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Maximizing Performance of pH Measurements for Inline Conditioning Buffer Preparation for Chromatography

By Karolina Busson, Joanne Sheehy, Liz Anderson, Therese Westman, and Enrique Carredano

ABSTRACT

In biopharmaceutical manufacturing, buffer preparation is traditionally performed manually by dissolving solid salts in water for injection (WFI) followed by offline mixing and specification testing. This method requires large buffer volumes, extensive infrastructure, and significant labor, often creating bottlenecks in production. To address these limitations and support process intensification, inline buffer preparation technologies have emerged.

Inline dilution (ILD) involves diluting concentrated buffers with WFI at the point of use. Inline conditioning (IC) advances this concept by blending single-component stock solutions of acid, base, and salt with WFI, enabling flexible, on-demand buffer formulation. IC also allows real-time adjustment of buffer properties such as pH, conductivity, and concentration through multiple feedback control mechanisms.

A critical requirement for IC is robust and accurate inline pH measurement, which remains challenging due to limitations in conventional pH sensor designs. Issues such as salt memory, a temporary bias caused by ion diffusion across the reference junction, and sensor drift, from exposure to sodium hydroxide or pure water, can compromise measurement reliability. The effects of sodium hydroxide are particularly pronounced during the chromatography runs where concentrated sodium hydroxide solutions are used to clean the chromatography column. Ideally, pH probes should remain in the system throughout these steps while the current recommendation is to take them out of the system.

To mitigate these effects, sensor manufacturers have introduced design enhancements including ion traps, pressurized potassium chloride reservoirs, and varied junction pore sizes. In this study, we evaluated several pH sensor types under conditions typical of chromatography applications. Sensors with pressurized potassium chloride demonstrated strong resistance to salt memory and sodium hydroxide exposure but showed reduced performance in low-conductivity buffers under dynamic flow and pressure.

Based on these findings, we propose a dual-sensor module: one sensor used for salt-free buffers, another for buffers containing sodium chloride and with the capability to be exposed to hydroxide during column cleaning-in-place (CIP). This configuration enables uninterrupted and inline pH monitoring, and continuous buffer release in line with a process analytical technology (PAT) approach, enhancing efficiency, sustainability, and cost-effectiveness in buffer preparation.

1. Introduction

In large-scale biopharmaceutical production, traditional buffer preparation—dissolving salts in water for injection (WFI) in tanks—poses significant challenges.[1,2] Buffers are typically dilute and required in large volumes, demanding substantial tank capacity, floor space, and operational investments. Additionally, a buffer formulation is complex and must meet multiple specifications simultaneously. Any failure after preparation can result in discarding an entire buffer batch, leading to high costs and inefficiencies.

To address these issues, two alternative approaches have emerged: inline dilution (ILD)[3–5] and inline conditioning (IC).[1,6,7] ILD involves diluting buffer concentrates with WFI at the point of use, allowing limited control via a single feedback loop (*e.g.*, flow or conductivity). IC, a more advanced method, formulates buffers on-demand from single-component stock solutions of acid, base, and optional salt and additives. With multiple pumps and feedback controls (flow, pH, conductivity), IC enables precise adjustment of buffer properties and

supports continuous buffer release using process analytical technology (PAT). A simplified flow scheme showing the relative position of an IC system's main instrumentation is shown in **Figure 1**.

PAT strategies enhance process control by enabling real-time monitoring and adjustment, ensuring consistent output in accordance with a continuous validation approach.[8-9] While pH feedback is often viewed as less reliable due to sensor drift and handling challenges[10], there is limited guidance on how to mitigate these issues. Regardless of the control strategy, robust and accurate inline pH measurement is a prerequisite for successful IC implementation.

This study aims to investigate the factors that compromise pH sensor performance—such as drift, slow response, and measurement bias—and identify a reliable solution that supports continuous release with PAT. Ultimately, this enables leaner, more sustainable, and cost-efficient buffer production in biopharmaceutical manufacturing.

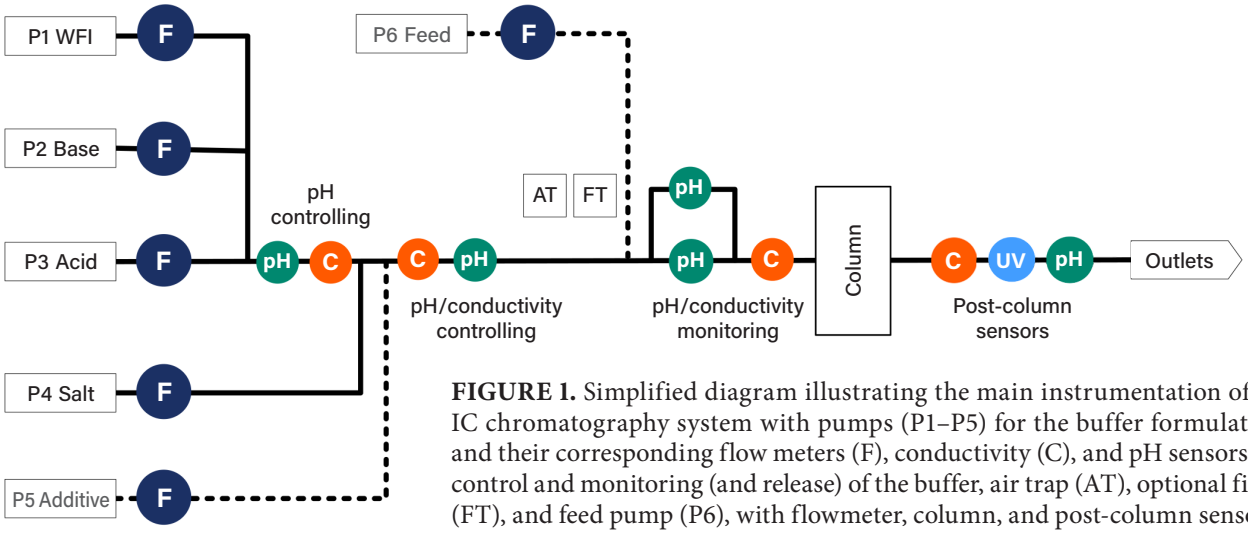


FIGURE 1. Simplified diagram illustrating the main instrumentation of an IC chromatography system with pumps (P1–P5) for the buffer formulation and their corresponding flow meters (F), conductivity (C), and pH sensors for control and monitoring (and release) of the buffer, air trap (AT), optional filter (FT), and feed pump (P6), with flowmeter, column, and post-column sensors.

2. Materials and Methods

2.1 Inline Conditioning Systems Used in This Study

Most experiments were conducted on an IC "demo system" located at Cytiva's Custom Engineering facility in Uppsala, Sweden. This system has a maximum flow rate of 600 L/h. Only one of the system pumps was used to let a premade buffer flow through the flow path equipped with four pH sensors. In this way, up to four different sensors could be tested at a time.

Experiments (detailed in section 2.5) were performed using three larger systems, each with a maximum flow rate of 5000 L/h, located at Johnson & Johnson Innovative Medicine's BioCork Suite 3 in Cork, Ireland.

