Gradient Verification for Biological Separations Using the VERITY® 1810 Conductivity and pH Monitor

Technical Note TRANS0616

The VERITY® 1810 Conductivity and pH Monitor is a simple and powerful tool for the verification of gradients commonly used in biological separations.

Introduction

Conductivity and/or pH monitoring are commonly used for biological separations and is necessary for the purification of oligonucleotides, monoclonal antibodies (mAbs), and other proteins. The VERITY® 1810 Conductivity and pH Monitor is a state-of-the-art detection instrument for biological purifications with a large dynamic range and rapid response time for real-time gradient monitoring.

This technical note highlights the ability of the VERITY® 1810 Conductivity and pH Monitor under TRILUTION LC® control to verify gradient composition during a chromatographic run.

Figure 1. VERITY® 1810 Conductivity and pH Monitor with Optional pH Flow Cell.

Materials & Methods

Standard Solutions

For conductivity monitoring, a solution of 100 mM phosphate buffer was prepared by dissolving powdered reagents in MilliQ purified water. MilliQ purified water was used as a solvent for the conductivity gradient.

For pH monitoring, solutions of 20 mM phosphate buffer and 0.1 M citric acid were prepared by dissolving powdered reagents in MilliQ purified water.

Instrumentation and Software

VERITY® 1810 Conductivity and pH Monitor with optional pH flow cell

322 HPLC Pump (H1 heads)

Minipuls® 3 (for washing the pump heads)

TRILUTION® LC v3.0 with Service Pack 6

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Conductivity Monitoring Methods

This experiment simulated use of a size exclusion or ion exchange column running a step gradient (Table 1) to mimic protein separation via buffers of varying ionic strengths. This experiment was conducted at room temperature and in a refrigerated unit (~10°C).

Table 1. Conductivity Monitoring Gradient

Conductivity Monitoring	
Mobile Phase	**A:** MilliQ-purified water **B:** 100 mM phosphate buffer
Gradient	0–5 min: 0% B 5–15 min: 0% B to 20% B 15–20 min: 25% B 20–30 min: 25% B to 50% B 30–35 min: 50% B 35–45 min: 50% to 100%B 45–50 min: 100% B 50.02–60.02 min: 0 %B
Flow Rate	10 mL/min

pH Monitoring Methods

The experiment simulated the use of a 1 mL protein column to run a buffer exchange, loading with five column volumes of binding buffer, eluting with five column volumes of elution buffer, and equilibrating with ten column volumes of binding buffer (Table 2). The VERITY® 1810 Conductivity and pH Monitor was used to monitor the mobile phase pH change with buffer composition change. This experiment was run at room temperature and in a refrigerated unit (~12°C).

Table 2. pH Monitoring Gradient

pH Monitoring	
Mobile Phase	**A:** 20 mM phosphate buffer (binding buffer; pH 8.101) **B:** 0.1 M citric acid (elution buffer; pH 2.054)
Gradient	0–5 min.: 0% B 5.05–10.05 min: 100% B 10.10–20.10 min: 0% B
Flow Rate	1 mL/min

