BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Demonstrating the Equivalence of Traditional Versus Automated Buffer Preparation Methods Using In-Line Conditioning Control Modes to Manage Incoming Stock Solution Variability

By Karolina Busson, Robbie Kamperveen, and Enrique Carredano

Abstract

In-line conditioning (IC) is a form of dilution where a process buffer is formulated in-line from concentrated stock solutions of acids, bases, and salts that are mixed with the correct amount of water-for-injection (WFI). This new buffer preparation strategy must prove its equivalency to buffers made the traditional way (*i.e.*, weighing salts, stirring in water, titrating with acid or base). In this paper, such a demonstration is presented using two control modes: (1) ratio control with flow feedback; and (2) pH/conductivity feedback. To obtain the necessary parameters for an error propagation analysis, a robustness study has been performed. Our analysis showed that with low incoming variability, or when the uncertainty of the stock solutions is below 2%, the two modes of control give comparable performance. When the uncertainty increases, so does the uncertainty of ratio control with flow feedback, more with respect to conductivity than pH, while the precision of pH/conductivity feedback remains at the same level. The choice of control should therefore take into consideration the critical process parameters, their tolerances, and the input variability in the stock solution concentration. In situations where there are higher variabilities in stock solution concentrations or process temperatures, this study suggests that pH/conductivity feedback might be a better option.

1.0 Introduction

The traditional buffer management approach requires a lot of equipment and floor space. Therefore, it is a limiting factor in the design of facilities and subsequent implementation of flexible plant operations.[1] Preparing buffers using traditional methods involves dissolving buffer components and water-for-injection (WFI) in tanks, transferring contents into bags, and transporting them to their points of use. Buffers are usually prepared in advance and stored for a few hours or days before use.

Higher bioproduction demands and/or evolving processes typically require larger areas dedicated to buffer management. In order to better accommodate anticipated process and capacity changes, Janssen Biologics BV decided to research alternative buffer management options. In-line conditioning (IC) technology[2] was investigated as a possible solution to increasing buffer capacity without the high equipment occupancy.

With IC, buffers can be produced on-demand and just-in-time (JIT), at the point-of-use (POU), from single-component stock concentrates of acid, base, and salt, diluted to the correct proportions. How this is done (*i.e.*, the mode of IC control) can be a simple ratio control with flow feedback, or pH and/or conductivity feedback together with flow feedback. Independent of the control mechanism, a release strategy should be implemented that guarantees the quality of the buffer properties before it is released. The release criteria should match the established process parameters. Understanding how the variability of incoming parameters affects the precision of the output, in any step of a process, is crucial for process understanding. It helps in determining the right control strategy and derivation of the input parameter tolerances needed for an output within specifications. This article focuses on the buffer formulation step performed by IC conditioning and how variabilities in the stock solution concentrations can be managed. Often there are multiple pieces of lab equipment used when prepping concentrated stock solutions (*e.g.*, a scale or meter). Along with human error, they all contribute to the variance of the output.

In the previous study[1], the understanding of chemical equilibria, along with the IC chromatography equipment design parameters, were identified as critical for securing system performance in an automated environment. The purpose of this study is to: (1) determine if equivalency and

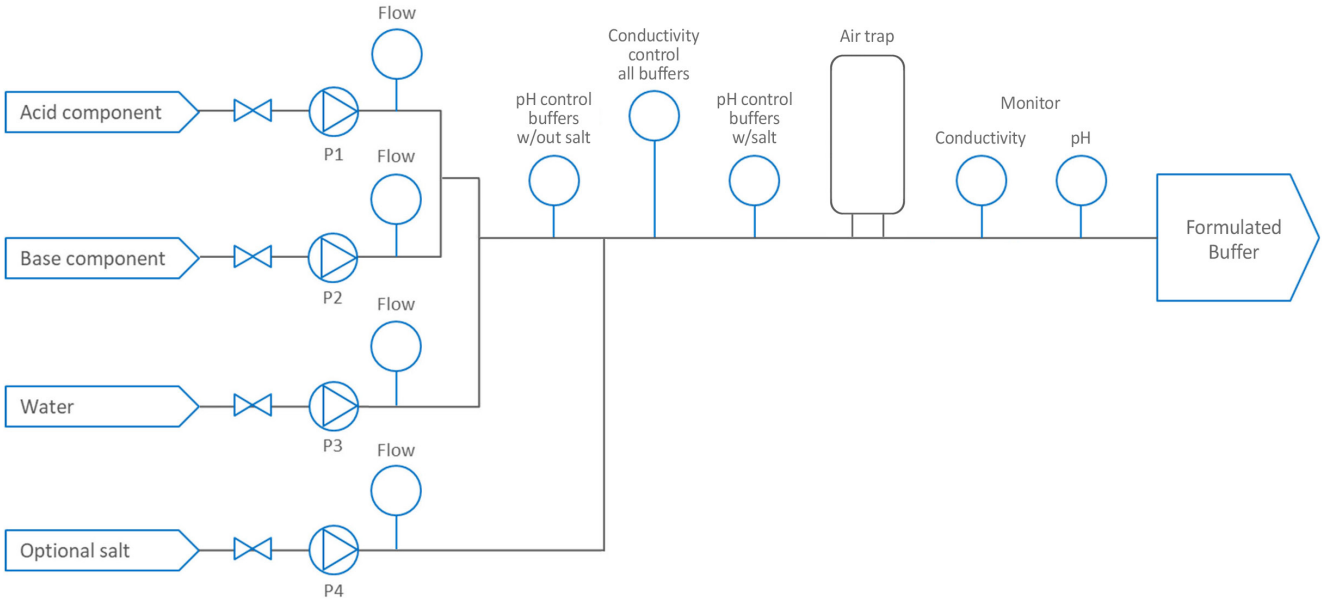


FIGURE 1. Flow scheme of the IC system used with inlets and pumps for acid and base buffer components, water, and optional salt (or additives) that can be included in the buffer recipe.

consistency can be confirmed between buffers produced traditionally and by IC; and (2) establish the recommended control and release strategy for IC. To meet the first objective, buffers already being used for purification purposes were formulated and analyzed. For the second objective, we sought to understand how variabilities in stock solution concentrations were propagated to the resulting pH and conductivity. Thus, a robustness study was conducted where the stock solutions were deliberately prepared at ±10% of a target concentration. Assuming a linear response, the results can be used to predict the error propagation when a smaller deviation is expected.

Published literature is available on the use of in-line dilution to reduce the space needed for buffer preparation.[3–5] Systems may or may not be equipped with additional pumps for pH and conductivity adjustments post-dilution. It must be pointed out that a buffer is often defined by additional parameters such as concentration of salt (as an alternative parameter to the conductivity), the flow rate at which the buffer is used, the buffer concentration, and the concentration of an optional additive. In contrast to the post-adjustment approach, IC controls and releases are based on all key parameters simultaneously, during buffer formulation.

2.0 Materials and Methods

2.1 The In-line Conditioning System

The IC system setup used in this study has four pumps and flow meters for controlled delivery of acid, base, WFI, and an optional salt solution when needed. The system is equipped with multiple pH and conductivity sensors for monitoring and dynamic feedback control. A simplified equipment diagram is shown in **Figure 1**. For the robustness study, an additional pair of pH and conductivity sensors were used, located in between the controlling sensors and the air trap.

