SOLUTE MIGRATION AND BAND BROADENING IN PRESSURE-ENHANCED LIQUID CHROMATOGRAPHY

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INTRODUCTION

Operating pressure in liquid chromatography (LC) can have an impact on solute retention, especially for large molecules. We recently introduced a new form of pressurized separations called Pressure-Enhanced Liquid Chromatography (PE-LC) [1]. An apparatus for the facile manipulation of column pressure was assembled consisting of a two-pump system and post-column flow restriction (*Figure 1*). Such a system enables combining mobile phase and pressure gradients in the same analytical run, or to work at any constant pressure independent on the flow delivered through the column. The PE-LC approach was already applied to improve peptides, proteins and oligonucleotides separations [2] and resulted in previously unseen selectivities (*Figure 2*).

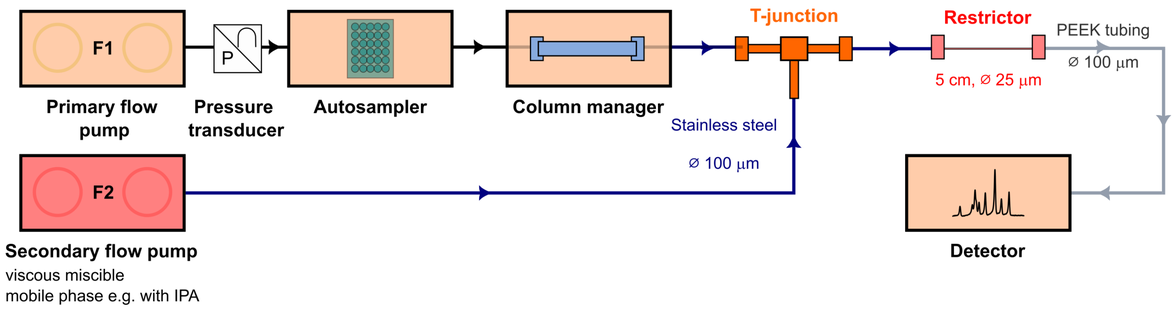

Figure 1. Schematic illustration of the PE-LC system

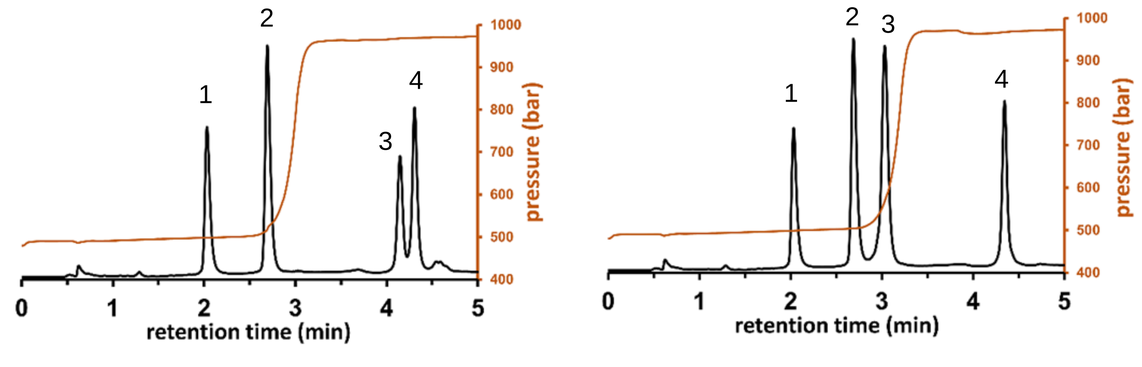

Figure 2. Gradient elution separations of oligonucleotides performed with a 450-bar pressure step. Peaks: dT40 (1), dT60 (2), dT80 (3), dT100 (4).

Upon recently studying the use of pressure gradients during liquid chromatography (PE-LC), it was noted that pressure differentials across a column can have a significant impact on peak width, not just retention as has been noted several times before. The aim of this work was to study this prediction and the effect of pressure gradients on solute migration velocity and band broadening/sharpening.

SOLUTE MIGRATION IN PE-LC

Figure 3 shows an illustration on the evolution of natural pressure drop (p_{col}), programmed pressure (p_{prog}), and the total pressure (p_{tot}) experienced by a solute and the local relative migration speed (u_{rel}) as function of migration distance (z) when assuming a positive concave pressure gradient and an L = 5 cm long column.