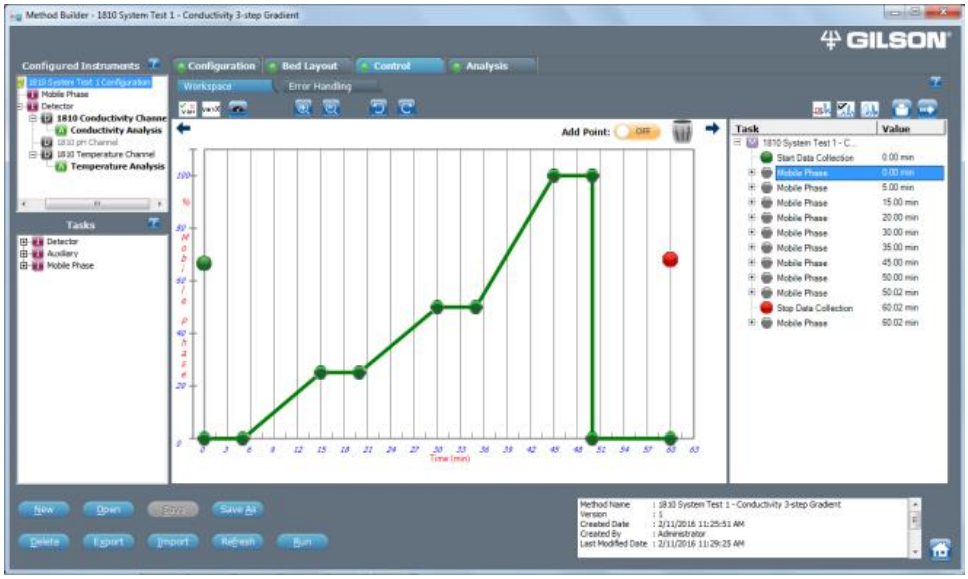

Figure 2. Example TRILUTION® LC method for conductivity monitoring

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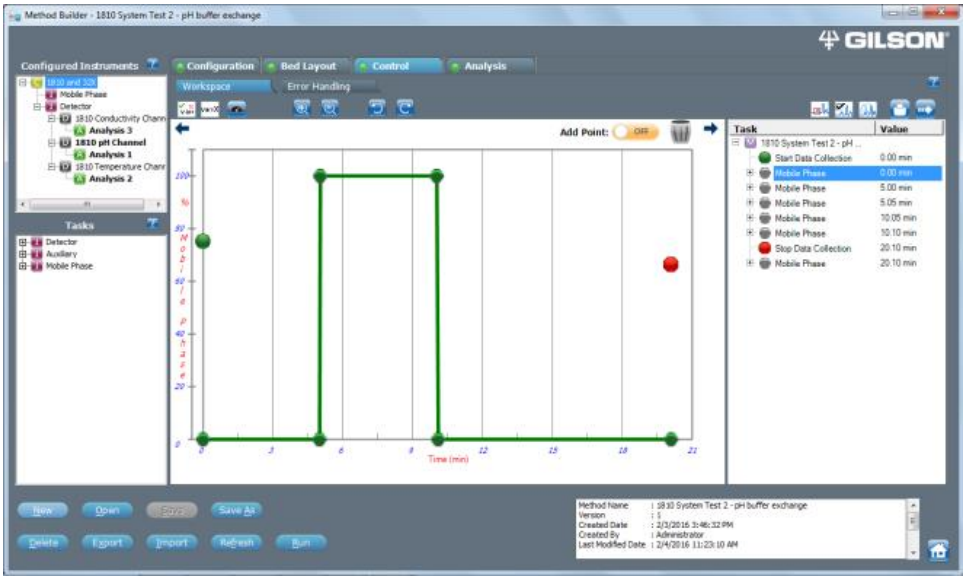

Figure 3. Example TRILUTION® LC method for pH monitoring

Results and Discussion

Conductivity Monitoring

At both room temperature and at 10°C, the VERITY® 1810 Conductivity and pH Monitor effectively monitored the changes to the mobile phase composition between 0% and 100% phosphate buffer (Figure 4). As the percentage of phosphate buffer increased, the VERITY® 1810 Monitor exhibited a corresponding increase in the conductivity signal. The VERITY® 1810 Monitor also indicated the return of the flow to effectively 0 mS/cm with the transition to 0% phosphate buffer. The results were similar at room temperature and at 10°C, demonstrating the ability of the VERITY® 1810 Monitor to collect conductivity data in different temperature environments without requiring recalibration. Conductivity and temperature data were collected simultaneously during the run (data not shown).

pH Monitoring

At both room temperature and at 12°C, the VERITY® 1810 Conductivity and pH Monitor effectively monitored the changes to the mobile phase composition, transitioning between 100% 20 mM phosphate buffer (pH 8) and 100% 0.1M citric acid (pH 2), and then returning to 100% 20 mM phosphate buffer (pH 8) (Figure 7). The VERITY® 1810 Monitor accurately followed the changes in pH, showing that the monitor can be used to detect the pH of the system. Results at room temperature were similar to results at lowered temperature, showing the ability of the VERITY® 1810 Monitor to collect pH data in different temperature environments. pH, conductivity, and data were collected simultaneously during the run (data not shown).

