



Factors affecting the accuracy and reproducibility of gradient formation for preparative liquid chromatography

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Factors Affecting the Accuracy and Reproducibility of Gradient Formation for Preparative Liquid Chromatography

Abstract

Solvent or buffer gradients can greatly improve peak separation and process economy in chromatography applications. An in-line quantitative sensor permits on-line mobile phase analysis, which with control feedback can guarantee accurate and reproducible gradients. Tests were run to compare controlled gradients (with detection and feedback) to non-controlled gradients. Parameters such as temperature, composition, and delivery method of stock buffers and solvents were varied to demonstrate the impact of these factors on gradient performance. When feedback control was used, the gradients were unaffected by these factors. Accurate and reproducible LC gradient formation can be achieved from laboratory scale through pilot scale to production with the capability of unattended automated GMP purifications.

Introduction

This study used a GE Healthcare automated pilot/production scale BioProcess™ HPLC Type II system that incorporates a specially designed mixing loop with feedback to form gradients (Fig 1). The mixing loop ensures proper solvent or buffer blending before delivery to the main system pump. When running in automated mode, the system controls gradient formation via PID feedback between a detector on the mixing loop and the proportioning valves. The user sets a bypass range (e.g. $\pm 1\%$). If the actual buffer or solvent composition falls outside of this range, the system will enter column bypass mode until the composition is correct again. This ensures accuracy and reproducibility of gradients delivered to the chromatography column. For this study, a NIR detector (1430nm) was used to form gradients of ethanol in H₂O. For other applications, different detectors such as conductivity can be used to measure ionic strength of buffer solutions.

Experimental

The properties of the solvent feeds were modified to replicate common occurrences in production liquid chromatography. The PID feedback was stopped and gradients were run using simple timed proportioning between the valves to form non-



Fig 1. BioProcess HPLC systems from GE Healthcare ensure accurate and reproducible gradients.

controlled gradients. The NIR signal was plotted over time to show the actual composition of ethanol. The same gradients were then run using the system's PID feedback control to form controlled gradients. To demonstrate the effect of inadequate mixing on gradient formation, runs were also performed with the mixing loop bypassed.

The NIR detector was calibrated using the stock solvents:

100% H₂O → 0% on NIR transmitter
95% ethanol → 100% on NIR transmitter

non-controlled gradients

- one run with both solvents at ambient temperature, no pressure differential between delivered solvents, and no adjustment made to solvent composition.
- one run with water temperature at 35°C.
- one run with ethanol diluted to 85%.
- one run with pressure of delivered H₂O lowered by 0.24 bar (3.5 psi).

controlled gradients

- one run with both solvents at ambient temperature, no pressure differential between delivered solvents, and no adjustment made to solvent composition.
- one run with water temperature at 35°C.
- one run with ethanol diluted to 85%.
- one run with pressure of delivered H₂O lowered by 0.24 bar (3.5 psi).

non-controlled gradient without mixing loop

- one run with both solvents at ambient temperature, no pressure differential between delivered solvents, and no adjustment made to solvent composition.
- one run with pressure of delivered H₂O reduced by 0.24 bar (3.5 psi).

All runs are 0–100% gradients of ethanol in H₂O over 30 minutes.

Results

The NIR signal was recorded over time at a collection rate of 10Hz. A reading of approximately 880mV corresponds to 100% on the NIR transmitter. Figure 1 illustrates how changing the temperature, delivery pressure, and stock solvent composition affects the accuracy and reproducibility of gradient formation. Figure 2 illustrates how controlled gradient formation with PID feedback can form accurate and reproducible gradients regardless of changing properties of the stock solvents. The spikes in the controlled gradient curves (Fig2) indicate portions of time when the system was in column bypass mode (spikes caused by drop in backpressure). Note that all of the controlled gradient curves have slightly different slopes depending on how often the system went into column bypass mode. The more often the system bypasses the column, the longer it takes for the gradient to reach 100%. This explains the decreased slope of the curve corresponding to decreased H₂O delivery pressure. The system was in column bypass mode for this run more often than any of the other runs. The curve corresponding to diluted ethanol levels of under 100% and remains in bypass mode at the end of the run as a result of the dilution.

