Frequently asked questions

Inline Conditioning system

Buffer preparation can be both time-and space-consuming and can easily become a challenge in biomanufacturing. In-line preparation of buffers from highly concentrated, single-component stock solutions saves both time and storage space. Compared with preparing one concentrate per buffer formulation, many different buffers can be prepared from the same stock solutions using GE Healthcare's Inline Conditioning system.

With the Inline Conditioning system, buffers can be formulated just-in-time in an automated manner. The system can be used off-line as a stand-alone central buffer preparation station to feed different processes in the facility or in-line connected to a chromatography column using a built-in chromatography functionality. Compared with preparation of buffers as a separate operation, integration of buffer preparation with the chromatography step both saves time and reduces facility footprint. Feedback control of the preparation process ensures accurate formulation of the final buffer, and the automation enables high consistency between batches. Compared with traditional buffer preparation, in-line conditioning can help increase efficiency in buffer production for biomanufacturing applications.

Technical capabilities

Q. During feedback control, extra buffer consumption is unavoidable. What is the additional consumption during feedback control?

A. The extra buffer consumption will vary. With an approximate time to reach a stable pH of about 1 min and at a flow rate of 600 L/h, the additional buffer consumption will be about 10 L that is diverted to waste.

Q. Based on buffer volumes, composition of stock solutions, and time to reach pH and/or conductivity targets, can I be confident that the Inline Conditioning system will produce the specified buffer within a reasonable time, without the need to waste thousands of liters of buffer before a stable pH/conductivity is achieved?

A. Yes. When the buffer recipes are known, the system can be programmed to produce the buffer using either the ***Recipe and flow*** or ***Flow*** feedback control mode at an initial stage of, for example, 0.5 min. Thereafter, the ***pH*** feedback control can be turned on to finetune the buffer formulation process using slow, so-called, PID parameters. When the buffer formulation is close to target, such a control strategy will be sufficient to reach the target within 1 min. There are users that prefer using ***Flow*** feedback as a general control strategy. However, the ***pH*** (and ***Conductivity*** if applicable) feedback will reduce the error in the final buffer formulation by reducing the dependence of the accuracy in composition of the stock solutions, the ambient temperature or water temperature.

Q. Can the Inline Conditioning system be set up to track run time of each pump?

A. Like in any chromatography system UNICORN™ doesn't provide direct reading of the run time of each pump. However, each pump has its own flow meter and readings are recorded during the run so that run time tracking data for each pump can later be retrieved from the flow curve in the result file (and logbook). The UNICORN system control software can handle up to 46 flow curves simultaneously.

Q. Is it possible to use the Inline Conditioning system for sample conditioning?

A. Yes. In such a case, the feed from the sample pump will be treated in the same way as a buffer component. The sample will be diluted and adjusted with buffer from one or more of the remaining inlets. This operation will be performed in ***Flow*** control mode, otherwise additional instrumentation for sample monitoring and control will be required.

Q. Considering scalability, are the same algorithms used for the Inline Conditioning system as in ÄKTA™ avant?

A. The systems use different algorithms, but they are scalable in the sense that buffer recipes in ***BufferPro*** can be used in ***Flow*** feedback control with the Inline Conditioning system to produce the same formulation. In addition, the Inline Conditioning system can derive buffer formulations using ***pH*** feedback control and the percentages of each single component solution can be mixed in the ÄKTA avant using a quaternary valve to produce the same buffer in laboratory scale.

Q. Are the Inline Conditioning systems used mainly in chromatography, for buffer preparation, or in filtration applications?

A. The systems are designed for downstream chromatography and filtration applications. Approximately 50% of the systems are used as stand-alone central buffer preparation stations to feed different processes in the facility and the rest are in-line connected to a chromatography column (built-in chromatography functionality).

Q. Can the Inline Conditioning system generate a pH gradient?

A. Yes. A pH gradient can easily be generated where the pH increases linearly between two values. The system will vary the percentage of acid and base based on a measured pH value.