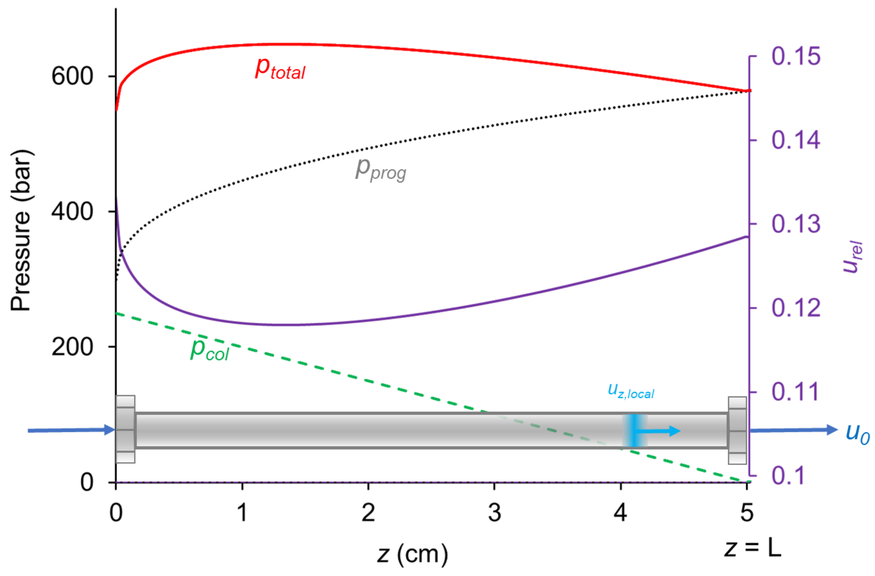

Figure 3. Schematic illustration of axial pressure changes and solute migration in PE-LC

PEAK BROADENING IN PE-LC

During pressure-controlled LC conditions, it is possible for observed peak widths to be very different from those observed in non-pressure-controlled (common LC) conditions. As noted above, pressure changes can significantly alter the velocity of the peak front and rear.

Figure 4 illustrates the change of pressure, solute retention and local migration velocity for the front and rear of a peak started migrating with Δz_{load} = 0.2 cm and when performing a linear positive pressure gradient from 0 to 600 bar in 5 min. *Figure 4 A* shows the programmed pressure (positive gradient), the pressure dropped only on the column (primary pump, negative gradient) and the actual pressures experienced by the peak front and rear (in this case these are positive gradients). *Figure 4 B* shows the change of the migration velocity of the peak front and rear as a function of time. The peak front leaves the column at t = 3.67 min while the rear exits at t = 4.10 min (Δt_{peak}= 0.43 min). *Figure 4 C* illustrates the change of local retention as a function of migration distance. The peak front starts migrating with $k_{inlet,front}$ = 4.48 and leaves the column with $k_{out,front}$ = 10.18. The rear part of the peak experiences $k_{inlet,rear}$ = 4.67 and leaves the column with $k_{out,rear}$ = 11.35. *Figure 4 D* shows the calculated peaks (normalized to retention time) for non-pressure-controlled condition and for the applied pressure gradient.

