Single-use bioprocessing

Gradient performance of the DynaChrom Single-Use Chromatography System

Keywords

DynaChrom Single-Use Chromatography System, chromatography, isocratic gradient, linear gradient

Introduction

Here we evaluate the performance of the Thermo Scientific™ DynaChrom™ Single-Use Chromatography System in generating gradients. The unit operations of chromatography may be discrete (step) and/or continuous (linear) gradients to accomplish various process objectives. Utilizing a linear gradient can effectively separate challenging impurities closely associated with the molecule of interest, such as aggregates for antibodies or empty capsids for viral vectors. Conversely, employing a step gradient to dilute buffer concentrates enhances buffer volume management and reduces the necessary footprint for the unit operation. These design-focused solutions aim to introduce micro-efficiencies into the manufacturing workflow process, leading to time and cost savings throughout the production life cycle.

The DynaChrom Single-Use Chromatography System provides extensive process-scale flexibility through the use of modular single-use fluid transfer assemblies (FTAs). These FTAs are available in four different inner diameters (1/4 in., 3/8 in., 1/2 in., and 3/4 in.) and can be equipped with up to three high-performance, low-pulsation pumps. The specific types and combinations of pumps are determined based on the processing requirements. Moreover, the system allows for the implementation of binary and tertiary gradients within the range of system flow rates.

Materials and methods

The experiments evaluated 1/4 in., 3/8 in., 1/2 in., and 3/4 in. FTAs for step and linear gradient performance using a DynaChrom Single-Use Chromatography System configured with either two Quattroflow 1200SU 5° pumps (1/4 in. FTA) or two Quattroflow 1200SU 5° pumps and one Quattroflow 2500SU 5° pumps (3/8 in., 1/2 in., and 3/4 in. FTAs). Solutions used during the testing of isocratic gradients were 2 M NaCl (aq) and deionized (DI) water/ambient water for injection (AWFI). Testing of linear gradients used the following solutions: 0.5 M NaCl (aq), 1% acetone, and DI water/AWFI. Additional details about the component specifications and system hold-up volume are listed in the DynaChrom system recipe process transfer document [1]. The automated pump linearization operation was used to set flow rates. No column was used. Simulated column back pressure of 2 bar was achieved by an automated Equilibar® control valve during the 1/4 in. FTA testing (single-use component part number SDO3NZA2; valve casing part number SDO3-CA-G3) and by a manual restrictor during the 3/8 in., 1/2 in., and 3/4 in. FTA testing. The bubble trap was inline and the guard filter was bypassed (Figure 1).

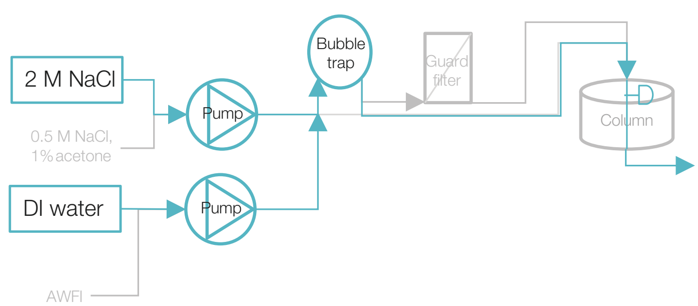


Figure 1. Simplified schematic of binary gradient flow path (step gradient example highlighted). The trap was inline, the guard filter was bypassed, and tubing with a manual restrictor was used to simulate column back pressure as a surrogate column.

1/4 in. FTA gradient results

1.1 Step gradients

The step gradients were performed on the DynaChrom system using a two-pump unit (PU-10 and PU-20) configuration utilizing Quattroflow 150SU pumps. Step gradients were performed using the 1/4 in. FTA according to Table 1. Experiments were performed at 147 L/hr and 210 L/hr. Steps of 10%, 25%, and 50% were performed based on the overall system flow rate. For example, a 10% step at 147 L/hr would be a PU-10 output of 14.7 L/hr and a PU-20 output of 132.3 L/hr. The system was operated at each condition for 5 min.

Table 1. Step gradient test conditions for 1/4 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 150SU) solution: 2M NaCl PU-20 (Quattroflow 150SU) solution: AWFI	
System flow rate (L/hr)	Step as %PU-10 of system flow rate (%)
147	10
	25
	50
210	10
	25
	50

Thermo Scientific™ BioProcess Containers (BPCs) filled with concentrated NaCl and AWFI were connected to the corresponding pumps. Inline conductivity values at each gradient baseline were compared to offline dilutions as the controlled variable. Offline dilutions were tested using a Thermo Scientific™ Orion™ Versa Star Pro™ pH/ISE/Conductivity/Dissolved Oxygen Multiparameter Benchtop Meter with a Thermo Scientific™ Orion™ DuraProbe™ 4-Cell Conductivity Probe.