Q. Can the Inline Conditioning systems be used alone to operate a chromatography column or is a separate ÄKTA system or pump required?

A. Yes, the system can be used to operate a chromatography column (built-in chromatography functionality). In the cases where the system is used as a central buffer prep station, the column connection is not needed.

Q. If the Inline Conditioning systems can be used on their own, what is the difference from using an ÄKTA system? Are the Inline Conditioning systems more like pumps not providing, for example, process data?

A. The Inline Conditioning systems are chromatography systems that provide process data like any other chromatography system. The difference between an ÄKTAprocess™ system and an Inline Conditioning system is the additional buffer preparation capabilities of the Inline Conditioning system. With an ÄKTA system, you can run gradients but buffers need to be prepared in a separate step prior to the run. With the Inline Conditioning system, these two steps can be integrated, starting from the stock solutions.

Q. How much can an Inline Conditioning system evolve with the varying needs of a multiproduct facility?

A. If the buffer formulation needs would change at the facility, an analysis of the stock concentration required for the new formulations should be conducted. Inline Conditioning is a flexible system with several inlets and pumps of different sizes, so that for similar processes and column sizes, it should be possible to produce the new formulations. When changing buffer formulations, the trade-off is buffer formulation flow rate versus single component concentration.

Q. How much help do I need from GE Healthcare when changing the buffer system?

A. Changing buffer system can be conducted by the help of Buffer organizer. This is a spreadsheet tool to design stock solution concentration setup based on actual system pump configuration. Knowing the molar buffer composition, this is a rather easy exercise.

Reliability and robustness

Q. How can I be sure that the wrong buffer will not be directed to the column?

A. On top of the ***pH*** and ***Conductivity*** feedback control that, when activated, will work to keep the pH and conductivity tight around the target, there will be two layers of security to make sure that the wrong buffer will not be directed to the column:

A pH and conductivity interval can be defined so that if the buffer is outside this interval, the system can be programmed, for example, to bypass the column or direct the buffer to waste. When within range again, the buffer can be redirected to the column. If, for example, the buffer is out of range because one of more stock solutions are empty, the pumps will stop and the operator will be notified. When the empty bag is exchanged for new stock solution, the operator presses ***Continue*** to get the buffer formulation process to restart. When within range, the buffer is redirected to the column.

Alarm functions. On top of these precautions, it is still possible to set up alarms for any monitor including those used for the controlling. An alarm will put the system into paused when triggered.

Q. What control functionalities can be used with the Inline Conditioning system?

A. Three modes of feedback control can be used with the system:

1. In ***Recipe and flow*** feedback, a known buffer formulation is entered in the UNICORN software. The software adjusts the flow rates of the specified stock solutions to achieve desired formulation. This control mode is useful when the temperature is constant and the stock solutions are accurate. The pump percentages can be changed in steps or linearly to obtain a gradient. Flow feedback ensures that the correct flows from the concentrated stock solutions are combined. In-line conductivity and pH measurements can be used for monitoring of the buffer properties and for release.
2. In ***pH and conductivity*** feedback, the user enters the target pH and conductivity and the software uses feedback from flow, conductivity, and pH sensors to adjust flow rates of the stock solutions to achieve desired conductivity and pH. In this control mode, both the temperature and the stock solution concentration can vary without affecting accuracy of final buffer formulation. If the buffer contains salt, however, the operator needs to specify concentrations of the final buffer as well as the acid and base stock solutions.
3. In ***pH and flow*** feedback, the user enters target pH and the software adjusts the flow rates of the acid and base stock solutions to achieve desired pH in the final formulation. In this control mode, the pH of the buffer is compared to a target value to determine whether the base or the acid percentage should increase or decrease. Flow feedback ensures that the buffer concentration is kept constant and, in the case where the buffer contains salt, the salt concentration is, as desired, either constant or in a gradient. The percentage of water added is regulated to keep a constant overall flow rate. Using this control mode, the operator needs to specify concentrations of final buffer as well as acid and base stock solutions.

