Millipore®
Preparation, Separation,
Filtration & Testing Products

Mobius® FlexReady Solution with Smart Flexware™ Assemblies for Chromatography

An easy-to-use, innovative solution for clinical development and manufacturing of biopharmaceuticals

How to use the guide

This Performance Guide is a reference document that provides highlights of key performance aspects of the Mobius® FlexReady Solution with Smart Flexware™ Assemblies for Chromatography. This guide includes information from a number of applications and case studies that were designed and/or selected to provide a diverse overview of the system performance under a range of expected processing conditions.

The results included in this guide summarize outcomes and observations obtained in studies conducted using particular model feed streams and experimental conditions. It is important to note that results are intended as general examples and should not to be construed as product claims or specifications.

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MERCK

Introduction

The Mobius® FlexReady Solution with Smart Flexware™ Assemblies is a fully automated system designed to enable the development and clinical-scale operation of chromatography separations for the purification of MAbs, vaccines, plasma and therapeutic proteins. The system has the same functionality as traditional chromatography systems and by incorporating a completely single-use flow path it provides operational flexibility while eliminating concerns of carryover or cross-contamination.

With a flow rate range between 0.1 – 8.0 L/min and a pressure rating of 4 bar (58 psi), the Mobius® FlexReady Solution is suited to a wide range of chromatographic separation techniques and column sizes. The system is also compatible with membrane adsorbers, adding to the overall flexibility required by clinical development and manufacturing facilities. The separation techniques include ion exchange, affinity, hydrophobic interaction and gel filtration, in column diameters ranging from 50 mm to 450 mm, depending upon the application.

The Mobius® FlexReady Solution is available in two different scales: 0.1 to 2.2 L/min and 1.6 to 8 L/min flow rates. Both solutions use the same base carts and simply switch clamshells, pumps, flowmeters and instruments if multi-use, and flow path diameters to accommodate the different scales.

In addition, several options are available for optimal configuration by the user for any particular application. These include:

- **Gradient capability:** Users have the option of choosing gradient capability. If chosen, the system will include two pumps, two inlet manifolds and two flow meters versus one of each if gradient capability is not selected. The Common Control Platform® software (CCP® software) provides the capability to run either step or linear gradients with high accuracy across the flow range (10 – 90%) of the system. However, it is important to note that gradient and in-line dilution cannot be performed in the same step. In general, in-line dilution with purified water cannot be performed with the system as the flow meters are magnetic flow meters.
- **Bubble Trap:** The bubble traps perform two functions:
  - Removal of air bubbles from the feed stream.
  - Act as a mixing chamber for gradient formation. In order to facilitate efficient mixing, bubble traps are available for the following flow ranges — 0.1–0.5 L/min., 0.5–2.2 L/min., and 1.6–8 L/min. Each of the bubble traps has been designed to promote efficient mixing while minimizing pressure losses in its flow range.
- Pre and Post-Column Instrumentation:
  - Optional Pre-column instrumentation consists of pH and conductivity
  - Post-column instrumentation is standard and consists of pH, conductivity, and UV
  - UV wavelength options are 280 nm and 300 nm
  - Instrumentation can be in Multi-use or Single-use options
  - Post-column instrumentation has three options for optical path length — 1 mm, 2.5 mm, and 10 mm

The specific system size and configuration of options that was used to generate the performance data included in this guide will be noted in the Methods section for each study.

Summary of Studies

Hydraulic Performance

Objective

The system design allows the end-user to configure various flow paths; the most commonly employed flow path includes a bubble trap, pre-column filter (Opticap® XL 3 Gamma Compatible Capsule Filter with Millipore Express® SHC Hydrophilic Membrane) and a column operating in forward mode (flow path shown in blue in **Figure 1**). The objective of this test was to measure the pressure drop suffered in the system flow path in order to evaluate the suitability of the system for a given chromatography process.