Buffer transitions were analyzed based on the volume required to complete 95% of buffer conductivity transition for each flow rate, using the following steps:

1. Determine the low baseline (pre–step gradient start) and high baseline (steady-state step gradient) by taking the mean of the pre-column conductivity data for each baseline where the variance of data is less than 0.001 mS/cm.
2. Calculate the step width.

   $$\text{Step width} \left({}^{mS}/_{cm}\right) = \text{High baseline} \left({}^{mS}/_{cm}\right) - \text{Low baseline} \left({}^{mS}/_{cm}\right)$$

3. Calculate the conductivity at 95% of the buffer transition.

   $$\text{Conductivity}_{95} \left({}^{mS}/_{cm}\right) = 0.95 * \text{Step width} \left({}^{mS}/_{cm}\right) + \text{Low baseline} \left({}^{mS}/_{cm}\right)$$

4. Determine the start time for the low baseline ($\text{Time}_{\text{Low baseline start}}$) and the time when the conductivity is at 95% of the buffer transition (Time_{95}).
5. Calculate the 95% transition time.

   $$\text{Transition time}_{95} \text{ (hr)} = \text{Time}_{95} \text{ (hr)} - \text{Time}_{\text{Low baseline start}} \text{ (hr)}$$

6. Finally, calculate the step gradient volume to complete 95% of buffer transition.

   $$\text{Step gradient volume}_{95} \text{ (L)} = \text{Transition time}_{95} \text{ (hr)} * \text{System flow rate (L/hr)}$$

Figure 2 shows the inline conductivity reading at 10%, 25%, and 50% steps. The differences compared to the offline dilution readings are shown in Table 2. The volumes to complete 95% of the buffer transition are shown in Table 3. The DynaChrom System can provide efficient, consistent mixing and accurate conductivity readings within 2% of target for the tested conditions.

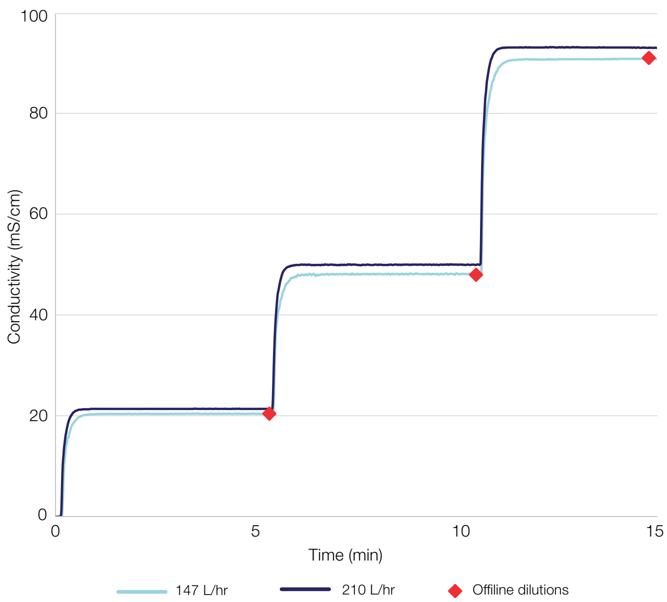


Figure 2. Inline conductivity reading of step gradients at 10%, 25%, and 50% PU-10 based on system flow rates of 147 L/hr (light blue), and 210 L/hr (dark blue) with 1/4 in. FTA and two Quattroflow 150SU pumps (PU-10 and PU-20); offline dilutions (red points) are shown for comparison.

Table 2. Percent differences between inline and offline conductivity readings for 1/4 in. FTA using two Quattroflow 150SU pumps (PU-10 and PU-20).

System flow rate (L/hr)	10% step error (%)	25% step error (%)	50% step error (%)
147	0.4	1.5	1.9
210	0.5	1.9	0.1

1.2 Linear gradients

The binary linear gradients were performed on the DynaChrom system using a two-pump configuration with Quattroflow 150SU pumps. Binary linear gradients for 30 and 60 min using the 1/4 in. FTA were performed according to Table 4. Experiments were performed at 147 L/hr and 210 L/hr. The range evaluated was 10%–90% on PU-10 based on the overall system flow rate. BPCs containing AWFI, and a NaCl solution with acetone, were connected to the corresponding pumps. Inline conductivity and UV values were confirmed to be accurate within the range of verification by running the conductivity/UV check operation as the control variable, which is further described in the software user guide [2]. Briefly, a solution with known conductivity and UV absorbance (2.5 mm optical path length) is used to verify proper functionality of the UV and conductivity sensors. The performance of the 30 and 60 min gradients was evaluated based on UV absorbance at 280 nm of an acetone solution.

Table 3. Step gradient volumes (in liters) to complete 95% of buffer transition for 1/4 in. FTA using two Quattroflow 150SU pumps (PU-10 and PU-20).

System flow rate (L/hr)	10% step PU-10 (L)	25% step PU-10 (L)	50% step PU-10 (L)
147	1.27	1.23	1.27
210	1.20	1.05	0.95

Table 4. 30 and 60 min linear gradient test conditions for 1/4 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 150SU) solution: 0.5 M NaCl, 1% acetone PU-20 (Quattroflow 150SU) solution: AWFI			
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)
147	30	10–90	90–10
210	30	10–90	90–10
147	60	10–90	90–10
210	60	10–90	90–10

Figures 3 and 4 show the normalized UV (normalized to the UV absorbance at the PU-10 90% baseline) for the 30 and 60 min gradients, respectively. The R^2 values for linear regression of all tested conditions are >0.99, confirming gradient linearity.

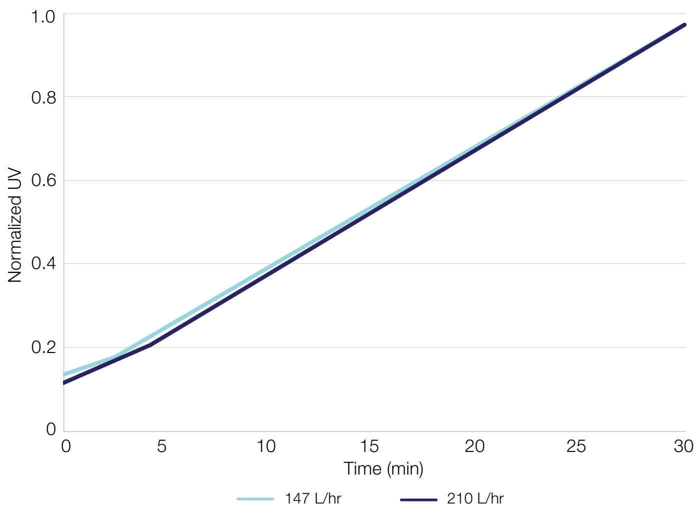


Figure 3. Normalized UV of 30 min linear gradients from 10% to 90% PU-10 based on system flow rates of 147 L/hr (light blue) and 210 L/hr (dark blue) with 1/4 in. FTA and two Quattroflow 150SU pumps (PU-10 and PU-20).