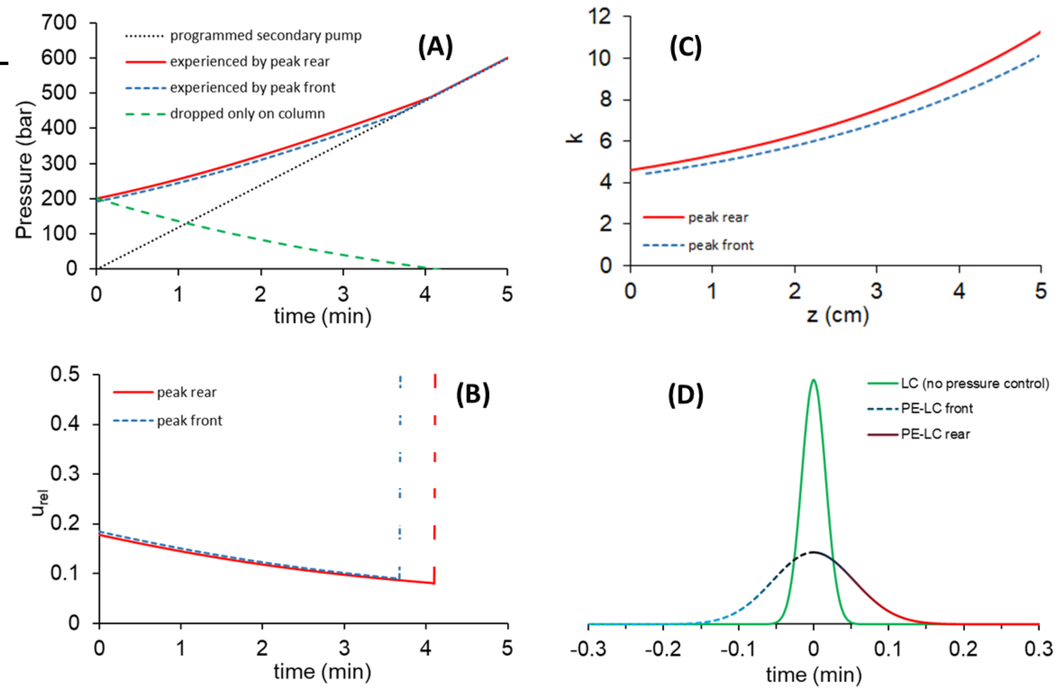

Figure 4. Hypothetical case study to illustrate band broadening in PE-LC: Pressure dropped on column: 200 bar, post column pressure gradient 0 to 600 bar in 5 min, column length: L = 5 cm, mobile phase velocity: u_0 = 10 cm/min and the initial band width is Δz_{load} = 0.2 cm. Panels: Pressure as a function of time for peak front and rear (A), the relative migration velocity of the front and rear in function of time (B), the actual retention of the peak front and rear as a function of migration distance (C) and calculated peaks for pressure controlled and regular LC (non-pressure controlled) conditions (D).

PEAK "SHARPENING" IN PE-LC

In this hypothetical case study, the same conditions were considered as for *Figure 4*, except the pressure program adjusted. Instead of a linear positive pressure gradient, a linear negative pressure gradient (600 to 0 bar in 5 min, induced by the secondary pump) was assumed.

Figure 5 shows the obtained plots and calculated peaks. As expected, in this case the peak front and rear experiences a steep negative pressure gradient in both time and spatial domain. Therefore, their migration velocity increases significantly as the peak travels through the column (positive migration velocity gradient). When setting this negative pressure gradient, the PE-LC peak will expectedly be sharper than the non-pressure-controlled (regular LC) peak. In other words, the PE-LC setup can be utilized to improve the apparent efficiency of a separation by creating sharper peaks.