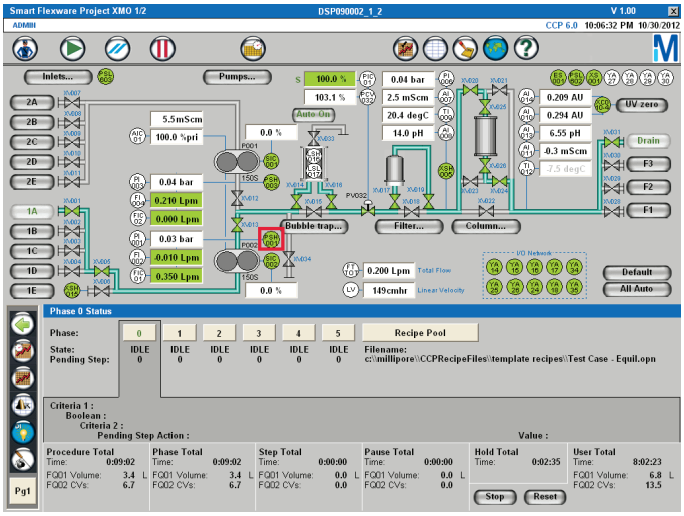


Figure 1. Chromatography P&ID.

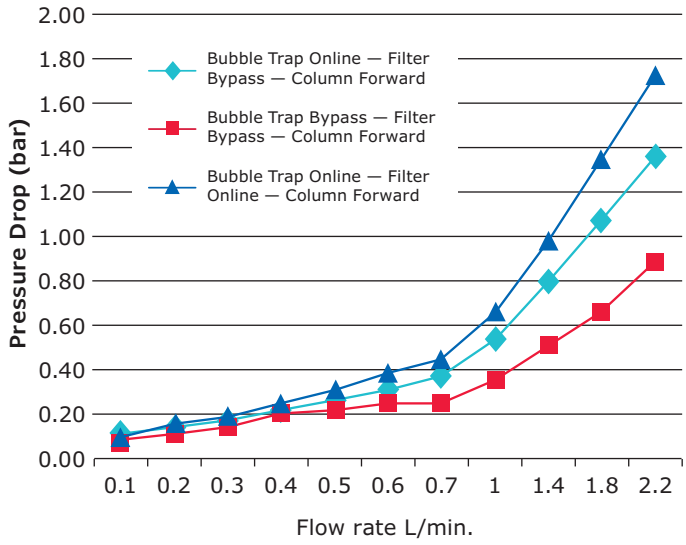
Materials and Methods

The system pressure drop with this flow path was measured across the operational flow range. RO water was employed as the process fluid. An appropriate amount of sodium chloride was added to achieve a conductivity of ~1mS/cm. This was necessary to ensure accurate functioning of the magnetic flow meters. The pressure was measured at PSH001 (outlined in red in **Figure 1**); the results are shown in **Figure 2**.

Results and Conclusions

The Mobius® FlexReady Solution offers three different bubble traps. The data in **Figure 2A** was generated with the smaller bubble trap. If the system pressure drop at higher flow rates is deemed excessive for a particular chromatography application, it is strongly recommended that the larger bubble trap available on the 2.2 L/min. system be employed for that application.

A: System Pressure Drop — Chrom. 2.2 L/min.



B: System Pressure Drop — Chrom. 8.0 L/min.

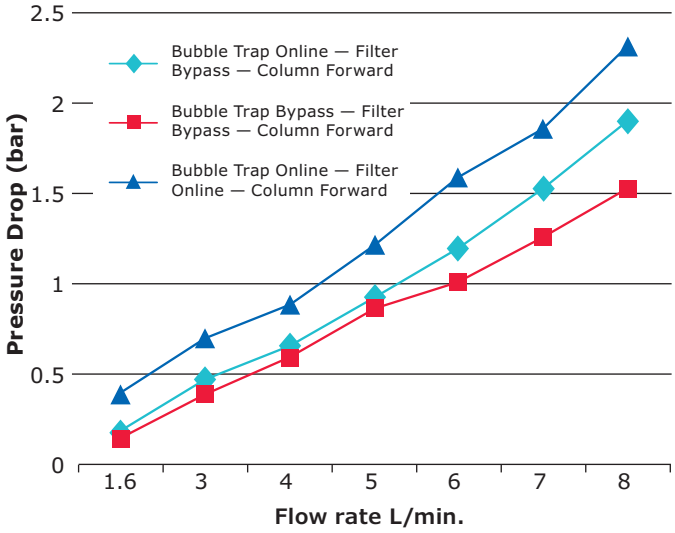


Figure 2A & 2B. System pressure drop with the flow path shown in Figure 1. The test was carried out at room temperature.