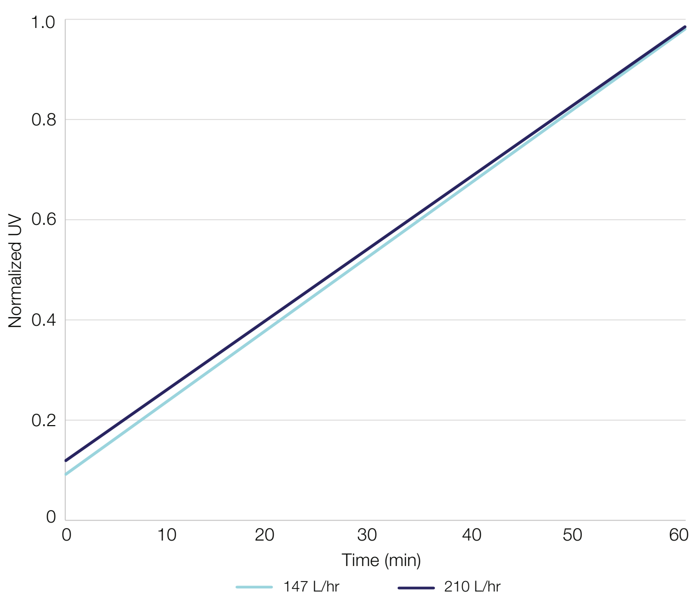


Figure 4. Normalized UV of 60 min linear gradients from 10% to 90% PU-10 based on system flow rates of 147 L/hr (light blue) and 210 L/hr (dark blue) with 1/4 in. FTA and two Quattroflow 150SU pumps (PU-10 and PU-20).

3/8 in. FTA gradient results

1.3 Step gradients

The step gradients were performed on the DynaChrom system using a two-pump configuration utilizing Quattroflow 1200SU pumps. Step gradients using the 3/8 in. FTA were performed according to Table 5. Experiments were performed at 270 L/hr and 420 L/hr. Steps of 10%, 25%, and 50% were performed based on the overall system flow rate. The system was operated at each condition for 5 minutes. BPCs filled with concentrated NaCl and DI water were connected to the specified pumps. Inline conductivity values at each gradient baseline were compared to offline dilutions. Offline dilutions were tested using an Orion Versa Star Pro pH/ISE/Conductivity/Dissolved Oxygen Multiparameter Benchtop Meter with an Orion DuraProbe 4-Cell Conductivity Probe. The transition analysis is described in section 1.1.

Table 5. Step gradient test conditions for 3/8 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 2 M NaCl PU-20 (Quattroflow 1200SU) solution: DI water	
System flow rate (L/hr)	Step as %PU-10 of system flow rate (%)
270	10
	25
	50
420	10
	25
	50

Figure 5 shows the inline conductivity reading at 10%, 25%, and 50% steps. The differences compared to the offline dilution readings are shown in Table 6. The volumes to complete 95% of the buffer transition are shown in Table 7. The DynaChrom system can provide efficient, consistent mixing and accurate conductivity readings within 4% of target for the tested conditions.

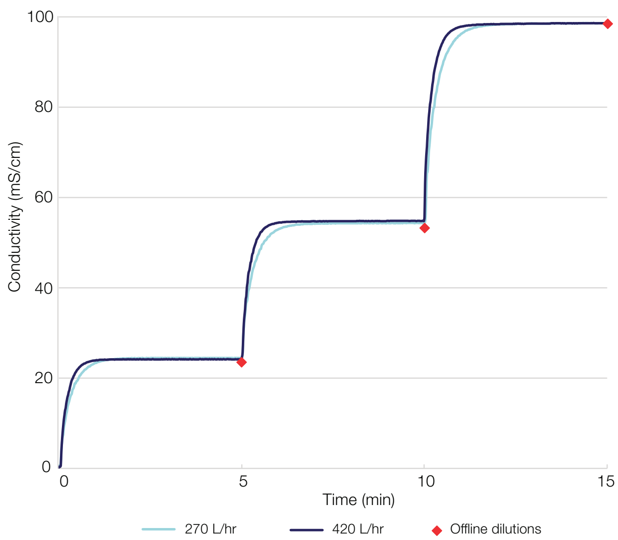


Figure 5. Inline conductivity reading of step gradients at 10%, 25%, and 50% PU-10 based on system flow rates of 270 L/hr (light blue) and 420 L/hr (dark blue) with 3/8 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20); offline dilutions (red points) shown for comparison.

Table 6. Percent differences between inline and offline conductivity reading percent difference for 3/8 in. FTA using two Quattroflow 1200SU pumps (PU-10 and PU-20).

System flow rate (L/hr)	10% step error (%)	25% step error (%)	50% step error (%)
270	4.0	2.1	0.1
420	2.8	2.1	0.2

Table 7. Step gradient volumes (in liters) to complete 95% of buffer transition for 3/8 in. FTA using two Quattroflow 1200SU pumps (PU-10 and PU-20).