2.2 Buffer Formulation

Target buffers with specific pH and conductivity values were formulated using two different control modes: (1) ratio control with flow feedback; and (2) pH/conductivity feedback. With flow feedback, the relative proportions of the components were maintained together with the total system flow rate setpoint. In this mode, the pH and conductivity meters were used for monitoring the in-line values. In the pH/conductivity feedback mode, the post- or pre-salt control pH sensor was used for feedback control, depending on whether the buffer contained salt or not. The corresponding controlling pH signal was used to automatically increase/decrease the flowrates of acid and base to reach the pH setpoint. At the same time, the controlling conductivity signal was used to adjust the buffer composition to reach the conductivity setpoint. Exactly how this was done depended on whether the buffer contained salt or not. When the buffer contained salt, the conductivity signal was used to automatically adjust the flow rate of the salt, and the acid and base flow rates were constrained to maintain the buffer concentration constant. In buffers without salt, the conductivity feedback adjusted the acid and base flow rates, whereas the pH feedback adjusted the relative proportions between the two. The operating range of the pumps used are 4–180 L/h for the acid, base, and salt, and 15–600 L/h

for the water. When operating within these ranges, the flow precision can be maintained within ±1% of the maximum pump flow rate or ±2% of reading, whichever is largest.

2.3 Equivalence Test Buffers

To demonstrate equivalency between IC-made buffers and buffers prepared manually, nine buffers were considered and then formulated from the stock solutions: sodium dihydrogen phosphate, citric acid, Tris hydrochloride, disodium hydrogen phosphate, trisodium citrate, Tris, and sodium chloride.

The ratios for the pumps in the runs using ratio control with flow feedback were calculated from the molar recipes currently used in production.

2.4 Robustness Test Buffers

Buffers of 20 mM sodium phosphate, at pH values of 6.6, 7.0, and 7.4, were formulated from stock solutions with nominal concentrations of 0.3 M NaH_2PO_4 (acid) and 0.3 M Na_2HPO_4 (base). At pH 7.0, buffers were also formulated with 0.5 M NaCl using a 3 M NaCl (salt) stock solution. The robustness testing consisted of varying the concentrations of the stock solutions at/around (±10%) the nominal concentrations. The following stock solutions were prepared: 0.3, 0.27, and 0.33 M for base and acid; and 3.5, 3.15, and 3.85 M for the salt. At pH 7.0, the center point without salt was run in duplicate and with salt, in triplicate.

The ratios for the pumps using ratio control with flow feedback were calculated from molar recipes determined with the algorithm and program "Buffalo," as described by Bjorkesten *et al*.[6]

When running in pH/conductivity feedback mode, the conductivity setpoint for each pH was determined by running in flow feedback with the center point stock solutions.

- pH 6.6→1.97 mS/cm
- pH 7.0→2.30 mS/cm
- pH 7.4→2.70 mS/cm
- pH 7.0 with 0.5 M NaCl→46.5 mS/cm

2.5 Error Analysis in Flow Feedback Control

When using ratio control with flow feedback, the precision in the pH and conductivity can be estimated with the following formula:

$$\sigma = \sqrt{{\sigma_{Formula}}^2 + {\sigma_{Pump}}^2 + {\sigma_{Stocks}}^2 + {\sigma_{meter}}^2} \quad \text{(Eq. 1)}$$

Here we borrow the symbol to represent a random error interval, which covers most probable outcomes. This would correspond to at least five times the standard deviation of each of the components.

The contribution $\sigma_{Formula}$ is the error in the recipe. If each buffer is considered individually, this is a systematic error that could be considered as such and pulled out from the calculation of the precision. Here, however, we are considering a general buffer selected randomly among a set of many buffers, and we are assuming that the formula error for this general buffer follows a normal distribution centered around zero error. If the recipe has been carefully worked out through hundreds of repeated experimental titrations, this error will be smaller and could be neglected, in the best of cases. However, if the formula was determined by one or very few experiments, this error could be larger, typically of the same magnitude as the error of a pH measurement. The σ_{Pump} and σ_{Stocks} contributions refer to the random error in pH or conductivity due to random errors in the amount of acid and base in the buffer formulated. In the first case, this is due to the uncertainty of the pumps, and in the second case, uncertainty of the stock solution concentrations. Finally, the σ_{meter} contribution refers to the uncertainty of the pH or conductivity meter itself (*i.e.*, as determined by the gage repeatability and reproducibility [R&R] of the meter).

2.6 Error Analysis in pH/Conductivity Feedback Control

In this case, the pH and conductivity are adjusted in the system. Most errors resulting from the preparation of stock solutions, pumps, or the formula can be neglected. In order to manage the risk of systematic errors, two meters are used, one for system control, and the other to monitor the solutions in-line before release. The total variance of the measurement reported by the monitoring and releasing electrode will be dependent on: (1) the buffer composition; and (2) the variance of the monitoring electrode. Assuming that the variance of the buffer composition is mostly due to the variance of the controlling electrode, the total variance of the measurement reported by the releasing electrode can be estimated as the sum of the variances of the two individual electrodes:

$$\sigma = \sqrt{2 * {\sigma_{meter}}^2} \quad \text{(Eq. 2a)}$$

The variance of the measurement reported by the controlling sensor is simply:

$$\sigma = \sigma_{meter} \quad \text{(Eq. 2b)}$$

This is the variance of the pH or conductivity in the buffer, regardless of whether there is a monitoring sensor or not. However, if, as in this case, an additional sensor is used for release, Equation 2a should be used, not because the buffer itself is less precise, but to take into consideration that the monitoring and releasing sensor measures pH and conductivity independently of the controlling one.

2.7 Estimation of Meter Precision

The gage R&R for both pH and conductivity meters used in the IC system provide parameters related to the precision

needed in the error analysis formulas. These parameters were estimated from the results of the robustness study. There were 31 runs without salt, each measured by four pH and four conductivity sensors, and 33 runs with salt, measured by three pH and three conductivity sensors. This made a total of 124 + 99 = 223 data points for the estimation of precision σ_{meter} with each data point consisting of a one-minute (average) continuous in-line measurement with a corresponding standard deviation. The precision σ_{meter} values for the pH and conductivity meters were estimated as five times the average of the standard deviations.

2.8 Calculation of the Error Propagation Contributions

The σ_{Pump} and σ_{Stocks} error propagation contributions on the pH were also estimated from the robustness study results using ratio control with flow feedback, without salt. The data points used, in this case, were when the errors in pH were reinforced (*i.e.*, either the acid concentration was 10% lower and the base concentration was 10% higher, or vice versa, as compared to the center points). The expected error in pH, due to 1% error in the stock solutions, was then estimated as the average of the total difference from the target for six cases divided by 20. The factor 20 was used instead of 10, since the two contributions added up. The corresponding σ_{Pump} and σ_{Stocks} contributions for the conductivity, as relative error in %, were assumed to be the same as the corresponding relative error in % for the concentration.

2.9 Calculation of the Precision as a Function of the Stock Concentration Variability

The precision of pH and conductivity were estimated for both control modes (ratio control with flow feedback and pH/conductivity feedback) using Equations 1 and 2 at a confidence level of five standard deviations. For σ_{Pump}, an uncertainty of 2% was assumed for the pumps, and for σ_{Stocks}, the error was calculated for a variable error of the stock concentration from 0–10%. The parameter $\sigma_{Formula}$ assumed to be 0 and 0.05 pH units for the pH and 0 and 2% for the conductivity to obtain the width of the uncertainty of the outcome due to an error in the recipe.

3.0 Results

3.1 Results of the Equivalence Tests

For the runs using ratio control with flow feedback mode (**Table 1)**, all buffers meet the ± 0.1 pH unit requirement consistently, as measured by two in-line pH meters. Only one buffer (0.05 M Tris, 1 M NaCl, pH 8.0) failed its tighter requirement (± 0.05 pH unit) for the two runs (0.07 and 0.08 pH unit difference). For the pH/conductivity feedback mode runs (**Table 2)**, all buffers met their process requirements of ± 0.05 pH unit, as measured by the in-line pH controlling sensor, and more importantly, the monitoring

TABLE 1. Equivalence experiments – flow feedback mode. Runs 1 and 2: maximum flow rate. Run 3: minimum flow rate. Average in-line values were taken for >1 minute after steady state.