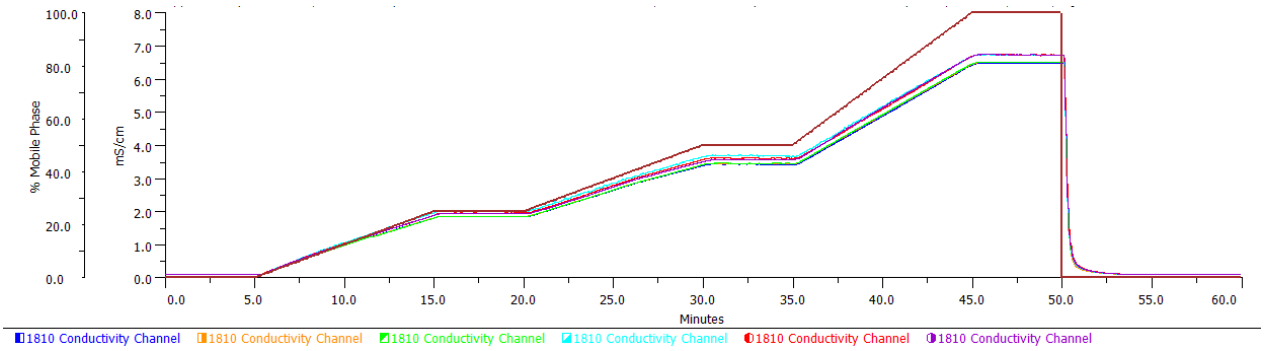

Figure 4. Conductivity monitoring at room temperature (aqua, pink, purple) and at 10°C (blue, orange, green) in relation to mobile phase concentration (dark red).

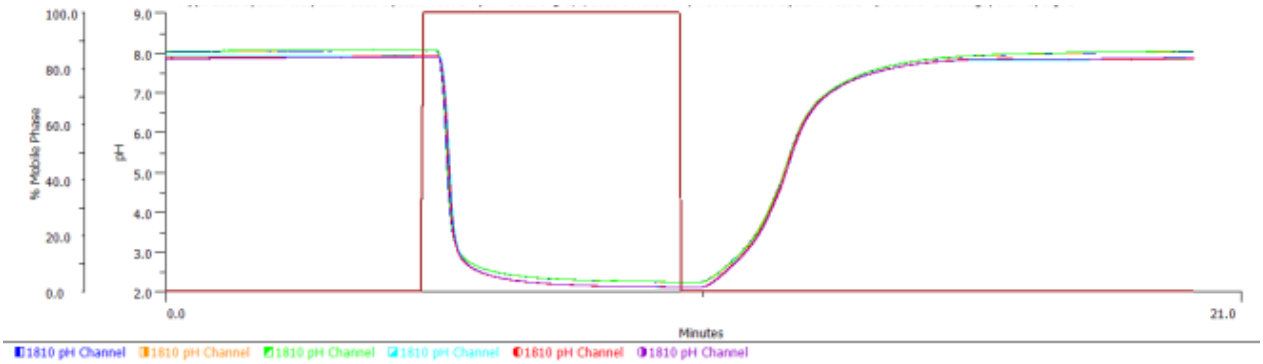

Figure 5. pH results at room temperature (aqua, red, purple) and at 12°C (blue, orange, green) in relation to mobile phase concentration (dark red)

Summary

With the Gilson VERITY® 1810 Conductivity and pH Monitor, real-time conductivity, pH, and temperature signals are monitored efficiently and effectively, which allows the user to protect their valuable samples, ensure product purity, and optimize the purification process.

Ordering Information

Item	Product Number
VERITY® 1810 Conductivity and pH Monitor	11100000
VERITY® 1810 pH Flow Cell	11100001
TRILUTION® LC v3.0	210631R30
TRILUTION® LC v3.0 Service Pack 6	210631R30P6

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