System flow rate (L/hr)	10% step PU-10 (L)	25% step PU-10 (L)	50% step PU-10 (L)
270	4.5	4.5	4.7
420	4.6	4.1	4.6

1.4 Linear gradients

The binary linear gradients were performed on the DynaChrom system using a two-pump configuration with Quattroflow 1200SU pumps. Binary linear gradients at 15, 30, and 60 min using the 3/8 in. FTA were performed according to Tables 8 and 9. Experiments were performed at 270 L/hr and 420 L/hr. The range evaluated was 10%–90% on PU-10 based on the overall system flow rate. BPCs containing DI water, and a NaCl solution with acetone or concentrated NaCl, were connected to the specified pumps.

The performance of the 15 and 30 min gradients was evaluated based on UV absorbance at 280 nm of an acetone solution. The 60 min gradient was assessed using conductivity. Although conductivity is not linear over the range evaluated, the experiment reflects a general processing use-case for difficult separations.

Table 8. 15 and 30 min linear gradient test conditions for 3/8 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 0.5 M NaCl, 1% acetone PU-20 (Quattroflow 1200SU) solution: DI water			
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)
270	15	10–90	90–10
420			
270	30	10–90	90–10
420			

Table 9. 60 min linear gradient test conditions for 3/8 in. FTA using using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 0.5 M NaCl PU-20 (Quattroflow 1200SU) solution: DI water			
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)
270	60	10–90	90–10

Figure 6 and Figure 7 show the normalized UV (normalized to the UV absorbance at the PU-10 90% baseline) for the 15 and 30 min gradients, respectively. Figure 8 shows the conductivity reading for the 60 min gradient at 270 L/hr. The R^2 values for linear regression of all tested conditions are >0.99, confirming gradient linearity.

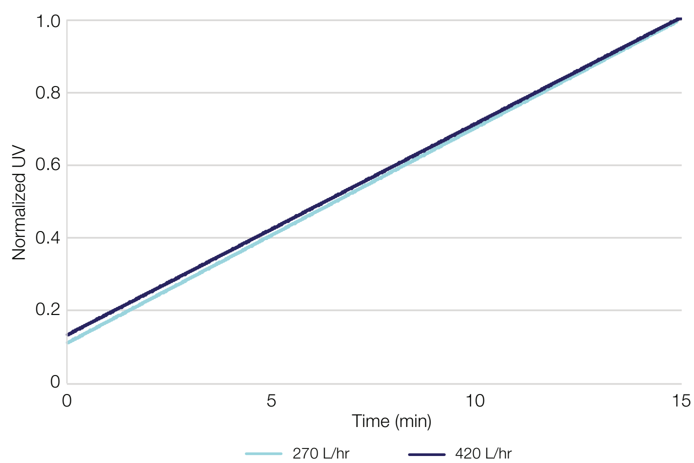


Figure 6. Normalized UV of 15 min linear gradients from 10% to 90% PU-10 based on system flow rates of 270 L/hr (light blue) and 420 L/hr (dark blue) with 3/8 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20).

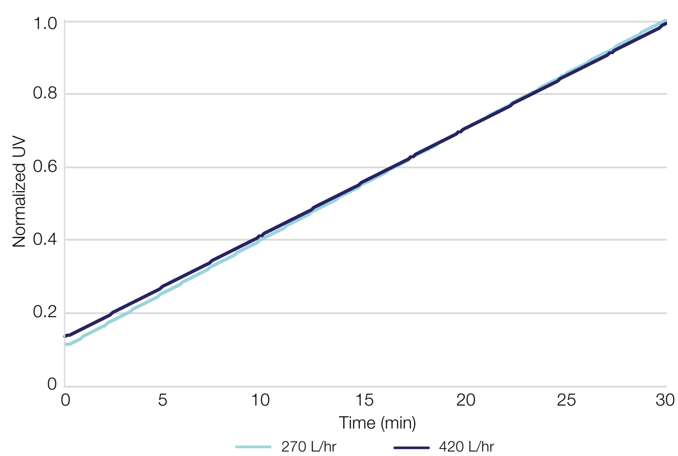


Figure 7. Normalized UV of 30 min linear gradients from 10% to 90% PU-10 based on system flow rates of 270 L/hr (light blue) and 420 L/hr (dark blue) with 3/8 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20).

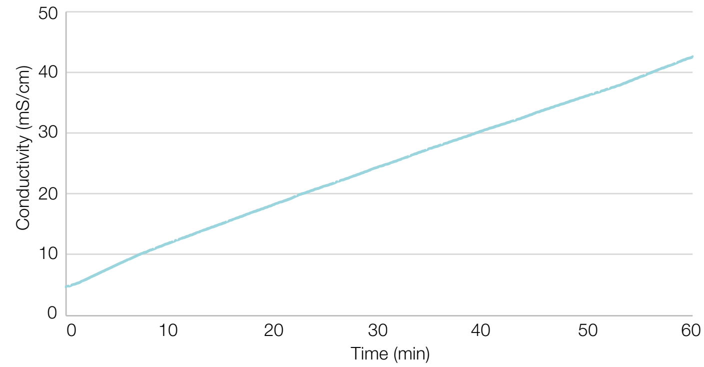


Figure 8. **Inline conductivity reading of 60 min linear gradient at 270 L/hr from 10% to 90% PU-10 based on system flow rate with 3/8 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20).**

1/2 in. FTA gradient results

1.5 Step gradients

The step gradients were performed on the DynaChrom system using a two-pump configuration utilizing Quattroflow 1200SU pumps. Step gradients using the 1/2 in. FTA were tested according to Table 10. Experiments were performed at 450 L/hr and 750 L/hr. Gradient steps of 10%, 25%, and 50% were performed based on the overall system flow rate. The system was operated at each condition for 5 min. BPCs filled with concentrated NaCl and DI water were connected to the specified pumps. Inline conductivity values at each gradient baseline were compared to offline dilutions. Offline dilutions were tested using an Orion Versa Star Pro pH/ISE/Conductivity/Dissolved Oxygen Multiparameter Benchtop Meter with an Orion DuraProbe 4-Cell Conductivity Probe. The transition analysis is described in section 1.1.