Experiment #	Buffer Description	pH (pH unit)				Conductivity (mS/cm)			
		Run 1	Run 2	Run 3	Acceptance Criteria	Run 1	Run 2	Run 3	Acceptance Criteria
1	0.1 M Na citrate, pH 3.5	3.46/3.48	3.46/3.48	3.49/3.51	3.45–3.55	98.11	5.42/5.46	5.52/5.58	4.94–6.04
2	0.05 M Tris, 1 M NaCl, pH 8.0	8.05/8.07	8.08/8.08	ND	7.95–8.05 @ 21.5–23.5°C	99.10	85.21/85.08	ND	76.3–93.3
3	0.9% NaCl	ND	ND	ND	ND	98.91	ND	ND	13.85–16.9
4	0.1 M Na citrate, pH 5.0	ND	ND	ND	4.95–5.05	98.19	ND	ND	11.06–13.52
5	0.05 M Tris, pH 8.0	7.86/7.86	7.87/7.86	7.88/7.87	7.65–8.04 @ 24.5–25.5°C	99.76	2.92/2.92	2.92/2.92	2.55–3.24
6	0.2 M Na phosphate, pH 6.8	6.83/6.83	6.81/6.82	6.80/6.81	6.75–6.85	98.89	18.10/18.21	18.12/18.23	16.38–20.02
7	0.05 M Na phosphate, 0.15 M NaCl, pH 7.3	ND	ND	ND	7.15–7.46	99.12	ND	ND	17.0–21.6
8	0.03 M Na phosphate, pH 6.5	6.57/6.57	6.53/6.53	6.54/6.54	6.35–6.64 @ 24.5–25.5°C	98.69	2.90/2.90	2.91/2.92	2.35–3.44
9	0.05 M Tris, 0.05 M NaCl, pH 8.0	7.89/7.90	7.89/7.90	7.90/7.90	7.75–8.04 @ 24.5–25.5°C	98.80	7.55/7.58	7.53/7.57	5.95–9.04

NOTE: Not determined (ND)

TABLE 2. Equivalence experiments – pH/conductivity feedback mode. Runs 1 and 2: maximum flow rate. Run 3: minimum flow rate. Average in-line values were taken for >1 minute after steady state.

Experiment #	Buffer Description	Controlling/Monitoring pH (pH unit)				Controlling/Monitoring Conductivity (mS/cm)			
		Run 1	Run 2	Run 3	Acceptance Criteria	Run 1	Run 2	Run 3	Acceptance Criteria
1	0.1 M Na citrate, pH 3.5	3.50/3.47	3.50/3.47	3.48/3.47	3.45–3.55	5.38/5.42	5.38/5.42	5.47/5.48	4.94–6.04
2	0.05 M Tris, 1 M NaCl, pH 8.0	7.99/8.02	8.00/8.03	8.00/8.03	7.95–8.05 @ 21.5–23.5°C	84.81/85.05	84.22/84.65	84.62/85.06	76.3–93.3
3	0.9% NaCl	ND	ND	ND	ND	15.39/15.50	15.48/15.47	ND	13.85–16.90
4	0.1 M Na citrate, pH 5.0	5.00/5.00	5.00/5.01	5.01/5.01	4.95–5.05	12.17/12.39	12.17/12.34	ND	11.06–13.52
5	0.05 M Tris, pH 8.0	7.85/7.86	7.85/7.85	7.86/7.84	7.65–8.04 @ 24.5–25.5°C	2.88/2.91	2.91/2.93	2.90/2.91	2.55–3.24
6	0.2 M Na phosphate, pH 6.8	6.80/6.81	6.82/6.82	ND	6.75–6.85	18.28/18.18	18.27/18.19	ND	16.38–20.02
7	0.05 M Na phosphate, 0.15 M NaCl, pH 7.3	7.30/7.30	7.30/7.30	ND	7.15–7.46	19.30/19.48	19.30/19.49	ND	17.0–21.6
8	0.03 M Na phosphate, pH 6.5	6.50/6.47	6.50/6.47	ND	6.35–6.64 @ 24.5–25.5°C	2.74/2.92	2.71/2.91	ND	2.35–3.44
9	0.05 M Tris, 0.05 M NaCl, pH 8.0	7.90/7.91	7.90/7.90	ND	7.75–8.04 @ 24.5–25.5°C	7.50/7.54	7.50/7.54	ND	5.95–9.04

NOTE: Not determined (ND)

sensor. For this same buffer (0.05 M Tris, 1 M NaCl, pH 8.0), it was necessary to recalibrate the monitoring electrode, since it failed in the first try (0.06 pH difference). All measured runs fulfilled the acceptance criteria with regard to in-line conductivity values independent of the control mode used. In general, the performance data obtained was very similar between the two modes.

3.1.1 Osmolality Measurements

To further confirm the equivalency of the buffers' osmolality, values were measured from grab samples of the runs and then compared to the acceptance criteria (**Table 3**). All measured runs fulfilled the acceptance criteria.

3.2 Results of the Robustness Tests

Ratio control with flow feedback mode results are shown in **Table 4** (buffers without salt) and **Table 5** (buffers with salt). Results from the pH/conductivity feedback mode experiments are shown in **Table 6** (buffers without salt) and **Table 7** (buffers with salt). The target values for pH/conductivity (pH 6.6/1.97 mS/cm, pH 7.0/2.3 mS/cm, and pH 7.4/2.70 mS/cm) for buffers without salt, shown in

TABLE 3. Osmolality measurement results compared to the acceptance criteria.

Experiment #	Buffer Description	Osmolality (mOsm/kg H_2O)								
		Flow Feedback Mode				pH/Conductivity Feedback Mode				Acceptance Criteria
		Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 1	Sensor 2	Sensor 3	Sensor 4	
1	0.1 M Na citrate, pH 3.5	0.185	0.182	0.182	0.181	0.180	0.180	0.177	0.180	0.155–0.189
2	0.05 M Tris, 1 M NaCl, pH 8.0	1.964	1.964	1.973	1.954	1.930	1.942	1.949	1.954	1.733–2.118
3	0.9% NaCl	ND	ND	ND	ND	0.281	0.282	0.281	0.281	0.257–0.314
4	0.1 M Na citrate, pH 5.0	ND	ND	ND	ND	0.244	0.244	0.241	0.242	0.221–0.271
5	0.05 M Tris, pH 8.0	ND	ND	ND	ND	ND	ND	ND	ND	NA
6	0.2 M Na phosphate, pH 6.8	0.398	0.399	0.400	0.398	0.400	0.398	0.393	0.398	0.351–0.429
7	0.05 M Na phosphate, 0.15 M NaCl, pH 7.3	ND	ND	ND	ND	ND	ND	ND	ND	NA
8	0.03 M Na phosphate, pH 6.5	ND	ND	ND	ND	ND	ND	ND	ND	NA
9	0.05 M Tris, 0.05 M NaCl, pH 8.0	ND	ND	ND	ND	ND	ND	ND	ND	NA

NOTES: Not determined (ND); not applicable (NA)

TABLE 4. Robustness experiments – flow feedback mode – buffers without salt. Runs 1 and 2: maximum flow rate. Run 3: minimum flow rate. Average in-line values from the last minute of each run are shown.