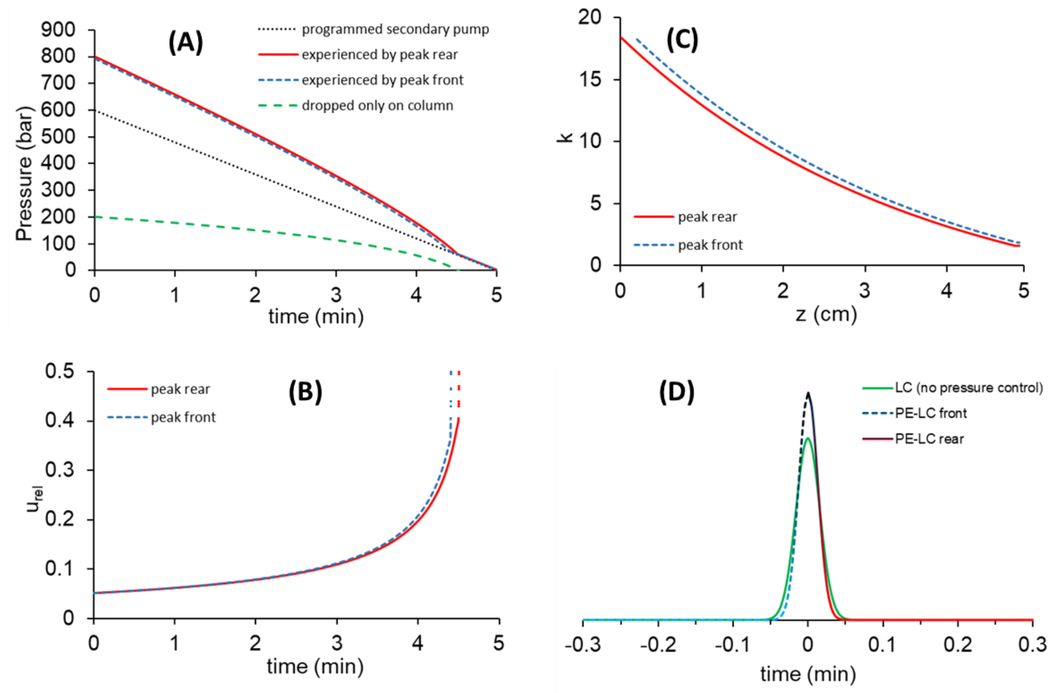

Figure 5. Hypothetical case study to illustrate band sharpening in PE-LC: Same conditions as for Figure 4, except the pressure gradient: 600 to 0 bar in 5 min (negative pressure gradient). Panels: Pressure as a function of time for peak front and rear (A), the relative migration velocity of the front and rear in function of time (B), the actual retention of the peak front and rear as a function of migration distance (C) and calculated peaks for pressure controlled and regular LC (non-pressure controlled) conditions (D).

EXPERIMENTAL VERIFICATION

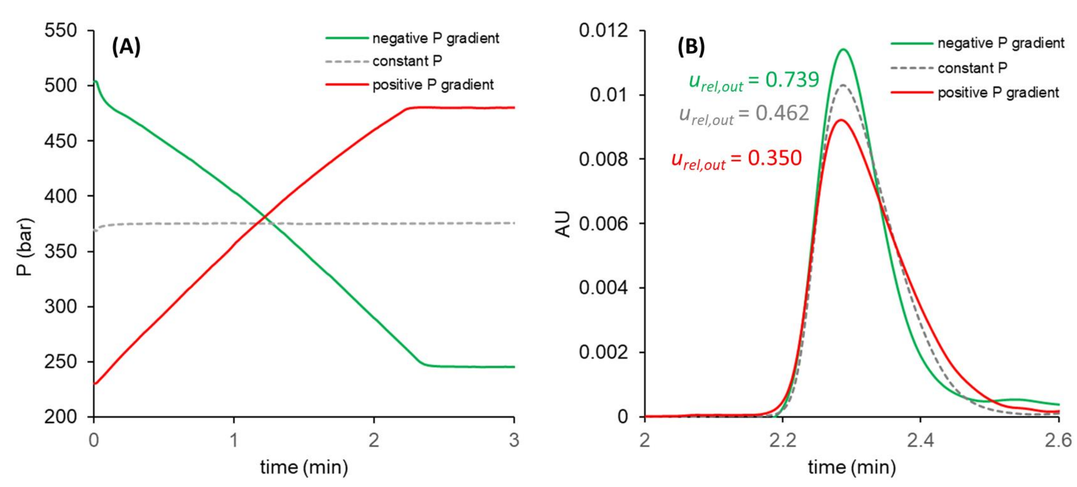

Figure 6. Experimental comparison of peak profiles when running constant pressure-, negative pressure gradient and positive pressure gradient experiments, maintaining the same average pressure. Measured pressure profiles (A) and corresponding chromatograms (B). Solute: insulin, column: ACQUITY™ Protein BEH™ C4 50 x 2.1 mm, mobile phase: 50% acetonitrile + 0.1% TFA, temperature: 35 °C, primary flow rate: F = 0.15 mL/min.

CONCLUSION

- **When solute's retention increases with pressure, it has been found that a positive pressure gradient will result in band broadening but improved selectivity**
- **A negative pressure gradient will help yield sharper peaks but limited selectivity**

References

1. S. Fekete, M. Fogwill, M. Lauber, Anal. Chem. 94 (2022) 7877-7884.
2. H. Lardeux, D. Guillarme, M. Imiolek, M. Lauber, S. Fekete, LCGC Advances in LC Column Technology, April 2023, p 28-34.

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