Table 10. Step gradient test conditions for 1/2 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 2 M NaCl PU-20 (Quattroflow 1200SU) solution: DI water	
System flow rate (L/hr)	**Step as %PU-10 of system flow rate (%)**
450	10
	25
	50
750	10
	25
	50

Figure 9 shows the inline conductivity reading at 10%, 25%, and 50% steps. The differences compared to the offline dilution readings are shown in Table 11. The volumes to complete 95% of the buffer transition are shown in Table 12. The DynaChrom system can provide efficient, consistent mixing and accurate conductivity readings within 2% of target for the tested conditions.

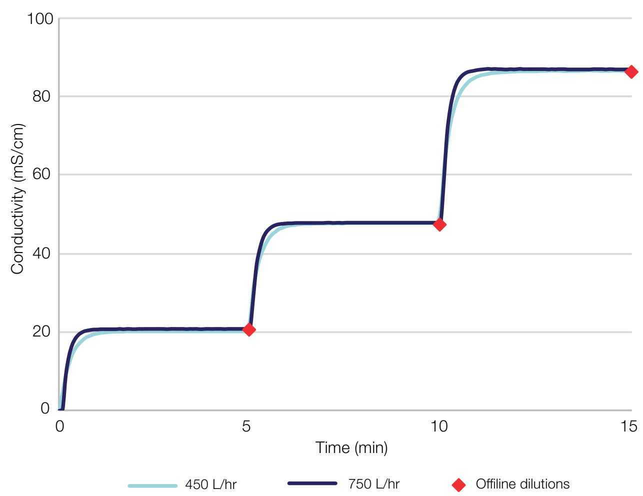


Figure 9. Inline conductivity reading of step gradients at 10%, 25%, and 50% PU-10 based on system flow rates of 450 L/hr (light blue) and 750 L/hr (dark blue) with 1/2 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20); offline dilutions (red points) are shown for comparison.

Table 11. Percent differences between inline and offline conductivity reading percent difference for 1/2 in. FTA using two Quattroflow 1200SU pumps (PU-10 and PU-20).

System flow rate (L/hr)	10% step error (%)	25% step error (%)	50% step error (%)
450	0.0	1.5	0.1
750	1.3	1.1	0.8

Table 12. Step gradient volumes (in liters) to complete 95% of buffer transition for 1/2 in. FTA using two Quattroflow 1200SU pumps (PU-10 and PU-20).

System flow rate (L/hr)	10% step PU-10 (L)	25% step PU-10 (L)	50% step PU-10 (L)
450	6.9	6.3	6.9
750	7.3	7.3	7.3

1.6 Linear gradients

The binary linear gradients were performed on the DynaChrom system using a two-pump configuration with Quattroflow 1200SU pumps. Binary linear gradients at 15, 30, and 60 min using the 1/2 in. FTA were performed according to Tables 13 and 14. Experiments were performed at 450 L/hr and 750 L/hr. The range evaluated was 10%–90% on PU-10 based on the overall system flow rate. BPCs containing DI water, and a NaCl solution with acetone or concentrated NaCl solution, were connected to the specified pumps.

Table 13. 15 and 30 min linear gradient test conditions for 1/2 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 0.5 M NaCl, 1% acetone PU-20 (Quattroflow 1200SU) solution: DI water			
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)
450	15	10–90	90–10
750			
450	30	10–90	90–10
750			

Table 14. 60 min linear gradient test conditions for 1/2 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 0.5 M NaCl PU-20 (Quattroflow 1200SU) solution: DI water			
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)
450	60	10–90	90–10

The performances of 15 and 30 min gradients were evaluated based on UV absorbance at 280 nm of an acetone solution. The 60 min gradient was assessed using conductivity. Although conductivity is not linear over the range evaluated, the experiment reflects a general processing use-case for difficult separations.

Figures 10 and 11 show the normalized UV for the 15 and 30 min gradients, respectively. Figure 12 shows the conductivity reading for the 60 min gradient at 450 L/hr. The R^2 values for linear regression of all tested conditions are >0.99, confirming gradient linearity.

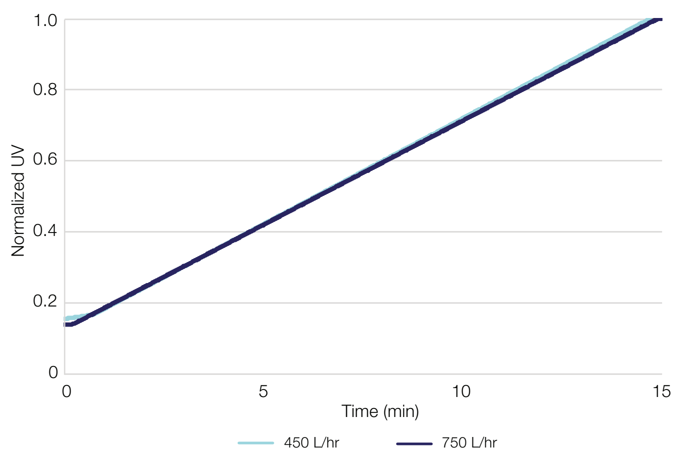


Figure 10. Normalized UV of 15 min linear gradients from 10% to 90% PU-10 based on system flow rates of 450 L/hr (light blue) and 750 L/hr (dark blue) with 1/2 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20).