   All control modes include flow feedback to maintain the total flow rate (approximated as the sum of all flow rates) constant by adjusting the flow of water. Whenever using pH feedback, it is possible to choose between three types of buffer mixing: weak acid/weak base, strong acid/weak base, or weak acid/strong base.

Q. Are the used probes, especially the pH probes, reliable or what precautions can I take to ensure they are? Are there specific maintenance or calibration plans for the probes?

A. Conductivity probes are commonly trusted, and conductivity feedback control of, for example, buffer gradients for ÄKTAprocess is widely used. pH probes are widely used in manual quality control and adjustment of buffer formulations. Due to their construction, pH probes are sensitive to bias, when changing from a solution containing salt to a solution without salt (salt memory effect). It usually takes some time before the equilibrium is established and the pH measurement is again free of bias.

With manual adjustments, pH can be allowed to stabilize before reading. In automated buffer preparation using the Inline Conditioning system, the following precautions can be taken:

1. The system features pH probes both upstream and downstream of the inlet for salt addition. Select the downstream option for buffers containing salt and the upstream option for buffers without salt to avoid the salt memory effect.
2. In addition to the controlling pH probe, the system features at least one monitoring pH probe to ensure reliable pH control even in the case one pH probe should be affected by bias.
3. An alarm can be set to control the flow rates of acid and base.
4. The pH probes should be regularly calibrated, for example, before each campaign and replaced, at least, at each service occasion.
5. If possible, the pH electrodes should be kept inside the potassium chloride caps when not in use. Incubating the electrodes in potassium chloride for a few minutes will remove bias due to salt. This procedure should be done before calibration to avoid later pH drifts.

Q. What are the flow rate ranges for different piping sizes?

A. Table 1 lists flow rate ranges for different Inline Conditioning systems.

Table 1. Flow rate ranges for Inline Conditioning systems from GE Healthcare

BioProcess™ Inline Conditioning system	Flow rate range (L/h)
10 mm PP, (½ inch)	120–600
16.2 mm PP, (¾ inch)	300–1000
20.4 mm PP (1 inch)	500–2000
32.6 mm PP (1 ½ inch)	1400–5000
40 mm PP (2 inch)	2700–10 000

Q. Is there a graph showing general performance of Inline Conditioning systems (not describing a specific setting)?

A. Performance is multidimensional, which would be difficult to present in a graph. However, this is captured in the buffer organizer (an Excel® based tool that is delivered along with the system)

Q. Conductivity is temperature dependent. Can it be controlled?

A. The conductivity of a solution is temperature dependent. When selecting conductivity control, the flow rates will be adjusted to meet the conductivity target value. This is preformed based on the readings of the controlling sensor by adjusting the flowrate of the components. In conclusion, at different temperatures the buffer composition (recipe) will be adjusted to reach the conductivity target.

Q. What is the precision of pH and conductivity that can be achieved, and what is the stability of pH and conductivity control over time?

A. When using pH feedback control of a buffer with good buffer capacity, precision is ± 0.1 pH units for the controlling pH electrode. For a monitoring electrode or for offline pH measurements, the variance of the additional electrode needs to be added, which results in ± 0.15 pH units. The specifications for the conductivity monitors when using conductivity feedback control are the same as for the ÄKTAprocess system and are listed in Table 2. These tolerances are also valid for stability over time.

Table 2. Precision of conductivity monitors

Conductivity range	Precision
0.1–100	± 2% or 0.5 mS/cm
100–300	± 4% or 0.25 mS/cm

Q. Does the flow have any influence on how fast the conductivity and pH reach their target value?

A. In general, the higher the flow rates the faster the conductivity and pH reach their target values. In addition, if the pH target has already been reached at one flow rate, a change to another flow rate will have no impact on the pH.