Gradient Performance

Objective

In some cases, chromatography processes require gradients in solution conditions to achieve their process objectives. These gradients may be continuous (linear) or discrete (step) in time. In either case, the accuracy of the gradients is critical to the success of the chromatography process. The Mobius® FlexReady Solution with Smart Flexware™ Assemblies for Chromatography employs dual pumps to deliver accurate and reproducible gradients. The necessary efficient mixing is achieved in the bubble trap.

Materials and Methods for 2.2 L/min. System

The gradient performance of the two Mobius® FlexReady Solutions was evaluated using RO water (an appropriate amount of sodium chloride was added to achieve a conductivity of ~1mS/cm) as the primary fluid and a 180 mg/L solution of L-Tryptophan in RO water as the secondary fluid. The gradient tests were executed using recipes developed in the CCP® 6 Recipe Editor. The resultant 280 nm UV trace was employed to characterize the gradient accuracy. The UV data was translated into gradient percent data using the UV absorbances of the primary and secondary fluids. The duration of the linear gradient test was 60 minutes.

Results

Figures 3 and **4** compare the actual and programmed linear gradient at 0.1 L/min. and 0.5 L/min. on the Mobius® FlexReady Solution.

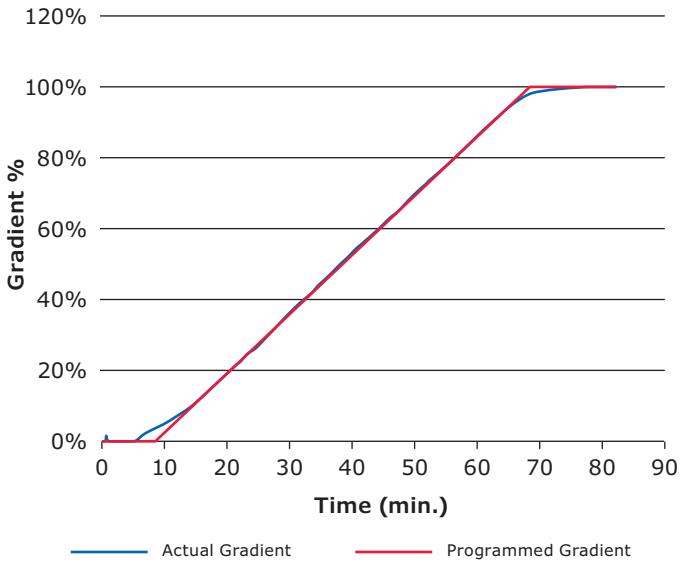


Figure 3. Linear gradient at 0.1 L/min.

The linearity of the gradients is clearly evident in **Figures 3** and **4**. The accuracy of the linear gradients was calculated by comparing the actual gradient percentage against the programmed gradient percentage. Thus, the error was computed as follows:

Gradient error (%) = Programmed gradient (%) – Actual gradient (%)

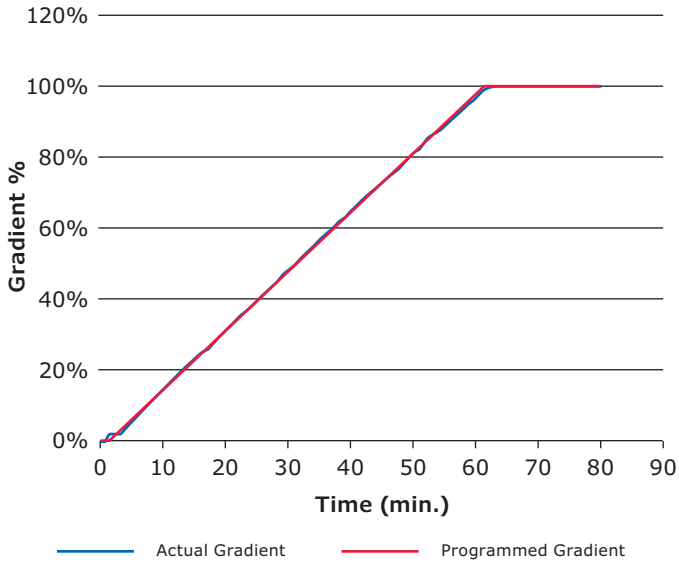


Figure 4. Linear gradient at 0.5 L/min.