Experiment #	Target pH (pH units)	Offset (%)		pH (pH unit)				Conductivity (mS/cm)			
		Acid Stock	Base Stock	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 1	Sensor 2	Sensor 3	Sensor 4
1	7.0	10	10	7.03	7.07	7.07	7.05	2.619	2.605	2.621	2.618
2	7.0	10	-10	6.86	6.89	6.85	6.88	2.000	1.983	1.995	1.994
3	7.0	-10	-10	6.96	6.98	6.94	6.96	1.879	1.864	1.875	1.873
4	7.0	0	0	7.03	7.06	7.03	7.04	2.398	2.378	2.402	2.399
5	7.0	-10	10	7.13	7.16	7.12	7.14	2.499	2.485	2.502	2.499
6	7.0	0	0	7.04	7.07	7.04	7.05	2.390	2.373	2.389	2.384
7	6.6	10	10	6.62	6.65	6.61	6.63	2.177	2.163	2.177	2.175
8	6.6	-10	-10	6.56	6.59	6.56	6.58	1.699	1.682	1.695	1.693
9	6.6	0	0	6.63	6.66	6.63	6.64	1.970	1.946	1.967	1.966
10	6.6	-10	10	6.71	6.73	6.70	6.72	1.987	1.971	1.984	1.982
11	6.6	10	-10	6.44	6.46	6.43	6.46	1.797	1.783	1.793	1.791
12	7.4	-10	-10	7.36	7.39	7.35	7.30	2.188	2.172	2.187	2.188
13	7.4	0	0	7.42	7.46	7.41	7.43	2.731	2.712	2.740	2.724
14	7.4	-10	10	7.51	7.54	7.50	7.51	2.935	2.920	2.946	2.944
15	7.4	10	-10	7.24	7.27	7.23	7.25	2.149	2.136	2.148	2.146
16	7.4	10	10	7.41	7.45	7.41	7.43	3.002	2.990	3.013	3.009

TABLE 5. Robustness experiments – flow feedback mode – buffers with salt (target pH 7.0). Average in-line values from the last minute of each run are shown.

Experiment #	Offset (%)			pH (pH unit)			Conductivity (mS/cm)		
	Salt Stock	Acid Stock	Base Stock	Sensor 2	Sensor 3	Sensor 4	Sensor 2	Sensor 3	Sensor 4
1	-10	-10	-10	6.98	6.99	7.00	42.998	43.445	43.414
2	0	-10	-10	6.93	6.96	6.95	46.230	46.692	46.664
3	10	-10	-10	6.92	6.94	6.95	49.433	49.921	49.894
4	-10	-10	10	7.17	7.18	7.19	43.545	43.991	43.956
5	0	-10	10	7.14	7.16	7.16	46.710	47.185	47.143
6	10	-10	10	7.11	7.14	7.14	49.934	50.634	50.429
7	-10	0	0	7.06	7.06	7.08	43.377	43.840	43.798
8	0	0	0	7.03	7.04	7.06	46.528	47.016	47.010
9	0	0	0	7.00	7.05	7.01	46.663	47.138	47.111
10	0	0	0	7.05	7.04	7.07	46.529	47.032	46.985
11	10	0	0	7.12	7.11	7.12	49.905	50.476	50.435
12	-10	10	-10	6.90	6.92	6.92	43.037	43.479	43.424
13	0	10	-10	6.92	6.95	6.94	46.469	46.762	46.907
14	10	10	-10	6.89	6.92	6.93	49.505	49.986	49.999
15	-10	10	10	7.09	7.11	7.12	43.565	43.843	43.803
16	0	10	10	7.03	7.07	7.05	46.843	47.200	47.184
17	10	10	10	7.04	7.05	7.08	49.906	50.401	50.372

TABLE 6. Robustness experiments – pH/conductivity feedback mode – buffers without salt. Average in-line values from the last minute of each run are shown.

Experiment #	Target pH (pH units)	Offset (%)		pH (pH units)				Conductivity (mS/cm)			
		Acid Stock	Base Stock	Sensor 1 (Controlling)	Sensor 2	Sensor 3	Sensor 4	Sensor 1	Sensor 2	Sensor 3	Sensor 4
1	7.0	10	10	7.00	7.03	6.99	7.02	2.310	2.296	2.314	2.311
2	7.0	10	-10	7.00	7.02	6.99	7.01	2.310	2.295	2.310	2.306
3	7.0	-10	-10	6.99	7.02	6.99	7.01	2.309	2.293	2.306	2.301
4	7.0	0	0	7.00	7.02	6.99	7.01	2.322	2.300	2.323	2.316
5	7.0	-10	10	7.00	7.03	6.99	7.02	2.311	2.299	2.312	2.310
6	7.0	0	0	7.00	7.03	6.99	7.01	2.318	2.296	2.321	2.316
7	6.6	10	10	6.60	6.63	6.60	6.62	1.982	1.970	1.983	1.981
8	6.6	-10	-10	6.60	6.61	6.59	6.61	1.936	1.916	1.935	1.917
9	6.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	6.6	-10	10	6.60	6.62	6.6	6.61	1.978	1.961	1.976	1.971
11	6.6	10	-10	6.60	6.61	6.6	6.61	1.979	1.964	1.976	1.972
12	7.4	-10	-10	7.38	7.43	7.39	7.39	2.691	2.675	2.694	2.679
13	7.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	7.4	-10	10	7.40	7.44	7.44	7.42	2.719	2.698	2.727	2.734
15	7.4	10	-10	7.40	7.43	7.44	7.41	2.707	2.693	2.713	2.706
16	7.4	10	10	7.40	7.44	7.39	7.42	2.710	2.699	2.718	2.715

TABLE 7. Robustness experiments – pH/conductivity feedback mode – buffers with salt (target pH 7.0). Average in-line values from the last minute of each run are shown.

Experiment #	Offset (%)			pH (pH unit)			Conductivity (mS/cm)		
	Salt Stock	Acid Stock	Base Stock	Sensor 2 (Controlling)	Sensor 3	Sensor 4	Sensor 2	Sensor 3	Sensor 4
1	-10	-10	-10	7.00	7.01	7.02	46.506	47.000	46.953
2	0	-10	-10	7.00	7.02	7.02	46.489	46.980	46.945
3	10	-10	-10	7.00	7.02	7.02	46.504	46.937	46.913
4	-10	-10	10	7.00	7.01	7.02	46.496	46.988	46.958
5	0	-10	10	7.00	7.02	7.02	46.495	46.947	46.911
6	10	-10	10	7.00	7.02	7.02	46.496	46.949	46.921
7	-10	0	0	7.00	6.99	7.01	46.502	46.998	46.875
8	0	0	0	7.00	7.04	7.02	46.505	46.976	46.956
9	0	0	0	7.00	7.01	7.02	46.510	46.972	46.940
10	0	0	0	7.00	6.99	7.00	46.509	47.003	46.951
11	10	0	0	7.00	6.99	7.00	46.496	46.993	46.950
12	-10	10	-10	7.00	7.01	7.02	46.494	46.999	46.955
13	0	10	-10	7.00	7.02	7.01	46.494	46.958	46.941
14	10	10	-10	7.00	7.02	7.02	46.506	46.953	46.903
15	-10	10	10	7.00	7.01	7.02	46.510	46.816	46.848
16	0	10	10	7.00	7.03	7.02	46.503	46.971	46.939
17	10	10	10	7.00	7.01	7.02	46.507	46.938	46.905

Figure 2 (pH) and **Figure 3** (conductivity), illustrate the mode's robustness, as compared to flow feedback (when 10% stock solution errors are taken into account). The same is seen for buffers with salt (pH/conductivity target values of 7.0/46.5 mS/cm), as shown in **Figure 4** (pH) and in **Figure 5** (conductivity).

3.3 Estimating Meter Precision

The results of the precision σ_{meter} estimation for both pH and conductivity are shown in **Table 8**. The obtained values agree nicely with the frequently used nominal precision of the meters (0.1 pH units and 2% for the conductivity, respectively)

3.4 Calculating the Error Propagation Contributions

The offset (or difference) in pH with the six flow feedback cases, where the separate effects of the acid and base stock solution deviations reinforce each other, is shown in **Table 9**.