Q. What is the impact of variation in flow rate on the stability of the pH and conductivity?

A. Essentially, there is no impact of variation in the total flow rate on the stability of the pH and conductivity.

Q. When the pH and conductivity are stable, the system will redirect the buffer from waste to the chromatography column (or the single-use buffer bag), which will prompt a change in the back-pressure. What is the influence of the change in back pressure on the stability of the pH and conductivity control?

A. Essentially, no influence. When changing the outlet to a manual valve that was half closed, no impact of the pressure increase on the pH and conductivity control was observed (Fig 1).

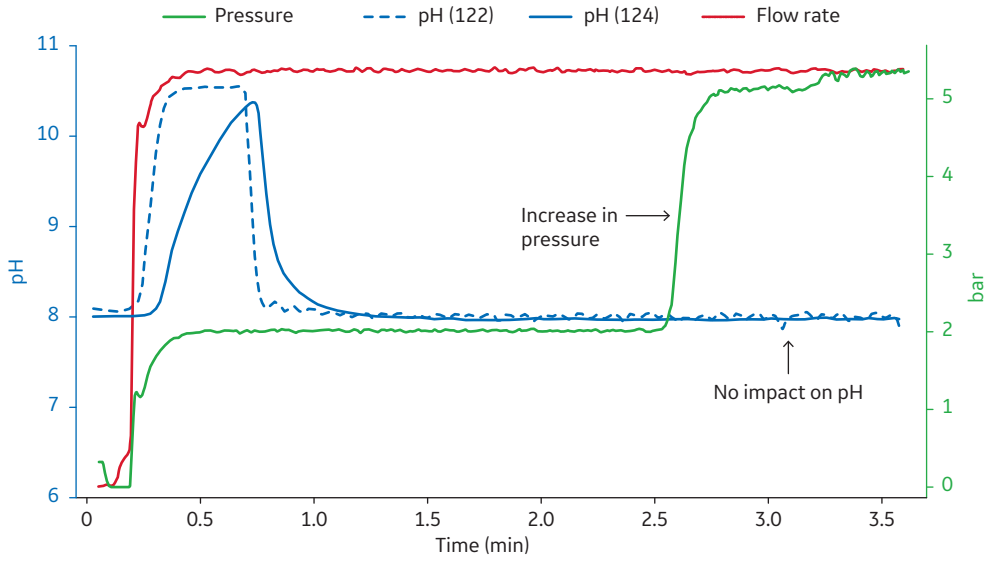
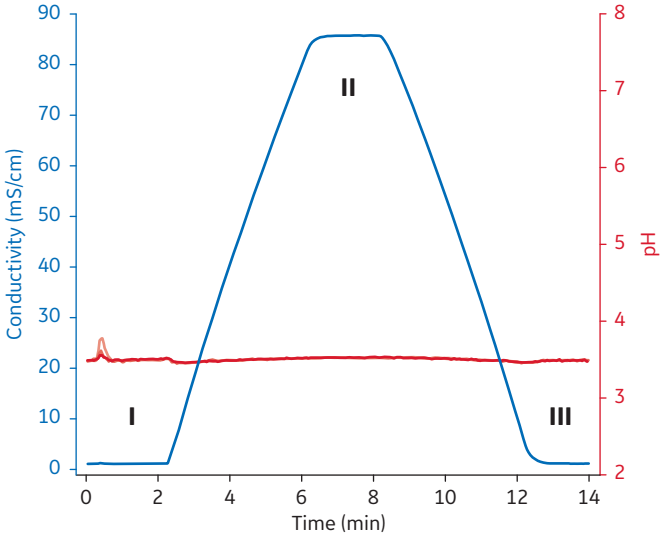


Fig 1. Pressure and pH readings during preparation of 16.0 mM Tris, 13 mM NaCl, pH 8.0 at 600 L/h.