The results for the two flow rates are illustrated in **Figures 5** and **6**. At both flow rates, the errors in the 10%–90% range are within 1%. At the gradient extremes (< 10% and > 90%), the errors increase to 2%–3.5% at the lower flow rate (0.1 L/min.). However, at the higher flow rate (0.5 L/min.), the errors at the gradient extremes are within 2% across the entire gradient range

Figure 7 compares the programmed and actual step gradients at 0.5 L/min. The errors in the step gradient are illustrated in **Figure 8**. As in the linear gradient case, the errors are within 2% in the 10%–90% gradient range.

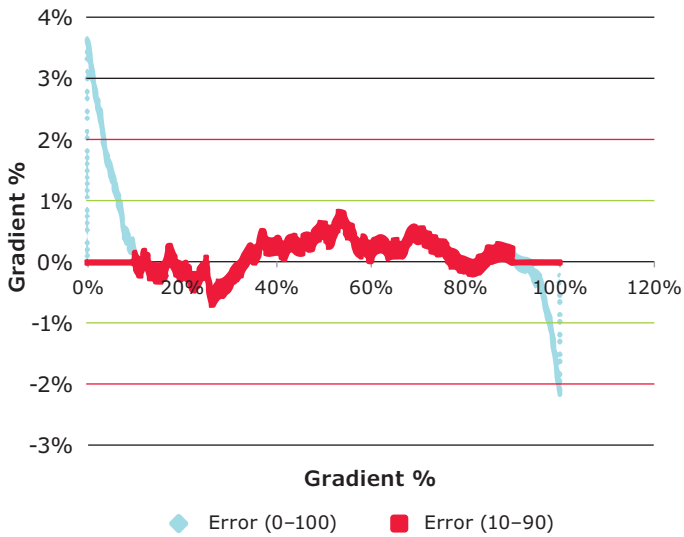


Figure 5. Errors in linear gradient at 0.1 L/min.

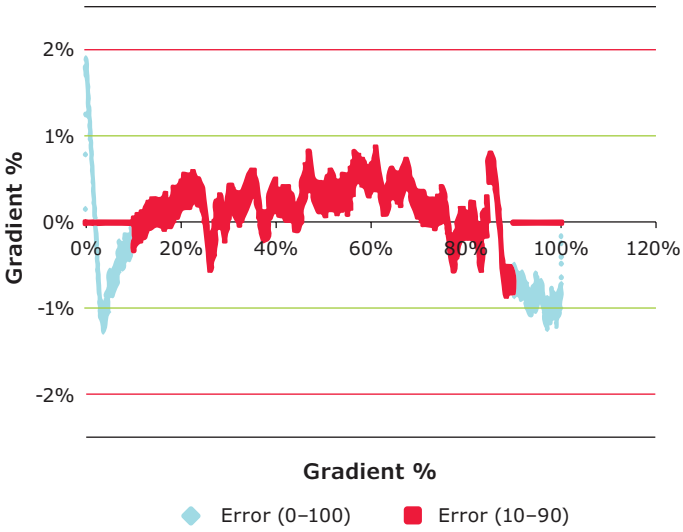


Figure 6. Errors in linear gradient at 0.5 L/min.

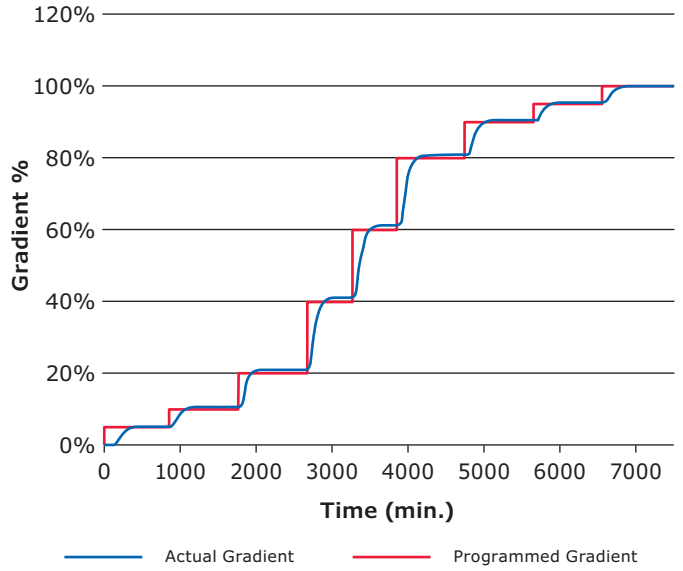


Figure 7. Step gradients at 0.5 L/min.