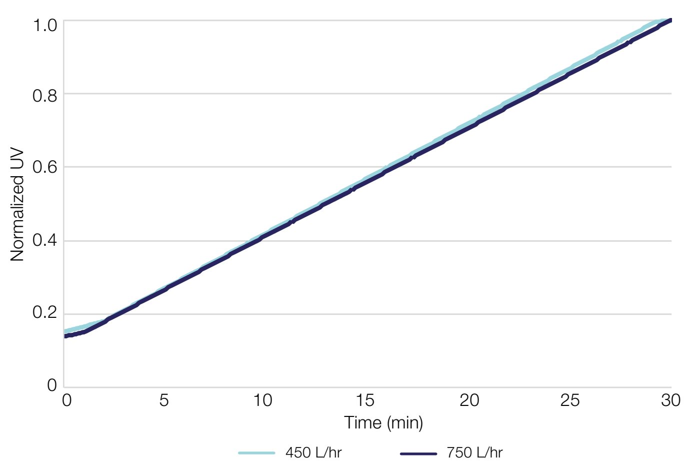


Figure 11. Normalized UV of 30 min linear gradients from 10% to 90% PU-10 based on system flow rates of 450 L/hr (light blue) and 750 L/hr (dark blue) with 1/2 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20).

3/4 in. FTA gradient results

1.7 Step gradients

The step gradients were performed on the DynaChrom system using a three-pump configuration with two Quattroflow 1200SU pumps and one Quattroflow 2500SU pump. Step gradients using the 3/4 in. FTA were performed according to Table 15 (PU-10 and PU-20) and Table 16 (PU-10 and PU-30). Experiments were performed at 1,080 and 1,500 L/hr. Steps of 10%, 25%, and 50% were performed based on the overall system flow rate. The system was operated at each condition for 5 min. BPCs containing concentrated NaCl and DI water were connected to the specified pumps. Inline conductivity values at each gradient baseline were compared to offline dilutions. Offline dilutions were tested using an Orion Versa Star Pro pH/ISE/Conductivity/Dissolved Oxygen Multiparameter Benchtop Meter with an Orion DuraProbe 4-Cell Conductivity Probe. The transition analysis is described in section 1.1.

Table 15. Step gradient test conditions for 3/4 in. FTA using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 2 M NaCl PU-20 (Quattroflow 1200SU) solution: DI water	
System flow rate (L/hr)	Step as %PU-10 of system flow rate (%)
1,080	10
	25
	50

Table 16. Step gradient test conditions for 3/4 in. FTA using PU-10 and PU-30.

PU-10 (Quattroflow 1200SU) solution: 2 M NaCl PU-30 (Quattroflow 1200SU) solution: DI water	
System flow rate (L/hr)	Step as %PU-10 of system flow rate (%)
1,500	10
	25
	50

Figure 13 shows the inline conductivity reading at 10%, 25%, and 50% steps. The differences compared to the offline dilution readings are shown in Tables 17 and 18. The volumes to complete 95% of the buffer transition are shown in Table 19. The DynaChrom system can provide efficient, consistent mixing and conductivity accuracy within 5% of target for the tested conditions.

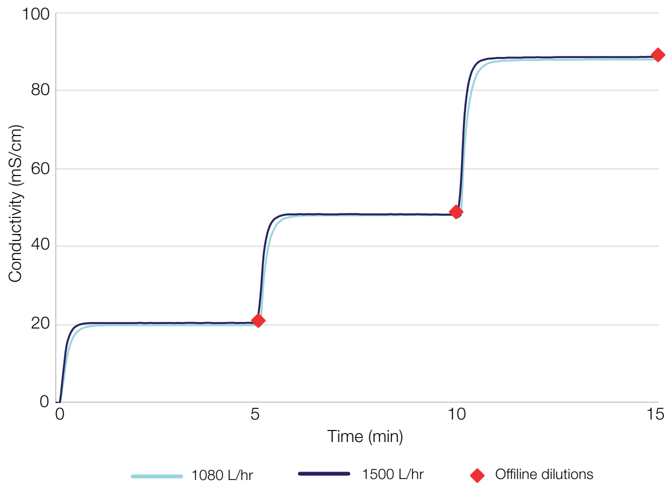


Figure 13. Inline conductivity reading of step gradients at 10%, 25%, and 50% PU-10 based on system flow rates of 1,080 L/hr (light blue; two Quattroflow 1200SU pumps for PU-10 and PU-20) and 1,500 L/hr (dark blue; Quattroflow 1200SU and Quattroflow 2500SU pumps for PU-10 and PU-30, respectively) with 3/4 in. FTA; offline dilutions (red points) are shown for comparison.

Table 17. Percent differences between inline and offline conductivity reading for 3/4 in. FTA using two Quattroflow 1200SU pumps (PU-10 and PU-20).

System flow rate (L/hr)	10% step error (%)	25% step error (%)	50% step error (%)
1,080	4.6	0.6	1.2

Table 18. Step gradient volumes (in liters) to complete 95% of buffer transition for 3/4 in. FTA using Quattroflow 1200SU and Quattroflow 2500SU (PU-10 and PU-30).