Figure 4 shows a comparison between using the mixing loop for solvent blending and bypassing the mixing loop.

Conclusion

- Using an in-line sensor and feedback control allows the formation of accurate and reproducible gradients at any scale regardless of deviations in temperature, delivery method, and composition of stock buffers and solvents. These deviations occur commonly in production scale chromatography and can drastically affect gradient accuracy and reproducibility when controlled gradient formation is not used.
- Using a high-speed mixing loop to blend solvents and buffers creates a smooth and reproducible gradient curve.

- Actual on-line monitoring of the chemical composition of blended buffers and solvents is much more significant than measuring physical parameters like mass flow alone. It allows the generation of a batch report that confirms actual gradient composition and inhibits incorrect solvent use. It also permits correction of changing feedstock solvent makeup (as shown in diluted ethanol experiment).
- Gradient elution liquid chromatography can be used to improve process scale separations in automated GMP purifications.

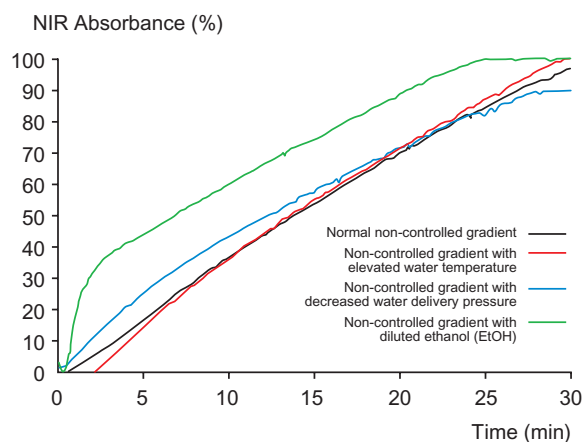


Fig 2. The affect of temperature, delivery pressure, and stock solvent composition on accuracy and reproducibility of gradient formation.

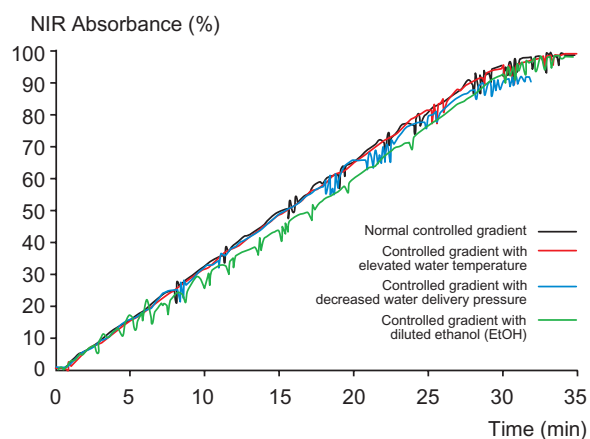


Fig 3. Controlled gradients eliminate the effects of changing properties of the stock solvents.

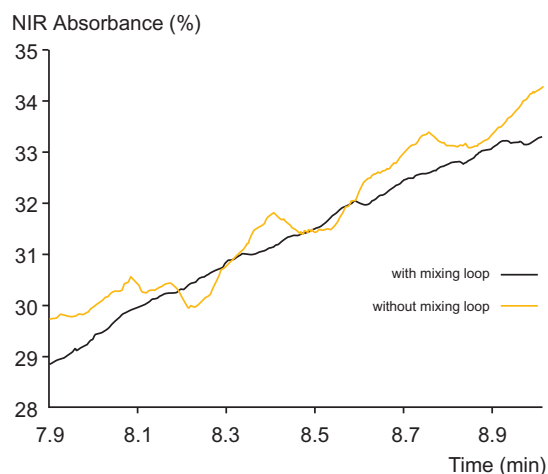


Fig 4. A mixing loop for solvent blending forms a much smoother gradient.

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