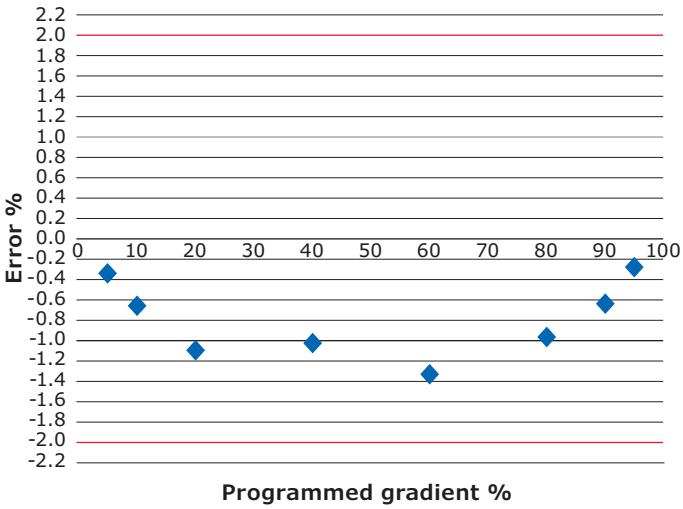


Figure 8. Step gradients at 0.5 L/min.

Materials and Methods for 8 L/min. System

Similar tests were executed on the 1.6–8 L/min system. This system has an operational flow range of 1.6–8 L/min. The gradient tests were carried out on a beta version. However, the gradient forming capability of the beta system was expected to be reflective of the final design.

Results

Figures 9 and **10** compare the actual and programmed linear gradient at 1.6 L/min and 8.0 L/min.

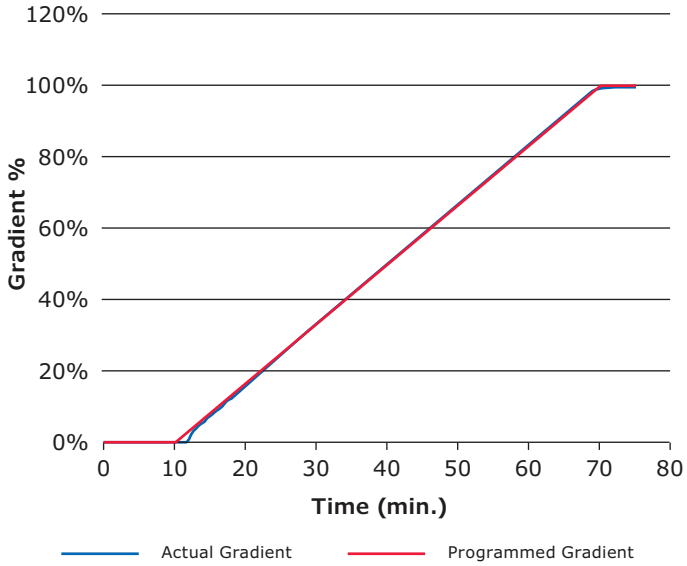


Figure 9. Linear gradient at 1.6 L/min.

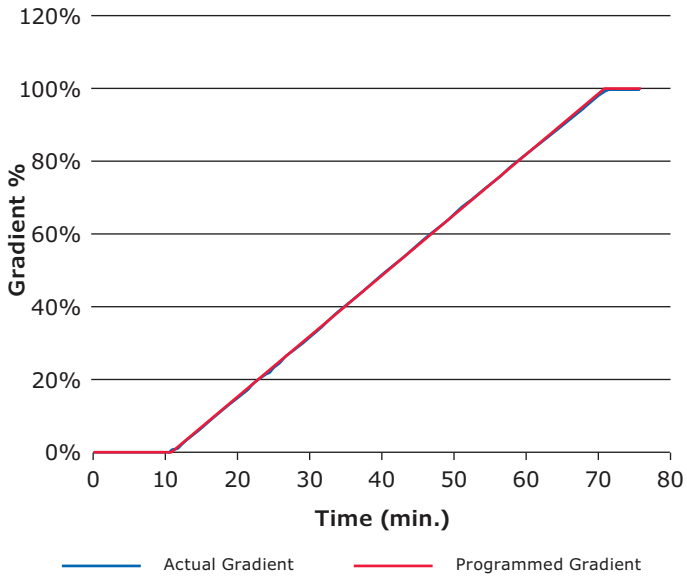


Figure 10. Linear gradient at 8.0 L/min.