System flow rate (L/hr)	10% step error (%)	25% step error (%)	50% step error (%)
1,500	0.2	4.1	1.4

Table 19. Step gradient volumes (in liters) to complete 95% of buffer transition for 3/4 in. FTA using two Quattroflow 1200SU pumps (PU-10 and PU-20) at 1,080 L/hr, and Quattroflow 1200SU and Quattroflow 2500SU pumps (PU-10 and PU-30) at 1,500 L/hr.

System flow rate (L/hr)	10% step PU-10 (L)	25% step PU-10 (L)	50% step PU-10 (L)
1,080	12.0	12.0	12.0
1,500	12.5	10.4	12.5

1.8 Linear gradients

The linear gradients were performed on the DynaChrom system using a three-pump configuration with two Quattroflow 1200SU pumps and one Quattroflow 2500SU pump. Binary linear gradients at 15, 30, and 60 min using the 3/4 in. FTA were performed according to Table 20 and Table 21.

Table 20. 15 and 30 min linear gradient test conditions for 3/4 in. FTAs using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 0.5 M NaCl, 1% acetone PU-20 (Quattroflow 1200SU) solution: DI water			
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)
1,080	15	10–90	90–10
	30	10–90	90–10

Table 21. 60 min linear gradient test conditions for 3/4 in. FTAs using PU-10 and PU-20.

PU-10 (Quattroflow 1200SU) solution: 0.5 M NaCl PU-20 (Quattroflow 1200SU) solution: DI water			
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)
1,080	60	10–90	90–10

Tertiary linear gradients at 15 and 30 min were performed according to Table 22. Experiments were performed at 1,080 L/hr and 1,500 L/hr. The range evaluated was 10%–90% PU-10 (binary) or PU-10 plus PU-20 (tertiary) based on the overall system flow rate. BPCs containing DI water, and a NaCl solution with acetone or concentrated NaCl solution, were connected to the specified pumps (Tables 20–22).

Table 22. Linear gradient test conditions for 3/4 in. FTA using PU-10, PU-20, and PU-30.

PU-10 (Quattroflow 1200SU) solution: 0.5 M NaCl, 1% acetone PU-20 (Quattroflow 1200SU) solution: 0.5 M NaCl, 1% acetone PU-30 (Quattroflow 2500SU) solution: DI water				
System flow rate (L/hr)	Gradient duration (min)	Range as %PU-10 of system flow rate (%)	Range as %PU-20 of system flow rate (%)	Range as %PU-30 of system flow rate (%)
1,500	15	5–45	5–45	90–10
	30	5–45	5–45	90–10

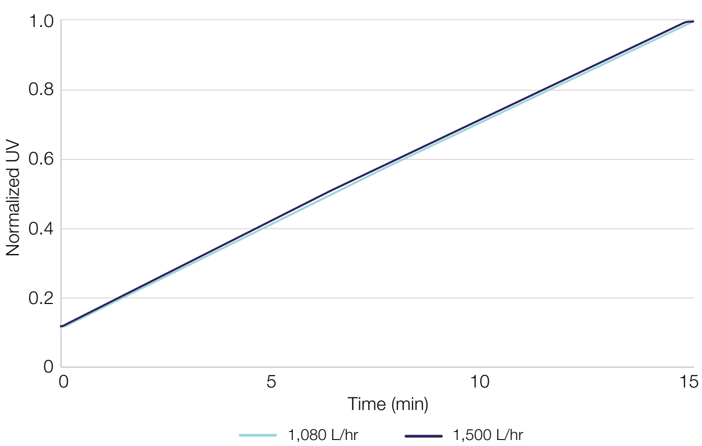


Figure 14. Normalized UV of 15 min linear gradients from 10% to 90% PU-10 based on system flow rates of 1,080 L/hr (light blue; two Quattroflow 1200SU pumps for PU-10 and PU-20) and 1,500 L/hr (dark blue; two Quattroflow 1200SU and Quattroflow 2500SU pumps for PU-10, PU-20, and PU-30, respectively) with 3/4 in. FTA.

The performances of 15 and 30 min gradients were evaluated based on UV absorbance at 280 nm of an acetone solution. The 60 min gradient was assessed using conductivity. Although conductivity is not linear over the range evaluated, the experiment reflects a general processing use-case for difficult separations.

Figures 14 and 15 show the normalized UV for the 15 and 30 min gradients, respectively. Figure 16 shows the conductivity reading for the 60 min gradient at 1,080 L/hr. The R^2 values for linear regression of all tested conditions are >0.99, confirming gradient linearity.

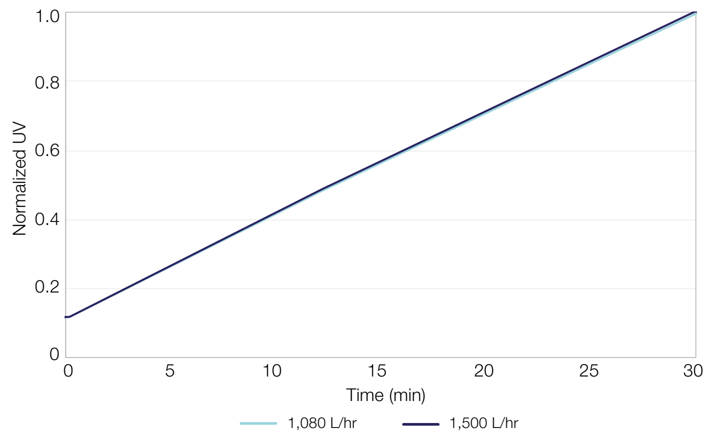


Figure 15. Normalized UV of 30 min linear gradients from 10% to 90% PU-10 based on system flow rates of 1,080 L/hr (light blue; two Quattroflow 1200SU pumps for PU-10 and PU-20) and 1,500 L/hr (dark blue; two Quattroflow 1200SU pumps and one Quattroflow 2500SU pump for PU-10, PU-20, and PU-30, respectively) with 3/4 in. FTA.