2.2 Methodology and Rationale of Tests Performed

Six types of pH sensors (listed in **Table 1**) were initially selected for testing. Vendors supplied the sensors, and their support is acknowledged. The first test, referred to

TABLE 1. Types of pH sensors evaluated.

Type	Sensor	Product Number	Junction Pore Size (µm)	Junction Material
1	Endress+Hauser CPS11	OPS111BA02ESAGEH	~5.0	PTFE
2	Hamilton 1611 with ion trap and pre-pressurized potassium chloride	EasyFermBioPHI 243632-1116	Unknown	Unknown
3	Endress+Hauser CPS11 with ion trap	CPS11-2BT2ESA	~5.0	PTFE
4	Endress+Hauser CPS71 with ion trap	CPS71-2TC2ESA	~0.5	Ceramic
5	Endress+Hauser CPS71 with ion trap and pre-pressurized potassium chloride	CPS71 2TP2ESA	~0.5	Ceramic
6	Endress+Hauser CPS171/CPS61 with ion trap and pre-pressurized potassium chloride	CPS171-AA7NTP2/ CPS61	~0.2	Ceramic

TABLE 2. Different combinations of equilibration buffer, CIP solutions, and contact times tested.

Condition	Equilibration Buffers	CIP Solution/Water	Contact/Incubation Time (min)
A	0.05 M sodium phosphate, 0.15 M sodium chloride, pH 7.4	0.1 M sodium hydroxide	90
B	0.10 M sodium acetate, pH 5.0	1.0 M sodium hydroxide	120
C	0.05 M sodium acetate, pH 5.0	Deionized water	180
D	0.05 M sodium acetate, pH 5.0	1.0 M sodium hydroxide	120
E	0.10 M acetic acid, pH ~3.0	1.0 M sodium hydroxide	120

as the "Tris test," assessed sensor performance in low-concentration buffer under varying pressure and flow conditions. One of the sensor types performed so poorly in this first test that it was excluded from further testing.

The remaining five sensor types were evaluated for their resistance to column cleaning-in-place (CIP) solutions, primarily sodium hydroxide. These tests included incubation in CIP solutions followed by exposure to different equilibration buffers, again under pressure and flow variations (see **Table 2**).

Based on the CIP test results: sensor type 6 was identified as the most robust and selected for further evaluation in a large-scale inline test during a manufacturing campaign. Additionally, sensor type 6 was subjected to a salt memory test and an extended pure water exposure test, alongside sensor type 1, which is the default sensor type in bioprocess chromatography systems from Cytiva.

Interestingly, the results (elaborated upon in section 3.0) demonstrated that:

- Type 6 failed the Tris test but passed the CIP test.
- Type 1 passed the Tris test but failed the CIP test.

To explore a more resilient setup, a drift study was conducted using sensor type 1 to determine how long a combination of type 1 and type 6 could operate effectively without requiring recalibration or removal from the system.

For each run, hundreds of pH measurements were captured inline at several readings per second, providing strong statistical resolution. Where possible, multiple sensors of the same type were used to confirm reproducibility. Sensor placement within the skid was consistent across tests, but local flow and pressure differences were considered as potential contributors to performance variability and were challenged. CIP resistance was assessed by incubating sensors in sodium hydroxide followed by equilibration under dynamic flow and pressure. While the number of runs was reduced for practical reasons, representative data from different sensor types are included.

2.3 Tris Test

To evaluate sensor performance under challenging conditions, the Tris test was conducted on six different pH sensor types (see **Table 1**). The test involved measuring pH in a low-concentration Tris buffer (0.01 M Tris, pH 8.0, conductivity ~0.6 mS/cm) while subjecting the sensors to variations in pressure and flow.

This specific buffer was chosen because, in our experience, it is known to be particularly difficult for inline pH measurement, making it a suitable benchmark for assessing sensor reliability and accuracy in demanding applications.

After the Tris test, one sensor type was excluded from further testing due to its exceptional instability (type 4, as explained in section 3.1).

2.4 The Effects of CIP Solutions

Following the Tris test, five of the pH sensor types (all of them except for type 4) were evaluated for their performance before and after exposure to CIP solutions (conditions A–E in **Table 2**). These solutions, primarily sodium hydroxide, are typically used for cleaning the column during chromatography runs. Type 6 sensors were only tested for conditions D and E due to late availability. These tests were conducted on different occasions and at various positions within the demo system.

Each sensor was tested under pressure and flow variations using five different equilibration buffer combinations, CIP solutions, and incubation times. Up to four sensors were tested simultaneously. All probes were calibrated before testing and verified against calibration solutions afterward.

The test protocol included:

1. A 10-minute (min) pressure-flow test using the equilibration buffer.
   a. Flow increased from 0–100 L/h at ~0.1 bar.
   b. The pressure was increased to ~2.5 bar by switching to outlet with a manual valve.
2. Sensors were then removed from the system flow path

and immersed in the CIP solution for the designated incubation time.
3. Re-equilibration: sensors were reinstalled, and equilibration was run with the same buffer used in step 1 at 100 L/h for three hours.
4. A second pressure-flow test was performed using the same equilibration buffer.

The absolute value of the difference between the measured pH at different stages and at the beginning of the run was used to assess sensor performance. This provided a measure of how each sensor responded before, during, and after exposure to the CIP solution.

Following the CIP test results, sensor type 6 was recognized as the most resilient and was chosen for further assessment in a large-scale inline application during a manufacturing campaign. In addition, sensor type 6 underwent both a salt memory evaluation and an extended water exposure test alongside sensor type 1.

2.5 Salt Memory Test

To evaluate the salt memory effect, pH sensors of type 1 and type 6 (see **Table 1**) were tested using two Tris buffers—one containing sodium chloride and one without.

After calibration and installation, the sensors were exposed to the following sequence:
1. Tris buffer without sodium chloride for approximately 8 min
2. Tris buffer with 1.0 M sodium chloride for about 5 min
3. Tris buffer without sodium chloride again for another 8 min

Throughout the test, inline pH readings were continuously recorded to assess both the quantitative offset and the qualitative behavior of each sensor type.

The key comparison was between the pH readings in the salt-free buffer before and after exposure to the salt-containing buffer. In the absence of salt memory, these readings should be identical. Any offset in the signal after salt exposure indicates the presence of salt memory. Additionally, the shape of the recovery curve provides insight into how long it takes for the sensor to return to its baseline (pre-salt) reading, offering a qualitative measure of the sensor's resilience to salt-induced bias.

2.6 Water Test

A water exposure test was performed on pH sensors of type 1 and type 6 to evaluate their stability and response after prolonged contact with pure water. The test consisted of three sequential phases:
1. Initial pressure-flow test using a buffer composed of 0.05 M Tris, 1 M sodium chloride, pH 8.0
2. Overnight immersion in pure water, with no flow or pressure applied
3. Final pressure-flow test using the same buffer, as in phase 1

This setup allowed for comparison of sensor performance before and after water exposure, helping to identify any drift, offset, or delay in response caused by prolonged contact with pure water.