The errors in the gradients for the two flow rates are illustrated in **Figures 11** and **12**. At both flow rates, the errors in the 10%–90% range are within 1%. At the gradient extremes (< 10% and > 90%), the errors increase to 2.5% at the lower flow rate (1.6 L/min.). However, at the higher flow rate (8.0 L/min.), the errors at the gradient extremes are still within 1%.

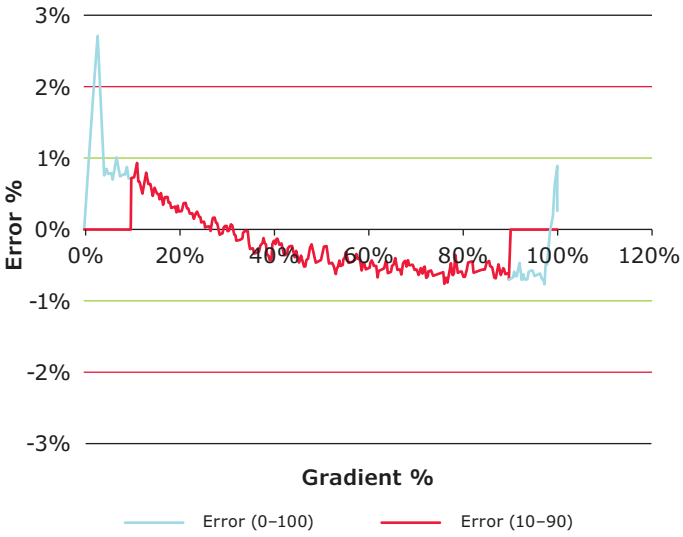


Figure 11. Errors in linear gradient at 1.6 L/min.

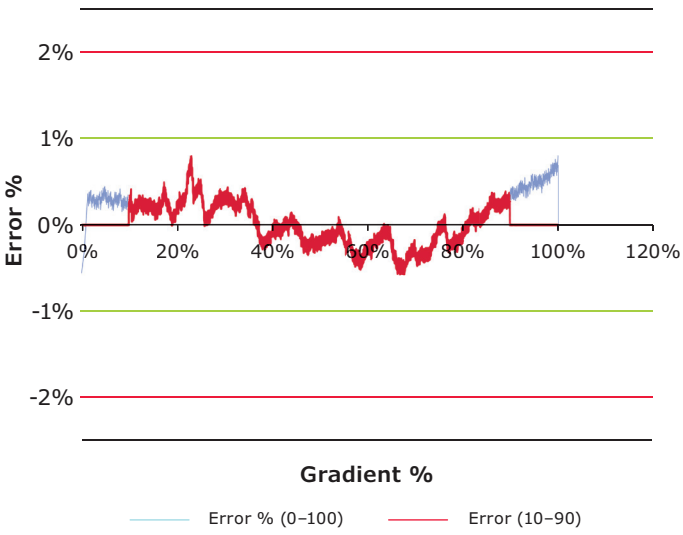


Figure 12. Errors in linear gradient at 8.0 L/min.

Figure 13 compares the programmed and actual step gradients at 4.0 L/min. The errors in the step gradient are illustrated in **Figure 14**. As in the linear gradient case, the errors are within 1% in the 10%–90% gradient range.