The average of the difference was calculated as 0.13376,

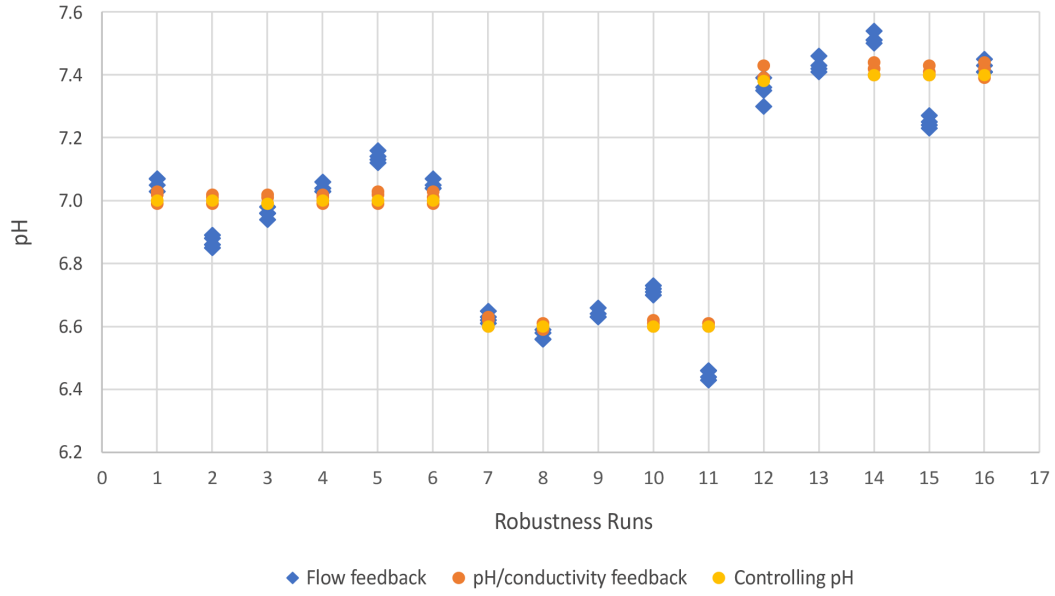


FIGURE 2. Average pH in the buffers without salt (see **Tables 4** and **6**).
Flow feedback mode uses four independent buffer monitoring sensors (blue rhombs). **pH/conductivity mode** utilizes three independent sensors to monitor (orange circles) and one to control (yellow circles) the buffer composition.

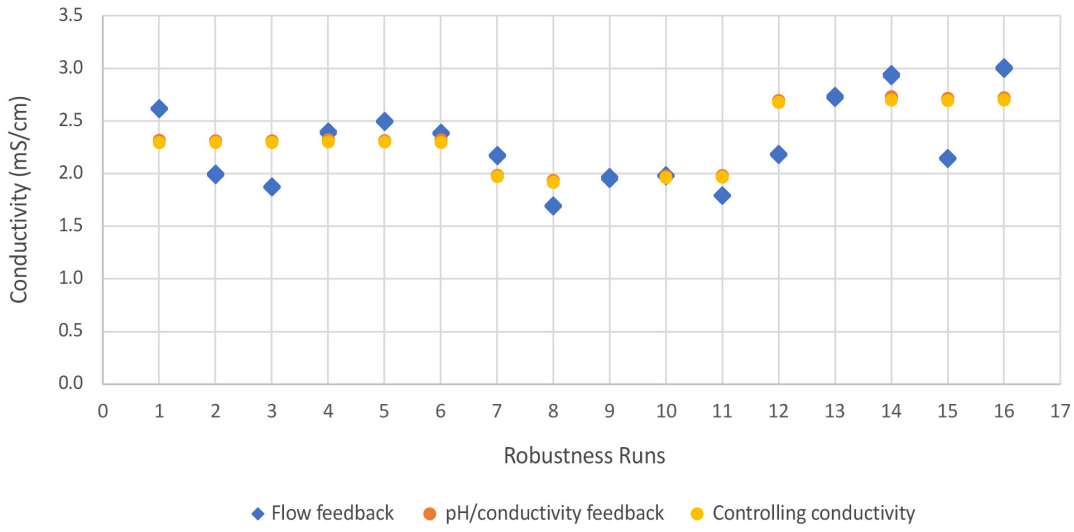
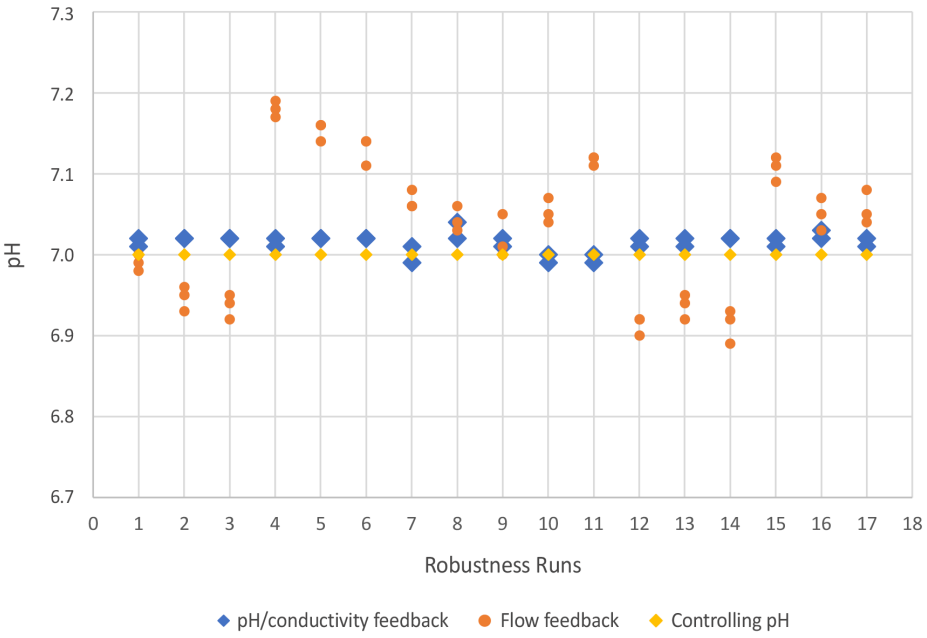


FIGURE 3. Average conductivity in the buffers without salt (see **Tables 4** and **6**).
Flow feedback mode uses four independent buffer monitoring sensors (blue rhombs). **pH/conductivity mode** utilizes three independent sensors to monitor (orange circles) and one to control (yellow circles) the buffer composition.

FIGURE 4. Average pH in the buffers with salt (see **Tables 5** and **6**).

Flow feedback mode uses three independent buffer monitoring sensors (orange circles).

pH/conductivity mode utilizes two independent sensors to monitor (blue rhombs) and one to control (yellow rhombs) the buffer composition.