2.7 Inline Tests During Large-Scale Manufacturing Campaign

Type 6 pH sensors were tested over a four-month, 13-batch manufacturing campaign at Suite 3 of Johnson & Johnson Innovative Medicine's BioCork facility in Cork, Ireland. The sensors were installed post-column, a location not critical to the process, allowing for continuous monitoring without interfering with production.

During each chromatographic cycle, the sensors remained in place throughout column CIP with sodium hydroxide followed by exposure to equilibration buffer. Each batch included three stages:
1. Capture: 5 cycles
2. Polishing 1: 3 cycles
3. Polishing 2: 2 cycles

The CIP conditions for each stage roughly corresponded to conditions A, B, and E in **Table 2**, with incubation times of 30, 70, and 70 min, respectively. The sensors were only removed during inter-batch storage and were excluded from testing during three capture and four cycles of the second polishing step, due to unrelated reasons.

After each cycle, a two-point verification was performed using calibration solutions at pH 4.0 and pH 10.0, ensuring the sensors remained within ± 0.1 pH units of expected values. Additionally, the time required for the pH reading to return to within specifications, after rinsing with equilibration buffer post-CIP, was recorded as a measure of sensor recovery and performance.

2.7.1 Additional High-Pressure Tests

The pressure at the pH sensor located post-column is lower than that of the controlling and monitoring pH sensors prior to the column. Therefore, additional high-pressure tests were conducted to challenge the sensors further. These tests involved rapid transitions between:
- 1.0 M sodium chloride (pH 10.0)
- WFI
- 0.1 M sodium acetate (pH 5.0)

These transitions helped evaluate the sensors' responsiveness and stability under more demanding pressure conditions.

2.8 pH Drift Test

A drift study of the type 1 sensors was performed by preparing a buffer of 0.05 M acetate buffer, pH 4.5 (conductivity ~1.5 mS/cm) and letting it recirculate in the system and recording the inline pH values by four different sensors. The method consisted of 23 one-hour long cycles and was run five times in total during five consecutive days. During the runs,

the flow rate of the liquid was stopped and started, and the outlet was changed back and forth between an unrestricted and a restricted outlet to obtain changes in pressure. In detail each cycle was described by the following steps:

1. Flow 200 L/h, no pressure, 10 min
2. Flow 200 L/h, ~2.5 bar, 10 min
3. Flow 0 L/h, 5 min
4. Flow 200 L/h, ~2.5 bar, 10 min
5. Flow 200 L/h, no pressure, 10 min
6. Flow 0 L/h, 5 min
7. Flow 200 L/h, no pressure, 10 min

The four pH sensors were of the type 1 and they were identified with numbers according to:

1. A several-months-old sensor
2. A new sensor
3. A new sensor that was subjected to a regeneration procedure by leaving it in the potassium chloride capsule for 10 min after each run of the method
4. A new sensor that was placed in a vessel containing the buffer

The several-months-old sensor was left in the calibration buffer, pH 7.0, over the weekend following the drift test and given a new measurement after seven days.

3. Results

3.1 Tris Test

Overall, the behaviour—observed not only in the Tris test but in all pressure flow variations—was that the pH tended to rise when the flow stopped and to fall again when the flow resumed. The correlation to pressure variations was not always the same. By defining the variation as (max–min)/2 during the test, it is possible to directly compare different sensors (**Figure 2**). Sensor type 4, which showed a variation of more than 0.3 pH units, was excluded from further investigations.

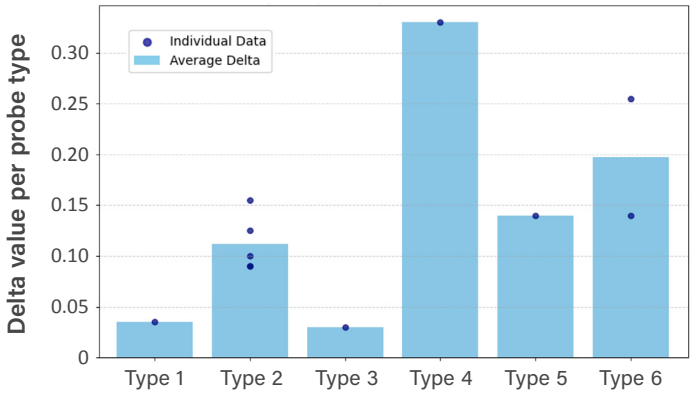


FIGURE 2. Visual representation of the pH variation of different pH sensors when using 10 mM Tris buffer as a delta value during a test where the flow rate and pressure are varied, calculated as: $\frac{pH_{max} - pH_{min}}{2}$ Types 2 and 6 were tested with more than one individual sensor (shown as multiple dots in the plot).

3.2 The Effects of CIP Solutions

Figure 3 A–F presents the results from evaluating the performance of five different sensor types under pressure and flow variations, both before and after exposure to the CIP solution. Conditions **A–C** were tested on four sensors, 1, 2, 3 and 5. Conditions **D** and **E** were also tested with type 6, which had just been made available by the vendor. The reference junction, and therefore, the pressure reservoir of one of the type 6 sensors tested on condition E had been open for six months before the testing.

3.3 Salt Memory Test

The results of the inline salt memory tests are shown in **Figure 4**. The type 1 sensor showed a maximum offset of 0.25 pH units due to salt memory, which after 8 min had gone down to 0.18 pH units. At this pace, it might take approximately 30 min to approach the baseline. The type 6 sensor, on the other hand, comes back into range (±0.1 pH units) in 1–2 min and back to the baseline within approximately five min, as experienced in our study.

3.4 Water Test

The results of the water test are shown in **Figures 5** and **6**. **Figure 5** is a pressure-flow test with 0.05 M Tris, 1 M sodium chloride, pH 8.0 buffer, performed *before* the sensors were left one night in water. **Figure 6** was a repeat of the **Figure 5** test *after* the sensor was stored in water for 12 hours. The results show how both type 6 sensors demonstrated a faster recovery in buffer than the type 1 sensor after storage in WFI.