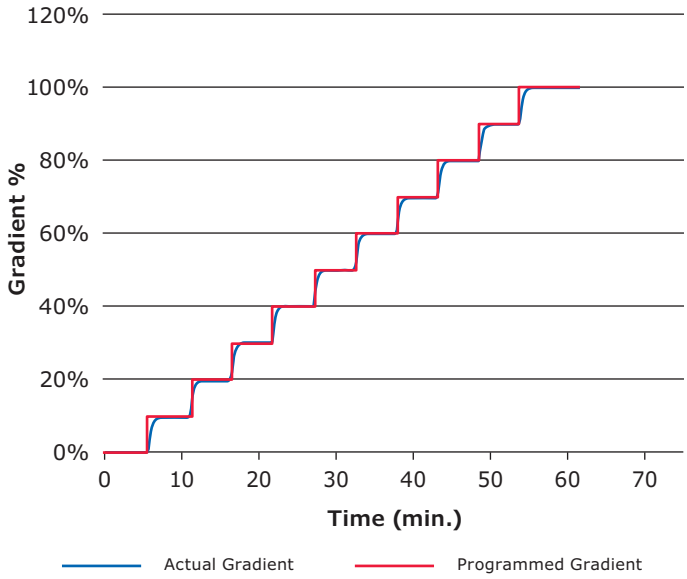


Figure 13. Step gradients at 8.0 L/min.

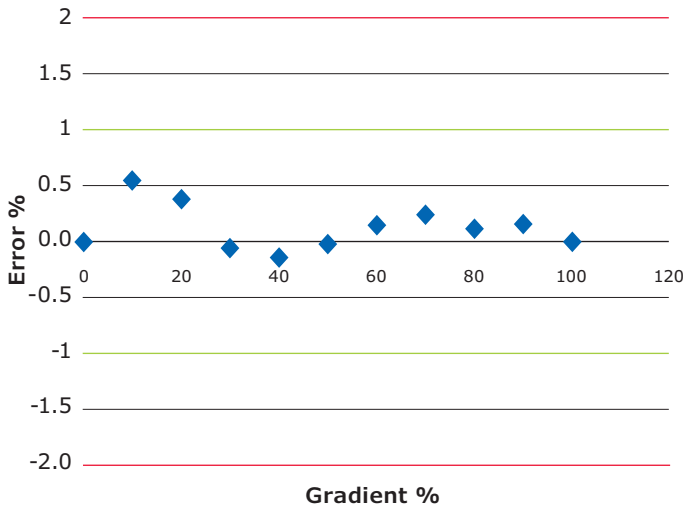


Figure 14. Errors in step gradient.

Conclusions

The Mobius® FlexReady Solution with Smart Flexware™ Assemblies for Chromatography specification claims a gradient accuracy of ± 2% over the 10%–90% gradient range. The results in Figures 3–14 clearly demonstrate that both versions of the system (0.1 to 2.2 L/min and 1.6 to 8 L/min. flow rates) can deliver gradients at this or better accuracy.

Column Qualification

Objective

A chromatography column is typically qualified with a non-retained tracer prior to use and qualification flow rates are often lower than the operational flow rates. The Mobius® FlexReady Solution has been designed to enable qualification and operation of a chromatography column with minimal modification.

Materials and Methods for 0.1 to 2.2 L/min Flow Rates

The Mobius® FlexReady 0.1–2.2 L/min. system spans a wider operational range than the 1.6–8 L/min. system. Thus, the potential for dispersion at the lower end of the flow rate is greater with the 0.1–2.2 L/min. system. In order to ensure proper qualification of a column at the lower end of the flow range of the 0.1–2.2 L/min. system, specific tubing sets are recommended depending on the qualification flow rate (catalogue no. XM0CMASM, XM1CMASM, XM2CMASM).

In addition, for qualification with the 0.1–2.2 system, it is recommended that the tubing from the column bottom be directly connected to the post-column instrumentation to minimize extra column dispersion. On the other hand, for the 1.6–8 L/min. system, the tubing from the column bottom can be connected directly to the "column bottom" port on the Smart Flexware™ container and does not need to be connected directly to the post-column instrumentation for qualification. In this case, due to smaller flow range, the extra column dispersion is minimal even at lower flow rates.

A 10 cm column was packed with Eshmuno® S resin. The column was qualified on a test stand and equilibrated with 400 mM NaCl solution followed by a 20 mL injection of 1 M NaCl. The qualification linear velocity was 160 cm/hr (~210 mL/min.). The Height Equivalent to a Theoretical Plate (HETP) and Asymmetry (As) of the column were reported to be 0.04 cm and 1.07, respectively.

Subsequently, the column was qualified using the 0.1–2.2 L/min. system. The appropriate tubing ID (3 mm) was employed and the tubing from the column bottom was connected directly to the post-column instrumentation.