Q. Is there any data on reproducibility of results?

A. An example of reproducibility and robustness when using the Inline Conditioning system is shown in Figure 2.



Whole int.	(min, average, max)
Run 1	(3.45, 3.50, 3.53)
Run 2	(3.45, 3.49, 3.53)
Run 3	(3.44, 3.49, 3.53)
Section I	(min, average, max)
Run 1	(3.47, 3.49, 3.51)
Run 2	(3.47, 3.49, 3.51)
Run 3	(3.49, 3.49, 3.51)
Section II	(min, average, max)
Run 1	(3.51, 3.52, 3.53)
Run 2	(3.51, 3.52, 3.53)
Run 3	(3.52, 3.52, 3.53)
Section III	(min, average, max)
Run 1	(3.46, 3.48, 3.50)
Run 2	(3.46, 3.48, 3.50)
Run 3	(3.47, 3.48, 3.50)

Fig 2. Overlay of three chromatograms from preparation of 20 mM citrate buffer, pH 3.5 with an increasing gradient of salt: 0 to 1 M NaCl at 400 L/h (flow feedback control). For this specific run, the accuracy was 0.1 pH units and the robustness 0.02 pH units.

Will my buffers work? Application experience

Q. Is there data showing correlation accuracy between in-line and off-line buffer preparation?

A. Based on an internal study done using different buffer families at different pH values, the correlation accuracy is 1:1 (Fig 3).

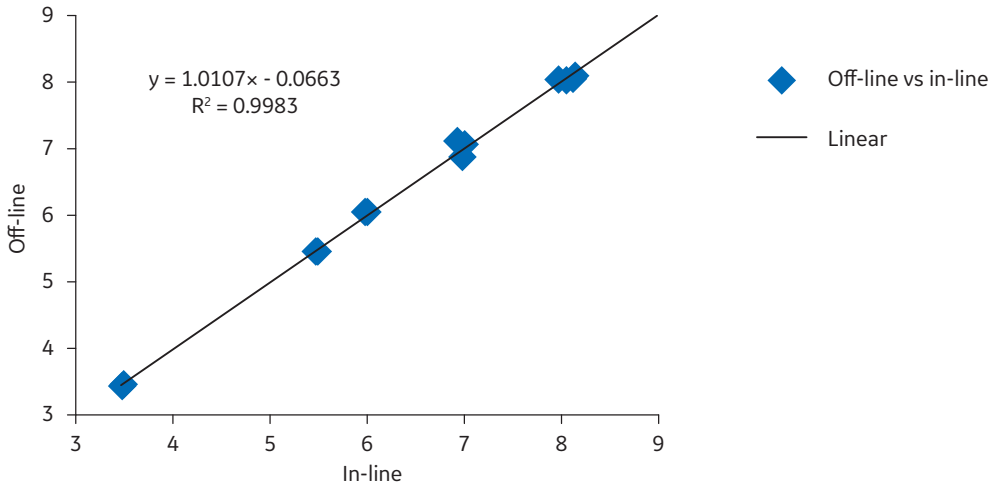


Fig 3. Correlation accuracy of in-line and off-line buffer mixing.

Q. What is the experience in using HyClone™ process liquids for buffer preparations?

A. HyClone single-component stock solutions have successfully been used in preparation of buffers for a mAb process (1).

Q. Can the Inline Conditioning system be used in all chromatography applications, for example, immobilized metal affinity chromatography (IMAC)?

A. To know if in-line conditioning is suitable for a specific application, one can start by looking at the list of buffers already tested (1). Also, buffers available in the ***BufferPro*** tool in the UNICORN software are good candidates for in-line conditioning. In general, buffers containing components of good solubility (e.g., imidazole) can be prepared by in-line conditioning.

Q. Has ammonium sulfate been used with the Inline Conditioning system.

A. Yes. Figure 4 shows an example of a preparation of a buffer that contains ammonium sulfate, formulated at two different flow rates.