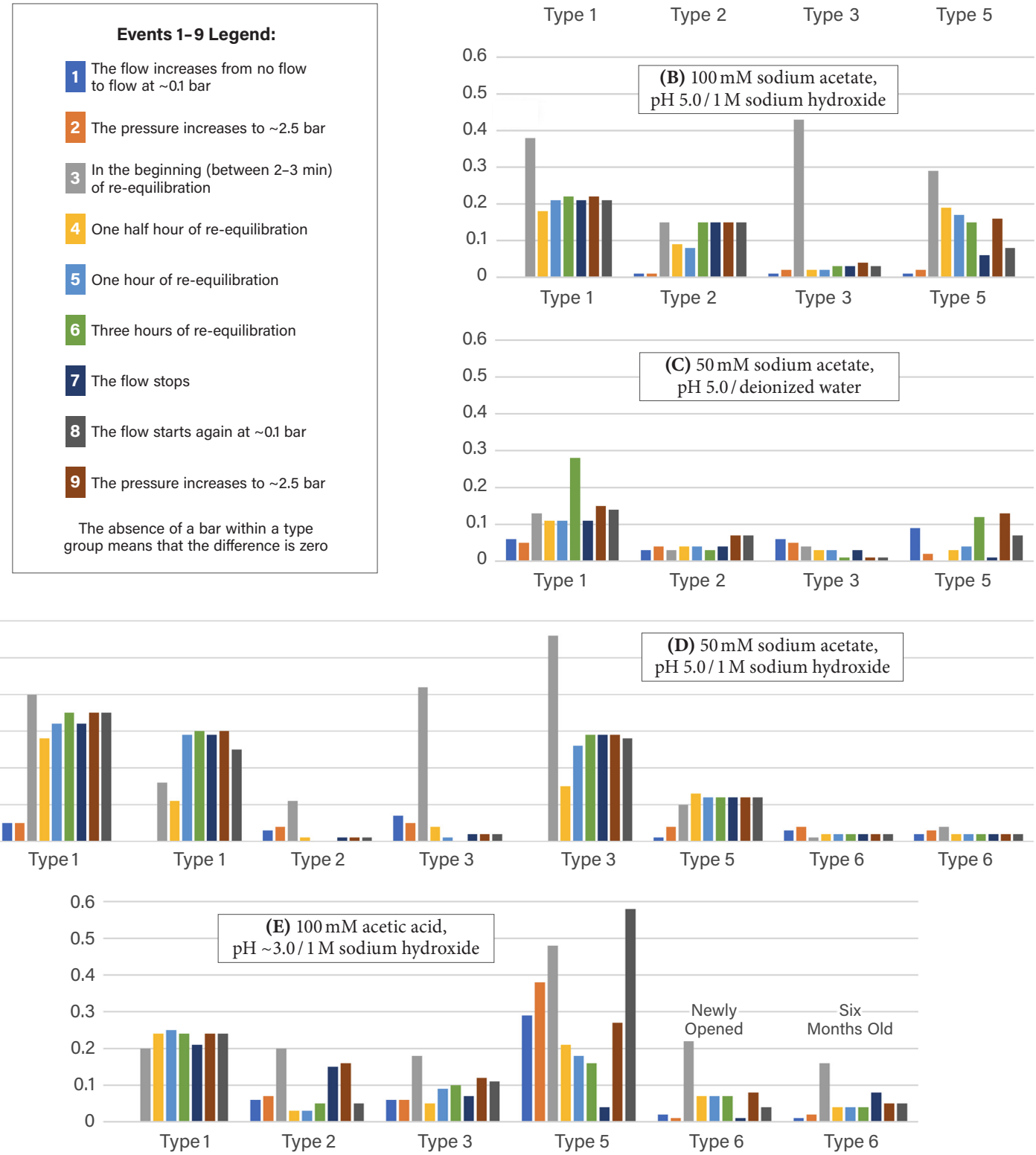
3.5 Inline Tests During a Large-Scale Manufacturing Campaign

The time required for the buffer pH to return to within the specification window during rinsing with equilibration buffer after column CIP was under 1 minute for the capture step and under 6 minutes for polishing steps 1 and 2, corresponding to conditions A, B, and E in **Table 2**. The results of the two-point verifications were (by ≤0.02 pH units) out of specification in two out of 50 occasions (5 cycles × 10 batches) for the capture step, five out of 39 occasions (3 cycles × 13 batches) for the polishing 1 step, and zero out of 18 occasions (2 cycles × 9 batches) for the polishing 2 step (touched on in section 2.7). The two-point verifications were performed post-CIP and equilibration, and they showed that the drift of sensor type 6 was very small, suggesting that calibration frequency can be reduced.

The high-pressure tests showed also less than 5 min recovery time from 1.0 M sodium chloride and less than 1 min recovery for the WFI, both with subsequent successful two-point verification.

Since the type 6 probes were in place during the entire

FIGURE 3.
Variations in pH measurement due to changes in conditions where the combination equilibration buffer/CIP solution was as described in the title of each subfigure. The height of the bars represents the absolute value of the difference between the actual pH value at each event ($i = 1–9$) and at the beginning of the run. The absence of a bar within a type group means that the difference is zero. Events 1 and 2 occur before the CIP and the rest occur afterward.



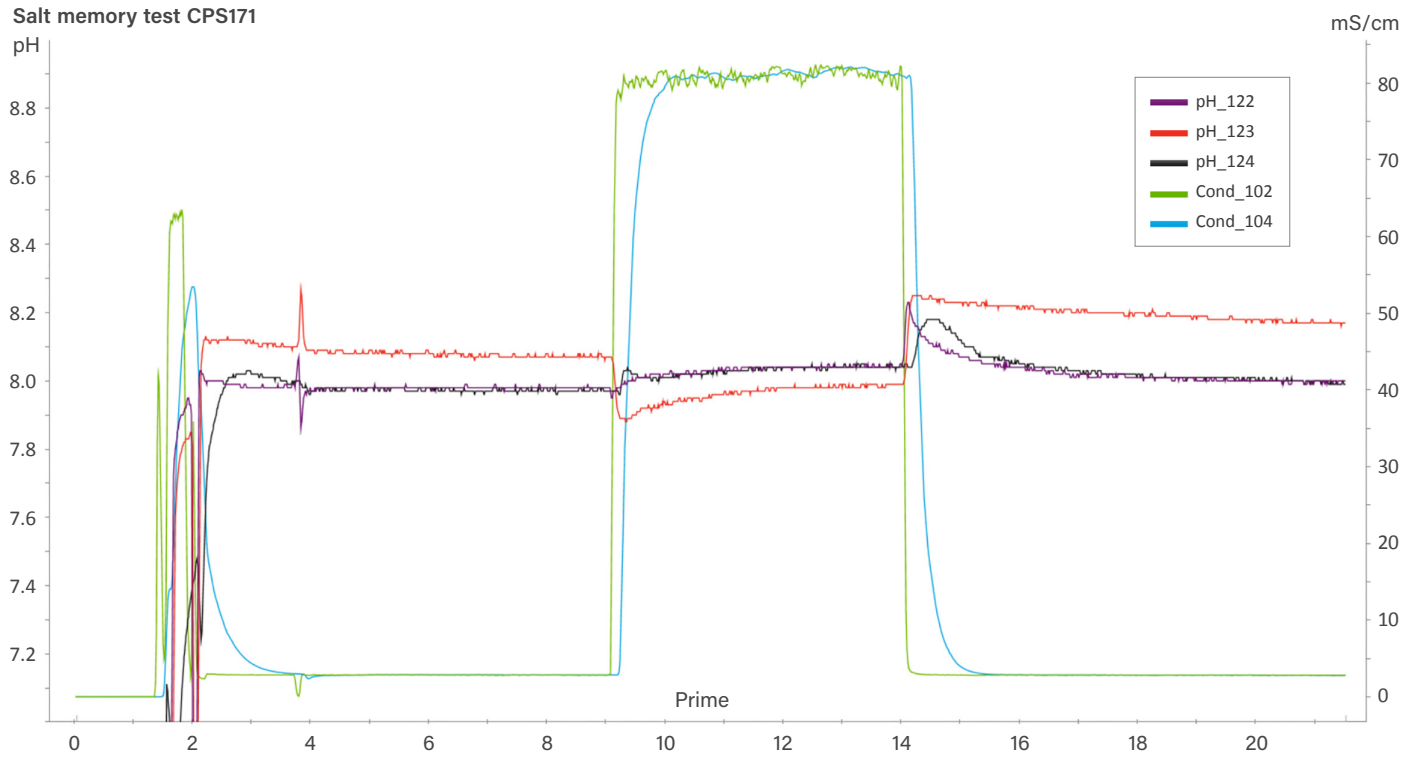


FIGURE 4. Salt memory test. pH_122: six-month-old type 6 sensor; pH_123: type 1 sensor; and pH_124: three-month-old type 6 sensor.

