain is supported by the pump HW/FW. It could be shown that the cst. P mode yields gradient performance comparable to that in cst. F mode in the C-term regime and better performance in the B-term regime. This is of particular interest for ultra-high resolution separations.

[1] K. Broeckhoven, D. Cabooter, F. Lynen, P. Sandra, G. Desmet, J. Chromatogr. A 1217 (2010) 2787-2795.