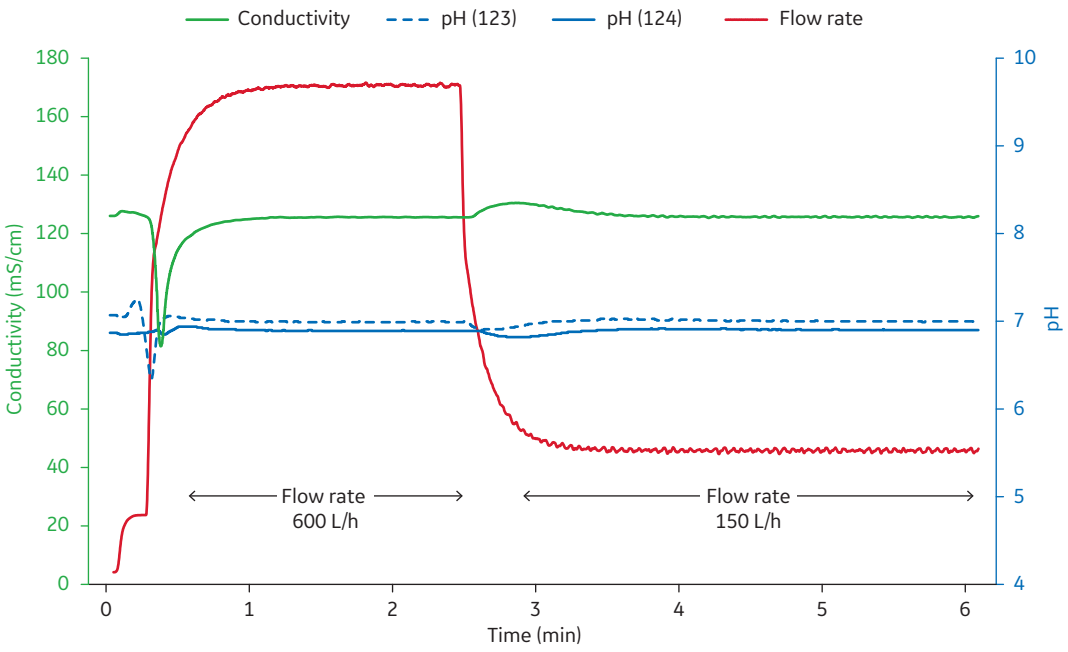


Fig 4. Preparation of 10 mM Tris buffer, 1 M $(NH_4)_2SO_4$, pH 7.0 from the stock solutions 0.1 M Tris-HCl, 0.01 M Tris base, and 3 M $(NH_4)_2SO_4$ at 600 and 150 L/h using pH-Flow feedback. Preparation time was 1.0 min for both flow rates.

Q. How are components that are highly viscous when concentrated (e.g., urea or sucrose) handled? Does the system consider the viscosity or solubility of the components when preparing the desired buffer formulation? Are mixers used in the Inline Conditioning systems?

A. For dilution applications, it is important to know the solubility and viscosity limits of the liquids involved. There might also be other limitations to consider, such as environmental, health, and safety limitations. The system itself does not keep track on such limitations. The system is provided with static mixers to facilitate the mixing of buffer components. However, for most of the aqueous buffers, the mixers are not needed. The static mixers can be replaced by spool pieces. Urea of concentrations up to 7 M have been tested with successful results in the system. With sucrose, stock solutions of a solubility higher than 1.25 M can be difficult to use due to viscosity.

CONTENT PAGE

Maintenance

Q. How is the system sanitized?

A. The Inline Conditioning system can be sanitized in the same automated manner as the ÄKTAprocess system, using the cleaning- in-place (CIP) block of valves.

Reference

1. Application note: Automated in-line buffer preparation from ready-made stock solutions in a mAb process step. GE Healthcare, 29260552, Edition AA, (2017).

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