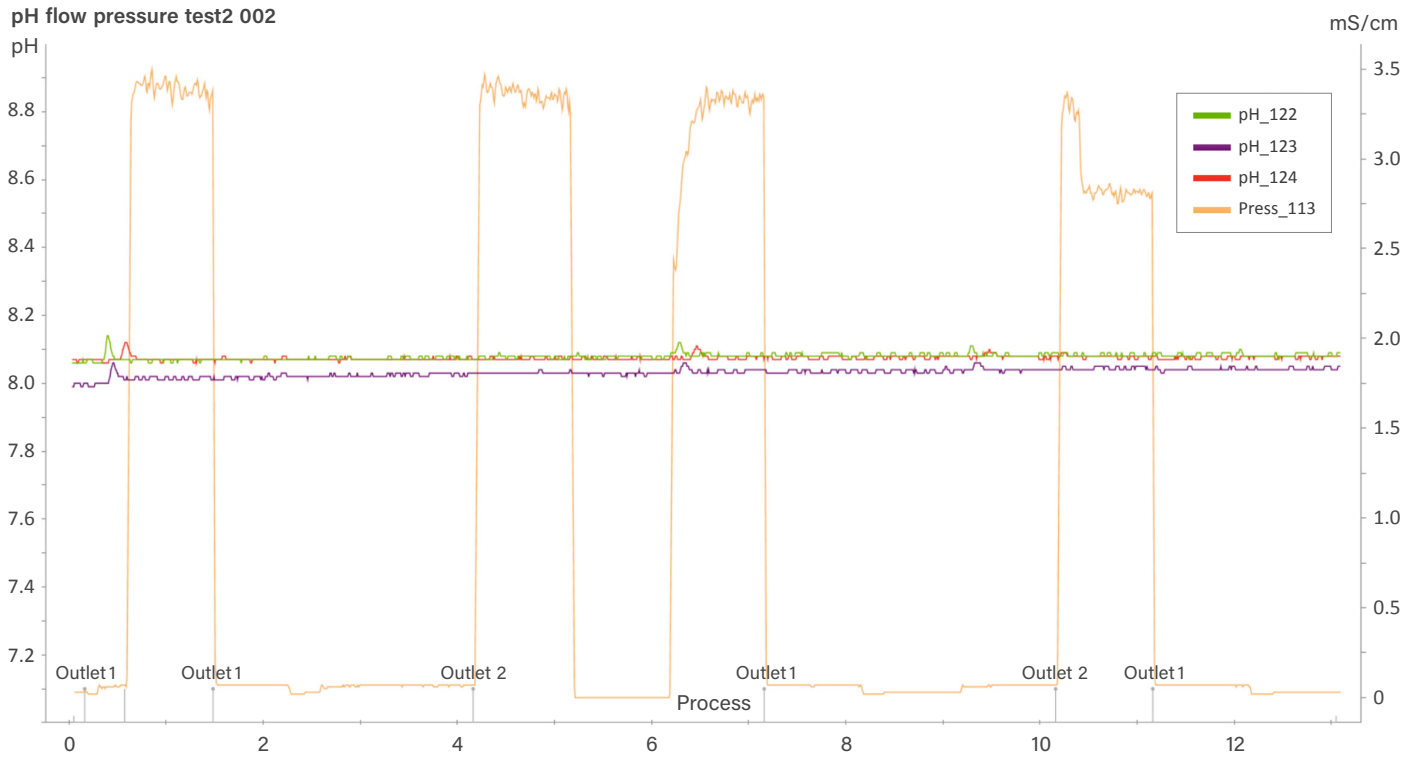


FIGURE 5. Pressure flow test with 50 mM Tris, 1 M sodium chloride, pH 8.0, before the sensors were left for one night in water. pH_122: six-month-old type 6 sensor; pH_123: type 1 sensor; and pH_124: three-month-old type 6 sensor.