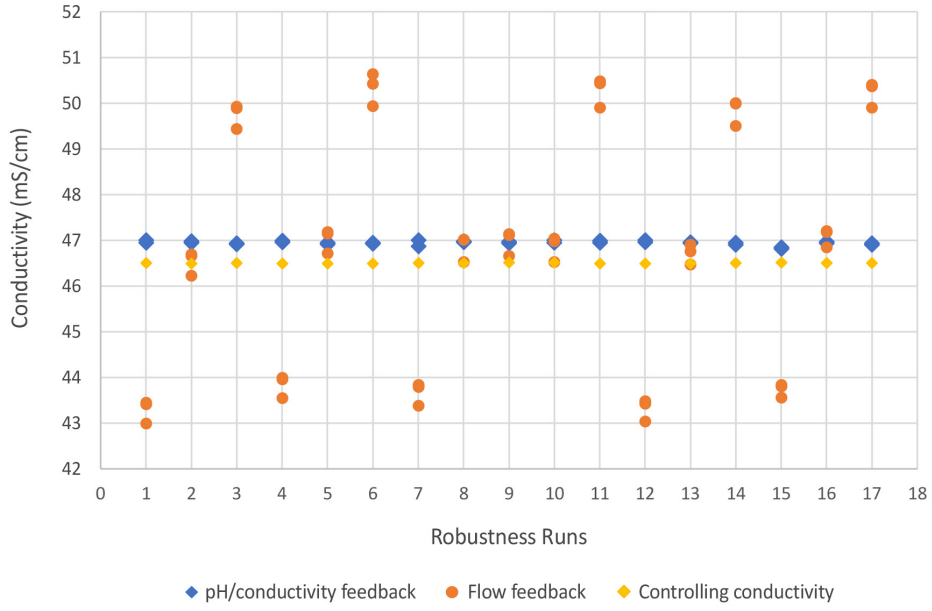


FIGURE 5. Average conductivity in the buffers with salt (see **Tables 5** and **7**).

Flow feedback mode uses three independent buffer monitoring sensors (orange circles).

pH/conductivity mode utilizes two independent sensors to monitor (blue rhombs) and one to control (yellow rhombs) the buffer composition.

TABLE 8. Estimated precision for pH and conductivity.

pH (pH units)	Conductivity Relative Error (%)
0.073	2.1

TABLE 9. Calculations used to estimate the average difference for 20% error in the stock solutions.

Target pH (pH unit)	Offset (%)		Average (%)		Difference (%)
	Acid Stock	Base Stock	Point	Centrum Point	
7.0	10	-10	6.8700	7.0450	0.1750
7.0	-10	10	7.1375	7.0450	0.0925
6.6	-10	10	6.7150	6.6400	0.0750
6.6	10	-10	6.4475	6.6400	0.1925
7.4	-10	10	7.5150	7.4300	0.0850
7.0	10	-10	7.2475	7.4300	0.1825

which, by dividing it by 20, showed 0.006688 pH units per % error in the stock solutions. This was then used to calculate the σ_{Pump} contribution after considering two pumps (from a total of three for acid, base, and salt) with 2% precision:

$$\sigma_{Pump}(\text{pH}) = \sqrt{2*(2*0.006688)^2} = 0.0189 \sim 0.02 \text{ pH units} \quad \text{(Eq. 3)}$$

Assuming a linear dependency between concentration and conductivity, the σ_{Pump} contributions for the conductivity, as relative error in %, were assumed to be the same as concentration and thereon for the pumps. When calculating σ_{Pump} while considering two pumps from three (acid, base, and salt) with 2% precision, the equation becomes:

$$\sigma_{Pump}(\text{conductivity}) = \sqrt{2*(0.02)^2} = 0.0189 \sim 0.028 = 2.8\% \quad \text{(Eq. 4)}$$

The σ_{Stocks} contributions for the pH and conductivity were calculated the same way as σ_{Pump}. The difference was that the uncertainty of each stock solution was allowed to vary from 0–10%, whereas σ_{Pump} remains constant. Finally, the $\sigma_{Formula}$ parameter was assumed to be 0.00 and 0.05 pH units (pH) and 0 and 2% (conductivity). In this way, all the contributions in the error analysis formulas could be estimated for both the pH and the conductivity.

3.5 Calculation of the Precision as Function of the Stock Concentration Variability

The precision calculated using Equations 1 and 2, with different assumptions as a function of the uncertainty in stock concentration from 0–10%, are shown in **Figure 6** (pH) and **Figure 7** (conductivity). What is common to both figures is that, whereas the uncertainty of pH/conductivity feedback is invariant to the precision of the stock solution concentrations, the corresponding uncertainty of ratio control with flow feedback increases with the uncertainty of the stock solution concentrations. What is different is that, for ratio control with flow feedback, the outcome precision of the conductivity is much more sensitive to the variability of the stock solutions than the pH. In other words, whereas the output uncertainty of the conductivity increases proportionally with the stock solution uncertainty, the output uncertainty of the pH remains quite unchanged for this control mode. This result is probably related to the buffering effect, which provides resistance toward a change in buffer pH (in the area of good buffer capacity) when a small amount of titrant is added to the solution. When the uncertainty of stock solutions is <2%, the two methods perform about the same, with respect to pH. In the absence of a formula error, the flow feedback methodology might have a slight advantage, with respect to the pH in this regime. The pH/conductivity feedback is, on the other hand, a better (and only) option if the allowed conductivity tolerance is ≤5% and the uncertainty of the stock solutions is >2%. On the other hand, if the uncertainty of the stock solutions is <2%, then the two methods are comparable in performance with regard to conductivity, with a slight advantage for pH/conductivity feedback.

3.6 pH Deviations in the Robustness Study Runs

The pH error calculated as the difference between the average of the last minute of each run compared to the

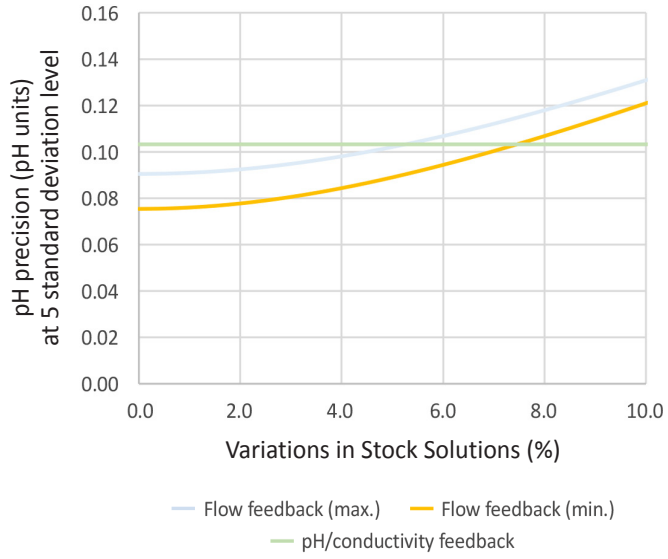


FIGURE 6. Calculated precision for pH as a function of the variability of the stock solutions. The relative error in the formula used in flow feedback is assumed to be 0.00 (minimum) and 0.05 (maximum) pH units.

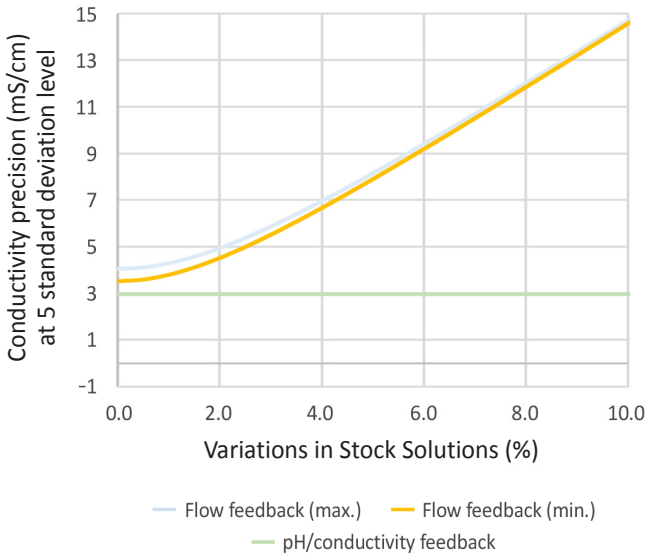


FIGURE 7. Calculated precision for conductivity as a function of the variability of the stock solutions. The relative error in the formula used in flow feedback is assumed to be 0% (minimum) and 2% (maximum).

corresponding targets are shown in **Table 10** (runs without salt) and **Table 11** (runs with salt). For the flow feedback runs without 10% deviation in the stock solutions, the maximum error found is 0.07, which is in fair agreement with **Figure 6** when the error in the stock solutions is <2%. The rest of the runs have similar (or larger) errors with the exception of runs where the acid and base offsets cancel each other. The highest deviations found are 0.17 pH units (without salt) and 0.19 pH units (with salt), corresponding to cases where deviations in acid and base reinforce each other. As mentioned in section 2.0, this corresponds to ~20% error in stock solutions and is outside of the graph range shown

TABLE 10. Robustness experiments – pH errors observed – buffers without salt.