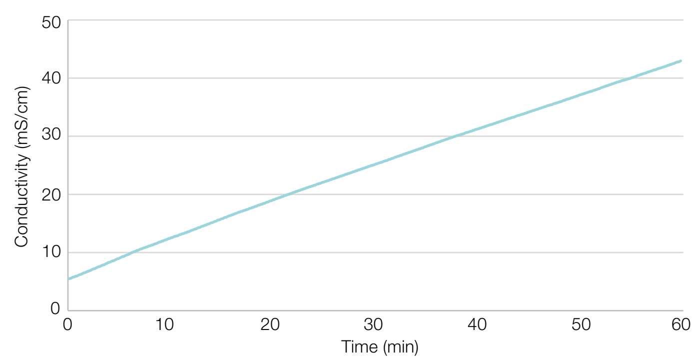
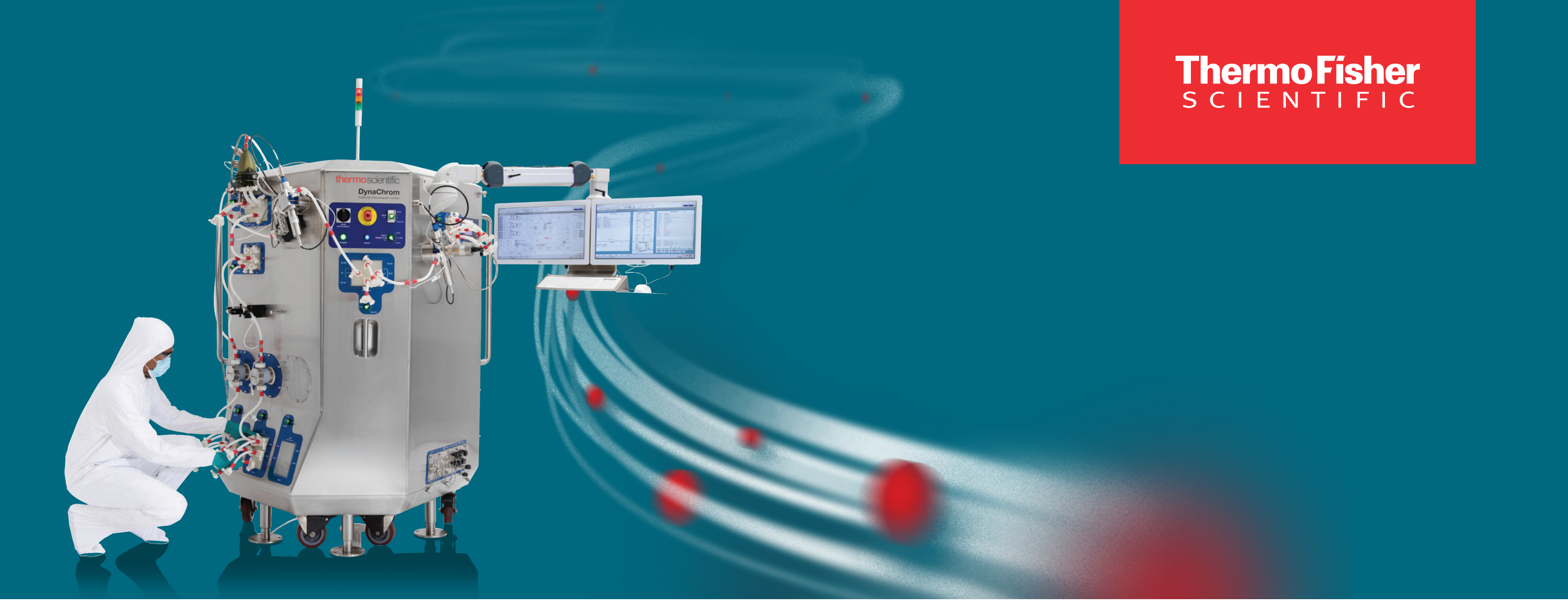


Figure 16. Inline conductivity reading of 60 min linear gradient at 1,080 L/hr from 10% to 90% PU-10 based on system flow rate with 3/4 in. FTA and two Quattroflow 1200SU pumps (PU-10 and PU-20).



Conclusion

These data demonstrate the step and linear gradient performance for the 1/4 in., 3/8 in., 1/2 in., and 3/4 in. FTAs of the DynaChrom system. Gradient capability is a pivotal factor supporting operational efficiency and performance of downstream purification processes by enabling tightly controlled separations to meet product quality requirements or alleviating buffer footprint constraints. The step gradient accuracy was controllable at less than 5% error for all tested conditions. The R^2 values for linear regression of gradients from 10% to 90% were > 0.99 for all tested conditions. These results show the capability of the DynaChrom Single-Use Chromatography System to produce accurate step and linear gradients.

Authors

Michelle Nolasco, Supervisor, R&D Innovation, Thermo Fisher Scientific; Kristina Pleitt, Sr Manager, R&D Innovation, Thermo Fisher Scientific; Krystle Prantil, Sr Manager, R&D Projects, Thermo Fisher Scientific.

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