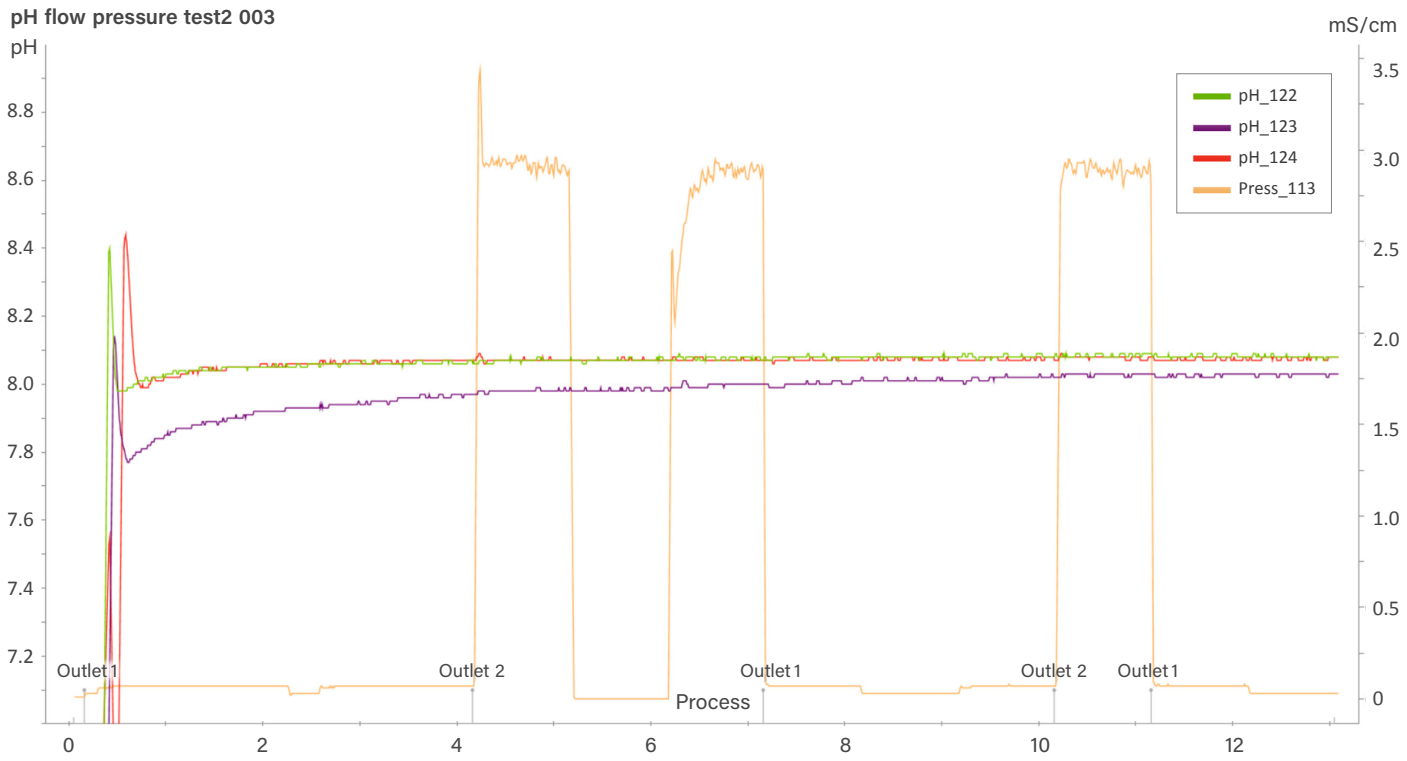


FIGURE 6. Pressure flow test with 50 mM Tris, 1 M NaCl, pH 8.0, after the sensors were left for one night in water. pH_122: six-month-old type 6 sensor; pH_123: type 1 sensor; and pH_124: three-month-old type 6 sensor.

process, it was possible to monitor their readings while other buffers involved were formulated and run in the systems. In all the instances, the tested probes reported stable values within specifications with two exceptions: when measuring in 0.1 M citric acid, pH ~2.15, conductivity ~3.3 mS/cm; and 0.1 M acetic acid, pH ~2.9, conductivity ~0.6 mS/cm, one sensor reported ~0.1 and ~0.3 lower pH values, respectively, when the flow rate was reduced. In the second case, the buffer was out of specifications before the flow rate reduction.

3.6 pH Drift Test

Figure 7 shows the pH continuously measured during the entire duration of the test (five days), and **Figure 8** shows the value reported by each sensor when in the pH 7.0 calibration solution at the end of each day. For every sensor, the changes in pH values were limited to ±0.02 pH units from the median, which is comparable to the noise level of an inline pH measurement.[5]

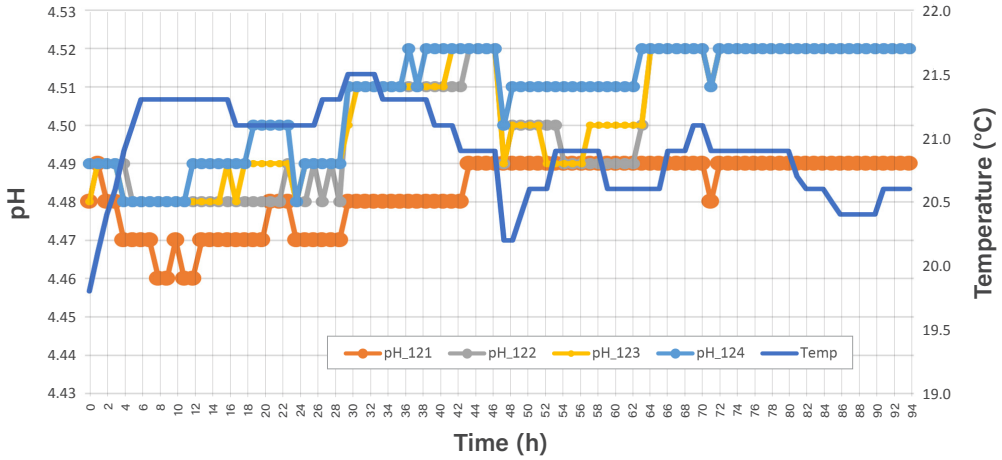


FIGURE 7. Measured pH in acetate buffer during the time of the drift study. All sensors were type 1 only. pH_121: a sensor several months old; pH_122: a new sensor; pH_123: a new sensor that was subject to a regeneration procedure; and pH_124: a new sensor that was placed in a vessel containing the acetate buffer.

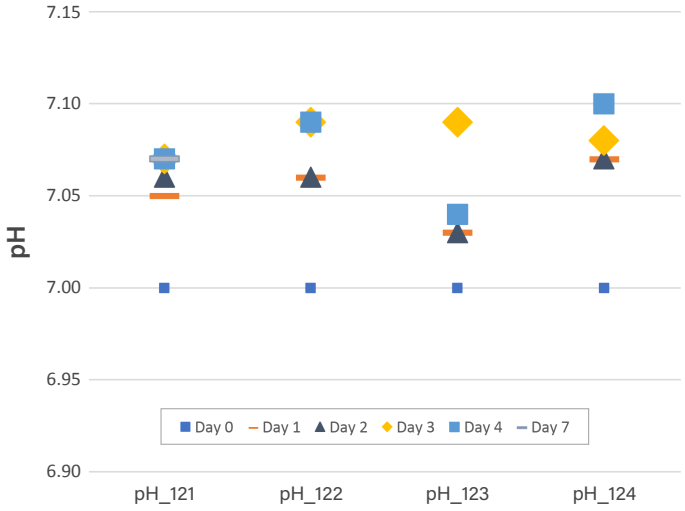


FIGURE 8. Measured pH in the calibration solution 7.0 at the end of each day of the drift study. All sensors were type 1 only. pH_121: a sensor several months old; pH_122: a new sensor: pH_123: a new sensor that was subject to a regeneration procedure; and pH_124: a new sensor that was placed in a vessel containing the buffer.

4. Discussion

4.1 Understanding Sensor Behaviour

To interpret the results of this study, it's helpful to review the basic structure of a combined pH electrode (illustrated in **Figure 9**). The sensing element is a glass bulb sensitive to protons, filled internally with a pH 7.0 buffer solution. Two silver chloride-coated silver wires are connected to a voltmeter:

- One wire is in the internal buffer.
- The other is in contact with an electrolyte—typically potassium chloride—contained within the reference electrode. This reference solution is separated from the process fluid by a porous junction, forming a salt bridge.

Chloride ions in both the internal and reference solutions are essential for efficient electron transfer between the liquid and the solid-state wires. The pH of the process fluid is determined by measuring the voltage difference across the glass membrane.

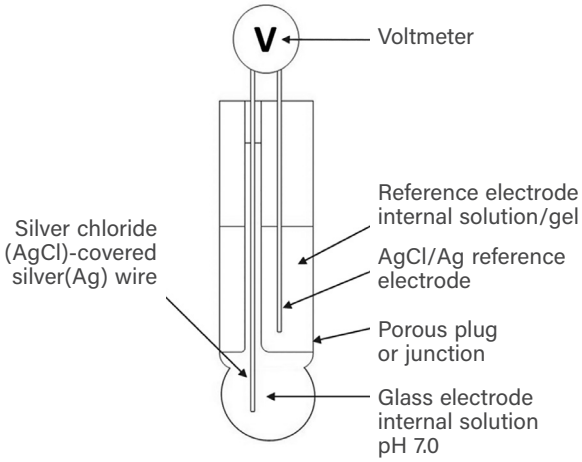


FIGURE 9. Simplified pH sensor diagram.