Experiment #	Target		Offset (%)		Flow Feedback Mode (pH units)				pH/Conductivity Feedback Mode (pH units)			
	pH (pH units)	Conductivity (mS/cm)	Acid Stock	Base Stock	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 1	Sensor 2	Sensor 3	Sensor 4
1	7.0	2.30	10	10	0.03	0.07	0.07	0.05	0.00	0.03	0.01	0.02
2	7.0	2.30	10	-10	-0.14	-0.11	-0.15	-0.12	0.00	0.02	0.01	0.01
3	7.0	2.30	-10	-10	-0.04	-0.02	-0.06	-0.04	0.01	0.02	0.01	0.01
4	7.0	2.30	0	0	0.03	0.06	0.03	0.04	0.00	0.02	0.01	0.01
5	7.0	2.30	-10	10	0.13	0.16	0.12	0.14	0.00	0.03	0.01	0.02
6	7.0	2.30	0	0	0.04	0.07	0.04	0.05	0.00	0.03	0.01	0.01
7	6.6	1.97	10	10	0.02	0.05	0.01	0.03	0.00	0.03	0.00	0.02
8	6.6	1.97	-10	-10	-0.04	-0.01	-0.04	-0.02	0.00	0.01	0.01	0.01
9	6.6	1.97	0	0	0.03	0.06	0.03	0.04	ND	ND	ND	ND
10	6.6	1.97	-10	10	0.11	0.13	0.10	0.12	0.00	0.02	0.00	0.01
11	6.6	1.97	10	-10	-0.16	-0.14	-0.17	-0.14	0.00	0.01	0.00	0.01
12	7.4	2.70	-10	-10	-0.04	-0.01	-0.05	-0.10	0.02	0.03	0.01	0.01
13	7.4	2.70	0	0	0.02	0.06	0.01	0.03	ND	ND	ND	ND
14	7.4	2.70	-10	10	0.11	0.14	0.10	0.11	0.00	0.04	0.00	0.02
15	7.4	2.70	10	-10	-0.16	-0.13	-0.17	-0.15	0.00	0.03	0.00	0.01
16	7.4	2.70	10	10	0.01	0.05	0.01	0.03	0.00	0.04	0.01	0.02

TABLE 11. Robustness experiments – pH errors observed – buffers with salt (target pH 7.0).

Experiment #	Offset (%)			Flow Feedback Mode (pH units)			pH/Conductivity Mode (pH units)		
	Salt Stock	Acid Stock	Base Stock	Sensor 1	Sensor 2	Sensor 3	Sensor 1	Sensor 2	Sensor 3
1	-10	-10	-10	-0.02	-0.01	0.00	0.00	0.01	0.02
2	-10	-10	10	0.17	0.18	0.19	0.00	0.01	0.02
3	-10	0	0	0.06	0.06	0.08	0.00	-0.01	0.01
4	-10	10	-10	-0.10	-0.08	-0.08	0.00	0.01	0.02
5	-10	10	10	0.09	0.11	0.12	0.00	0.01	0.02
6	0	-10	-10	-0.07	-0.04	-0.05	0.00	0.02	0.02
7	0	-10	10	0.14	0.16	0.16	0.00	0.02	0.02
8	0	0	0	0.03	0.04	0.06	0.00	0.04	0.02
9	0	0	0	0.00	0.05	0.01	0.00	0.01	0.02
10	0	0	0	0.05	0.04	0.07	0.00	-0.01	0.00
11	0	10	-10	-0.08	-0.05	-0.06	0.00	0.02	0.01
12	0	10	10	0.03	0.07	0.05	0.00	0.03	0.02
13	10	-10	-10	-0.08	-0.06	-0.05	0.00	0.02	0.02
14	10	-10	10	0.11	0.14	0.14	0.00	0.02	0.02
15	10	0	0	0.12	0.11	0.12	0.00	-0.01	0.00
16	10	10	-10	-0.11	-0.08	-0.07	0.00	0.02	0.02
17	10	10	10	0.04	0.05	0.08	0.00	0.01	0.02

in **Figure 6**. For the pH/conductivity feedback runs, the controlling pH average values show little to no error. This is not surprising, since they are controlling the flow of the pumps in their favour. The monitoring sensors, on the other hand, are still bound by a maximum error of 0.04 pH units, which is under the curve of the pH/conductivity feedback shown in **Figure 6**.

3.7 Relative Conductivity Error in the Robustness Study Experiments

The relative conductivity errors calculated for the average in-line values from the last minute of each run compared to the corresponding targets are shown in **Table 12** (without salt) and **Table 13** (with salt). For the runs in flow feedback mode without 10% deviation in the stock deviations, the

TABLE 12. Robustness experiments – conductivity errors observed – buffers without salt.

Experiment #	Target		Offset (%)		Flow Feedback Mode (%)				pH/Conductivity Mode (%)			
	pH (pH units)	Conductivity (mS/cm)	Acid Stock	Base Stock	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 1	Sensor 2	Sensor 3	Sensor 4
1	7.0	2.30	10	10	13.9	13.3	14.0	13.8	0.4	-0.2	0.6	0.5
2	7.0	2.30	10	-10	-13.0	-13.8	-13.3	-13.3	0.4	-0.2	0.4	0.3
3	7.0	2.30	-10	-10	-18.3	-19.0	-18.5	-18.6	0.4	-0.3	0.3	0.0
4	7.0	2.30	0	0	4.3	3.4	4.4	4.3	1.0	0.0	1.0	0.7
5	7.0	2.30	-10	10	8.7	8.0	8.8	8.7	0.5	0.0	0.5	0.4
6	7.0	2.30	0	0	3.9	3.2	3.9	3.7	0.8	-0.2	0.9	0.7
7	6.6	1.97	10	10	10.5	9.8	10.5	10.4	0.6	0.0	0.7	0.6
8	6.6	1.97	-10	-10	-13.8	-14.6	-14.0	-14.1	-1.7	-2.7	-1.8	-2.7
9	6.6	1.97	0	0	0.0	-1.2	-0.2	-0.2	ND	ND	ND	ND
10	6.6	1.97	-10	10	0.9	0.1	0.7	0.6	0.4	-0.5	0.3	0.1
11	6.6	1.97	10	-10	-8.8	-9.5	-9.0	-9.1	0.5	-0.3	0.3	0.1
12	7.4	2.70	-10	-10	-19.0	-19.6	-19.0	-19.0	-0.3	-0.9	-0.2	-0.8
13	7.4	2.70	0	0	1.1	0.4	1.5	0.9	ND	ND	ND	ND
14	7.4	2.70	-10	10	8.7	8.1	9.1	9.0	0.7	-0.1	1.0	0.9
15	7.4	2.70	10	-10	-20.4	-20.9	-20.4	-20.5	0.3	-0.3	0.5	0.2
16	7.4	2.70	10	10	11.2	10.7	11.6	11.4	0.4	0.0	0.7	0.6

TABLE 13. Robustness experiments – conductivity errors observed – buffers with salt (target pH 7.0).