Results for 0.1 to 2.2 L/min System

Figure 15 illustrates the resultant tracer peak. Analysis of the peak properties yielded an HETP and As of 0.05 cm and 1.2, respectively. This is in excellent agreement with the results obtained with the test stand.

Materials and Methods for 1.6 to 8 L/min. System

A QuikScale® 250 column was packed with Fractogel® SE HiCap (M) resin to a 20 cm bed depth. The recommended qualification velocity for this resin was 100 cm/hr (0.8 L/min.), which was below the flow range of the system. This column was qualified using the 1.6–8 L/min. system. The column was equilibrated with 200 mM NaCl solution followed by an 80 mL injection of 1 M NaCl.

Results for 1.6 to 8 L/min. System

The tracer peak is shown in Figure 16. The peak HETP and As were calculated to be 0.027 cm and 1.1, respectively. Given that the acceptance criteria for this resin are HETP < 0.05 cm and As between 0.7 and 1.4, the column would have been accepted for further use.

Conclusions

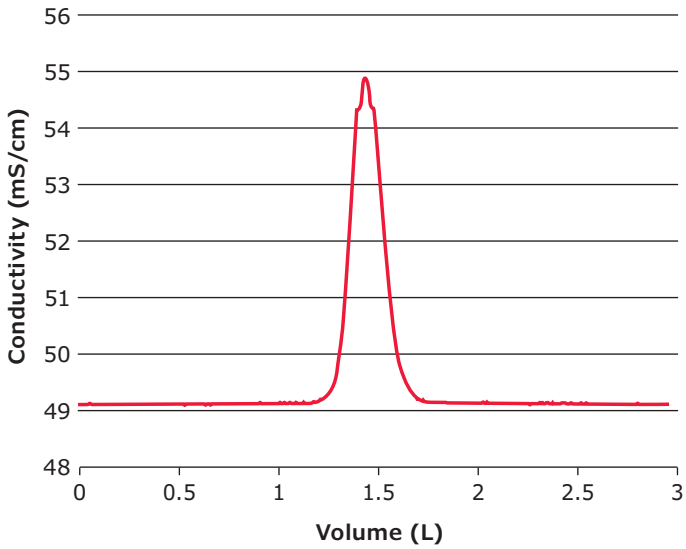


Figure 15. Tracer peak obtained with 0.1–2.2 L/min. system when qualifying a 10 cm ID column packed with Eshmuno® S resin.

Figures 15 and **16** clearly demonstrate that the Mobius® FlexReady Solution with Smart Flexware™ Assemblies for Chromatography may be employed for column qualification. In both cases, the recommended tubing sets should be employed. In the case of the 0.1–2.2 L/min. system, the tubing from the column bottom should be directly connected to the post-column instrumentation for qualification. In the case of the 1.6–8 L/min. system, the column tubing may be connected directly to the Smart Flexware™ container.

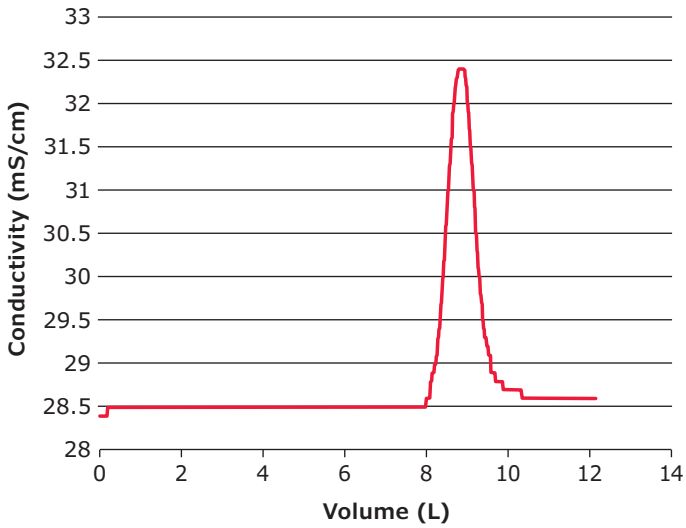


Figure 16. Tracer peak obtained with 1.6-8 L/min. system when qualifying a 25 cm ID column packed with Fractogel® SE/HiCap (M) resin.

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