Salt bridges using concentrated potassium chloride have been used for over a century to minimize liquid junction potentials in electrochemical measurements because potassium and chloride ions have similar size and therefore, similar mobility.[11] However, when other salts like sodium chloride diffuse through the junction, they can create an additional potential, leading to a temporary bias in the pH reading. This phenomenon is referred to in this study as "salt memory"—a lingering effect observed when a sensor previously exposed to a salt (or more specifically sodium chloride)-containing buffer is placed in a salt-free buffer.

If a sensor is recalibrated before this bias has dissipated, it may result in drift during subsequent measurements. Similarly, exposure to high concentrations of sodium hydroxide can flood the reference electrode with hydroxide ions, causing significant pH shifts until the ions diffuse out. Prolonged contact with pure water can also degrade measurement accuracy by reducing chloride ion concentration, which impairs the electrode's performance.

To address these challenges, sensor manufacturers have introduced design improvements such as:

- Ion traps to capture interfering ions
- Pressurized potassium chloride reservoirs to maintain consistent reference conditions
- Reference junctions with varied pore sizes to control diffusion rates (see **Table 1**)

A critical requirement for IC is robust and accurate inline pH measurement, which remains challenging due to limitations in conventional pH sensor design. Issues such as salt memory is one challenge since the sequence of buffers in chromatography applications include separate buffers with and without sodium chloride. The effect of sodium hydroxide is particularly pronounced during chromatography runs where concentrated sodium hydroxide solutions are used to clean the chromatography column. Ideally, pH probes should remain in the system throughout these steps, while the current recommendation is to take them out of the system. Furthermore, many buffers are very weak buffers with low conductivity and finally, the processes involve changes with respect to pressure and flow rate. In this study, we evaluated several pH sensor types with different constructions under conditions typical of chromatography applications to investigate the possibilities for obtaining an acceptable solution for bioprocess applications.

4.1 Sensor Performance and Limitations

Six types of probe types have been evaluated in this study, although not all have been evaluated on all tests. Type 4 was excluded as soon as it showed an outstandingly high instability in the Tris test. Type 6 was not tested in the first three CIP tests (conditions A–C, **Table 2**) since it was only

made available late in the study. However, when it showed outstanding results in the last two CIP tests (conditions D–E), it was subject to complementary tests like the salt memory and water tests to compare it to the current probe type 1. Type 6 was also tested during a large-scale manufacturing campaign.

Among all the sensors tested, only types 1 and 3 performed reliably in the Tris buffer test. Both share a common feature: large reference junction pores (~5 µm). The presence of an ion trap in type 3 did not appear to influence performance in this specific test.

In the CIP challenge tests **(Table 2)**, all sensors tested handled the mild CIP condition A (0.1 M sodium hydroxide) without significant deviation. However, during condition B, when exposed to stronger CIP conditions (1.0 M sodium hydroxide), most sensors showed deviations of ≥0.15 pH units or more, except for type 3, which stabilized quickly and maintained deviations <0.04 pH units after initial equilibration. Under water-based CIP conditions (condition C), type 3 again showed the smallest deviation, followed by type 2. Although type 3 appeared promising up to this point, it did not perform well under conditions D and E. The new equilibration buffers used in these conditions: 0.05 M sodium acetate, pH 5.0 (D); and 0.1 M acetic acid, pH 3.0 (E); did not support recovery of the type 3 sensors following 1.0 M sodium hydroxide exposure. Instead, only types 2 and 6, both equipped with ion traps and pressurized KCl reservoirs, maintained lower deviations after CIP. For condition E, where the equilibration buffer was 0.1 M acetic acid, pH 3.0, only type 6 kept deviations <0.1 pH units, although slightly higher than in previous tests. This increase correlated with the lower conductivity (~0.5 mS/cm) of the buffer, compared condition D (~2.6 mS/cm). Overall, type 6 demonstrated to be a promising candidate and was therefore subject to further evaluations.

In both the salt memory and water exposure tests, type 6 clearly outperformed type 1, showing faster recovery and minimal offset. Type 1 exhibited significant drift and slow return to baseline.

During the inline tests, in the manufacturing campaign, type 6 performed well overall. However, it struggled with two low-conductivity buffers:

- 0.1 M acetic acid, pH ~2.9, ~0.6 mS/cm
- 0.1 M citric acid, pH ~2.15, ~3.3 mS/cm

Type 6 sensors performed well in 0.05 M sodium acetate at pH 5.0, even though this buffer has lower conductivity than citric acid, demonstrating that conductivity alone does not explain poor performance—pH and buffer chemistry are critical factors. In strongly acidic, low-conductivity buffers, low ionic strength combined with high proton mobility near the junction creates steep concentration gradients and asymmetric ion transport when interacting with potassium chloride from the reference. These conditions amplify liquid junction potential (LJP) effects, leading to measurable pH offsets when flow slows.

Under dynamic flow, convective transport dominates, sweeping ions away at similar rates regardless of their individual diffusion coefficients. This minimizes concentration gradients near the junction and reduces LJP. When flow stops, convective mixing disappears, and transport is governed solely by diffusion. At this point, ions move according to their intrinsic mobilities, which depend on size, charge, and hydration shell. For example, the isolated hydrogen ion H^+ diffuses extremely fast, while bulky anions like citrate move slowly, creating uneven ion distributions and potential shifts.

Interestingly, the opposite effect was observed in the Tris test: with Tris-H^+ (a bulky organic cation), chloride ions diffuse faster than Tris-H^+, making the sample side relatively negative and causing the measured pH to increase when flow stops. The reference electrode depends on stable ion diffusion through the junction—when pores are small and the sample has low ionic strength, diffusion is restricted and LJP becomes significant.

In contrast, type 1 sensors with large pores allow faster ion exchange, keeping LJP closer to zero, even in low-ionic-strength samples. This design minimizes diffusion limitations, so even when flow stops and transport is governed by diffusion, the junction potential remains small because ion exchange is rapid and balanced. Bottom line: type 6 is optimized for chemical resistance and contamination control but is more sensitive to low ionic strength, low pH, and flow changes. Type 1, while less resistant to salt memory and hydroxide, maintains better stability in low-conductivity environments because its junction potential is less affected by diffusion limitations. Overall, pre-pressurized sensors with small junction pores (type 6) excel in resisting sodium hydroxide and pure water exposure, and recover quickly from salt memory, whereas non-pressurized sensors with large pores (type 1) are more prone to salt memory and hydroxide damage. Thus, the pressurized reference design prioritizes robustness and chemical durability over minimizing junction potential in challenging, low-conductivity buffers (acidic or with bulky cations), while large-pore designs trade chemical resistance for better performance under these conditions.