Experiment #	Offset (%)			Flow Feedback Mode (%)			pH/Conductivity Mode (%)		
	Salt Stock	Acid Stock	Base Stock	Sensor 1	Sensor 2	Sensor 3	Sensor 1	Sensor 2	Sensor 3
1	-10	-10	-10	-7.5	-6.6	-6.6	0.01	1.08	0.97
2	-10	-10	10	-6.4	-5.4	-5.5	-0.01	1.05	0.98
3	-10	0	0	-6.7	-5.7	-5.8	0.00	1.07	0.81
4	-10	10	-10	-7.4	-6.5	-6.6	-0.01	1.07	0.98
5	-10	10	10	-6.3	-5.7	-5.8	0.02	0.68	0.75
6	0	-10	-10	-0.6	0.4	0.4	-0.02	1.03	0.96
7	0	-10	10	0.5	1.5	1.4	-0.01	0.96	0.88
8	0	0	0	0.1	1.1	1.1	0.01	1.02	0.98
9	0	0	0	0.4	1.4	1.3	0.02	1.02	0.95
10	0	0	0	0.1	1.1	1.0	0.02	1.08	0.97
11	0	10	-10	-0.1	0.6	0.9	-0.01	0.98	0.95
12	0	10	10	0.7	1.5	1.5	0.01	1.01	0.94
13	10	-10	-10	6.3	7.4	7.3	0.01	0.94	0.89
14	10	-10	10	7.4	8.9	8.4	-0.01	0.97	0.91
15	10	0	0	7.3	8.6	8.5	-0.01	1.06	0.97
16	10	10	-10	6.5	7.5	7.5	0.01	0.97	0.87
17	10	10	10	7.3	8.4	8.3	0.02	0.94	0.87

maximum error found is 4.3% which is under the flow feedback max curve from **Figure 7** when the error in the stock solutions is <2%. The rest of the runs have larger errors. The highest deviations found are 20.4% for buffers without salt and 8.9% for buffers with salt. The higher relative error for buffers without salt might be attributed to the relatively low conductivity values of the buffers without salt, but in general, the magnitude of these errors complies with the right-hand side of **Figure 7**. For the runs in pH/conductivity feedback, the controlling conductivity average values show lower deviations as in the case for the pH. The monitoring sensors, on the other hand, are all below 2.7%, which is under the pH/conductivity feedback curve of **Figure 7**.

4.0 Discussion

The outcome of any successful process development undertaking includes a set of critical process parameters (CPPs) and their corresponding tolerance windows, guaranteeing that the critical quality attributes (CQAs) of the product are met. The allowed CPP tolerance intervals are a non-negotiable starting point for any new process control strategy. By using IC to manage and control buffer formulations, there is more time to focus on the different buffer properties corresponding to those specified in the CPPs. Taking into consideration input variability, the choice of the right control mode will ensure accuracy and robustness.

One purpose of this study was to determine if equivalency and consistency can be confirmed between buffers produced by IC and buffers produced using the current traditional makeup methods. With regards to conductivity and osmolality, equivalence was demonstrated with all the buffers tested falling within the specifications independent of the mode of control used. For the runs using the ratio control with flow feedback mode, only one buffer failed the pH requirement by a relatively small margin. The failing result illustrates that at ±0.05 pH units, it could be possible to fail one run. For the runs using pH/conductivity feedback mode, all buffers met process requirements, as measured by the in-line controlling and monitoring pH meters. Thus, the equivalency was fully demonstrated with the experiments using pH/conductivity feedback. The performance of the two control modes was, in general, similar to established process parameters while maintaining excellent control over the stock solution precision.

The second purpose of this study was to determine the recommended control and release strategy. To rationalize this further, it was necessary to make an error analysis for the two different control modes considering the expected: (1) input variability (recipe and the stock solutions); (2) precision of the pumps; and (3) pH and conductivity meter precision. Important parameters for the application of the error propagation formulas could be obtained from the results of the robustness study. The outcome of this work has shown that the expected variability of the incoming stock solutions is important when deciding on the right control strategy. While pH/conductivity feedback can be used to compensate for incoming variability, flow feedback cannot. With low incoming variability, the two modes of control give comparable performance. When the incoming variability increases, so does the uncertainty of ratio control with flow feedback, more with respect to conductivity than pH. Whereas, the precision of pH/conductivity feedback remains the same and should be the preferred control mode when there is an expected variation. Two examples of where pH/conductivity feedback is a better option are: (1) when there is expected uncertainty in the stock solutions of ≥2% and the expected conductivity variation tolerance is <5%; and (2) when a temperature-sensitive buffer like Tris is used and variations in temperature (as small as a few degrees Celsius) are expected. Where there is good control of the incoming stock solution variability and ratio control, as with the test buffers used in this study, either control mode will work well.

In-line osmolality measurement is not currently available, but osmolality could be validated out by testing a determined number of samples. The produced buffers will be released in-line, meaning that additional samples and testing will not be taken.

5.0 Conclusion

Equivalency and consistency were confirmed between buffers produced by IC and the current traditional makeup methods. Secondly, the results of this study show that the tolerances expected for the different CPPs are important in the choice of control mode. Input variabilities (*e.g.*, expected recipe precision, stock solutions, and temperature) should also be considered. With low incoming variability, the two modes of control give comparable performance. When the incoming variability increases, so does the uncertainty of ratio control with flow feedback mode, more with respect to conductivity than pH, while the precision of pH/conductivity feedback mode remains the same. The choice of control should therefore take into consideration the CPPs, their tolerances, and the input variability of stock solution concentration. In the equivalence tests with controlled and accurate stock solutions, the two methods are similar in performance.

References

[1] Tsai AM, Carredano E, Busson K. Deploying automated buffer production for cGMP use: Points to consider. *BioProcess J*, 2019; 18. https://doi.org/10.12665/J18OA-Tsai

[2] Carredano EN, Nordberg R, Westin S, Busson K, Karlsson TM, Blank TS, Sandegren H, Jagschies G. Simplification of buffer formulation and improvement of buffer control with in-line conditioning (IC). In: *Biopharmaceutical processing: development design and implementation of manufacturing processes*. Elsevier Ltd., Amsterdam, 2018, pp 513–25.

[3] Matthews T, Bean B, Mulherkar P, Wolk B. An integrated approach to buffer dilution and storage. *Pharma Manufacturing*, 2009. https://www.pharmamanufacturing.com/articles/2009/046/

[4] Malone T, Li M. PAT-based in-line buffer dilution. *BioProcess Int*, 2010. https://bioprocessintl.com/downstream-processing/separation-purification/pat-based-in-line-buffer-dilution-186362/

[5] Li M, Kamat V, Yabe H, Miyabayashi T, Jariwala S. Process analytical technology-based in-line buffer dilution in downstream bioprocessing. *PharmTech*, 2010. https://www.pharmtech.com/view/process-analytical-technology-based-line-buffer-dilution-downstream-bioprocessing

[6] Bjorkesten L, Carredano E, Malmquist G, Rodrigo G, Stafstrom N, inventors. Cytiva Sweden AB, assignee. *Preparation of liquid mixtures*. US patent US9446329B2. 2016 Sep 20. https://patents.google.com/patent/US20110039712A1/en

About the Authors

Karolina Busson, MSc, Application Specialist,
Customized Bioprocess Solutions (CBS), Cytiva, Uppsala, Sweden

Robbie Kamperveen, MSc, Staff Engineer, Janssen Supply Chain group,
Johnson & Johnson, Leiden, Netherlands

Enrique Carredano, PhD*, Application Specialist,
Customized Bioprocess Solutions (CBS), Cytiva, Uppsala, Sweden

Corresponding Author

Address: Björkgatan 30, 751 84 Uppsala, Sweden
Email: enrique.carredano@cytiva.com