Interestingly, the behavior of the reference junction differs markedly between sensor types. Type 1 sensors, which possess relatively large and open pores can, under some conditions, develop a persistent and spatially extended potential gradient within the reference. However, they are largely immune to the short-lived, diffusion-driven transient junction potentials that may arise in more confined geometries. In contrast, Type 6 sensors—with their small, tortuous junction structures—do not exhibit lingering gradients, but can be susceptible to transient liquid-junction potentials under specific conditions, particularly when the conductivity is low and the mobilities of the participating ions are strongly asymmetric.

4.1.1 Summary of Findings

- No single sensor met all performance criteria.
- Sensor type 1 is suitable for inline measurement of most water-soluble buffers but must be removed during column CIP and requires a workaround for salt memory.
- Type 6 can remain in place during column CIP and recovers quickly from salt memory but is not suitable for certain low-conductivity buffers.

4.2 Proposed Solution

Continuous release of the buffer being supplied to the application is a critical issue. For instance, it is not acceptable to have a reading error of 0.2–0.3 pH units every time the process switches from a salt-containing buffer to a buffer without salt. At the same time, it is desirable to avoid needing to take out the electrodes every cycle during column CIP.

The solution proposed here (shown in **Figure 10**) consists of two parts:

1. The use of two dual pH module arrangements, one for controlling and one for monitoring.
2. Use two types of sensors in each module, type 1 for buffers without salt and type 6 for buffers with salt, and sodium hydroxide for column CIP.

Sensor selection should be recipe-driven, based on buffer composition. Buffers with an estimated sodium chloride concentration ≤0.05 M should be routed through the type 1 path, covering all low-conductivity and low-pH buffers. Buffers with sodium chloride >50 mM should use type 6 sensors. (Buffers with ≤0.05 M sodium chloride do not cause salt memory; data not shown.) Column CIP (sodium hydroxide) and water or ethanol washes should also be routed through type 6 sensors. To ensure continuity, conductivity can be used to confirm when the CIP solution has been fully replaced by the equilibration buffer.

With this arrangement, it should be possible to measure, control, and monitor any water-soluble buffer and still have a route for column CIP solution to avoid taking the sensors out of the system for CIP reasons. In addition, the drift test demonstrated that sensors of type 1 can go one week without calibration, removing the need to calibrate the sensors at every cycle. Furthermore, the inline study during a large-scale manufacturing campaign showed that the type 6 sensor can function even longer without calibration. These results suggest that if the proposed solution (**Figure 10**) is implemented, the pH sensors would not need to be taken out of the system at every cycle for either column CIP or calibration reasons. Together the implication is that manual intervention, time, and risk for introducing contamination into the system can also be reduced. The dual-sensor approach enables continuous manufacturing by eliminating downtime during buffer transitions and column CIP cycles. Automatic switching ensures uninterrupted pH monitoring across diverse process conditions, while each sensor operates within its optimal range. This design supports flexible facilities by accommodating a wide range of buffer chemistries and cleaning protocols without manual intervention, reducing complexity in multi-product environments and improving overall process robustness.

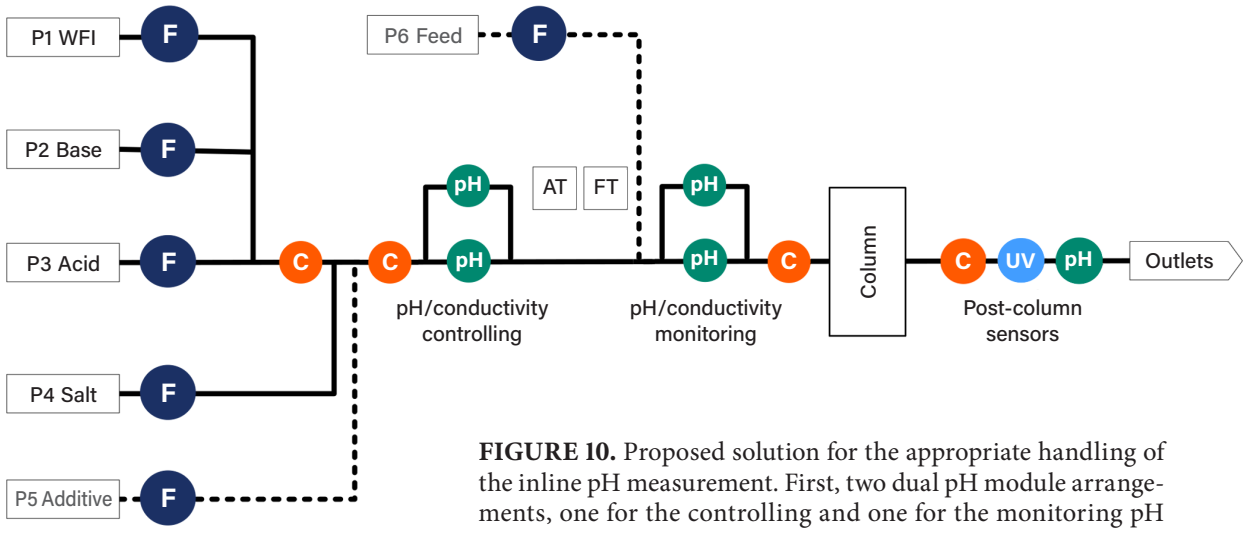


FIGURE 10. Proposed solution for the appropriate handling of the inline pH measurement. First, two dual pH module arrangements, one for the controlling and one for the monitoring pH sensors, are used. Second, for every dual pH module, two types of sensors are used, type 1 for buffers without and type 6 for buffers with sodium chloride.

5. Conclusion

This study shows that it is difficult to find one pH probe that fulfills all the necessary criteria for accurate inline measurement for chromatographic applications. We therefore suggest using two types of sensors, one for buffers without and another one for buffers with sodium chloride and hydroxide solutions exposure. To be able to switch between the sensor type a dual pH module arrangement is proposed where the choice can be done automatically with help of valves. Additional redundancy can be achieved by implementing a dual pH module arrangement for the controlling sensors as well as for the monitoring sensors in an IC system. The study further indicates that with this solution manual intervention, time, and risk for introducing contamination into the system also will be reduced. Finally, with the proposed set-up it should be possible to integrate inline pH measurements for both control and release in line with a continuous validation approach with PAT in buffer making, resulting in a leaner, sustainable, and more cost-efficient production of biopharmaceuticals.

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About the Authors

Karolina Busson, MSc[a], Senior Application Specialist; Joanne Sheehy[b], Principal Manufacturing Process Specialist; Liz Anderson, MSc[b], Principal Engineer; Therese Westman, MSc[a], Senior Automation Project Leader[a]; **Enrique Carredano, PhD**[c*], Senior Scientist, R&D, Start-to-Finish.

a. Custom Engineering, Cytiva, Björkgatan 30, 751 84 Uppsala Sweden

b. Johnson & Johnson Innovative Medicine Ireland UC, Barnahely, Ringaskiddy, P43 FA46, Co Cork, Ireland

c. R&D, Start-to-Finish, Cytiva, Björkgatan 30, 751 84 Uppsala Sweden

***Corresponding Author:** Email: enrique.carredano@cytiva.com