cytiva

Inline conditioning FAQs



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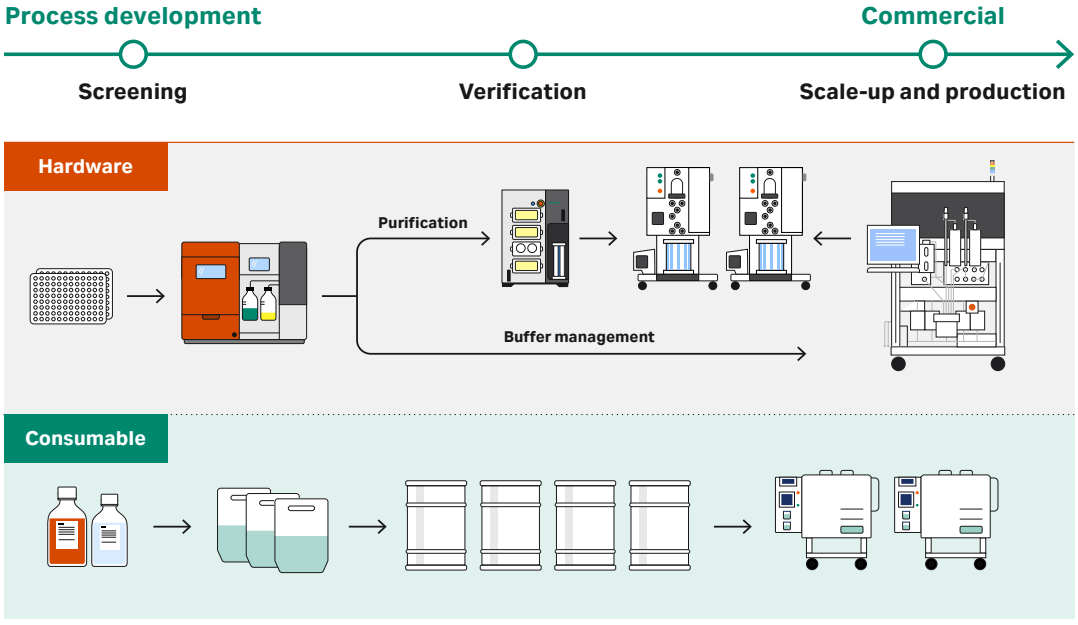
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Buffer preparation can be both time- and space-consuming and can easily become a challenge in biomanufacturing. Inline preparation of buffers from highly concentrated, single-component stock solutions saves both time and storage space (1). Compared with preparing one concentrate per buffer formulation, many different buffers can be prepared from the same stock solutions using Cytiva's Bioprocess™ inline conditioning system (2).

With the inline conditioning system, buffers can be formulated just-in-time in an automated manner. The system can be used offline as a stand-alone central buffer preparation station to feed different processes in the facility, or inline 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, inline 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 **pH control**, **Cond control no salt** instruction in **Init** mode, and/or **Cond control salt** instruction in **Conc** mode at an initial stage of, for example, about 1 min. Thereafter, the ***pH and/or conductivity*** feedback control can be turned on to fine-tune the buffer formulation process using slow, so-called, PID parameters. Activate the Buffer verification function here and it will release the buffer when it is within the set limits. When the buffer formulation is close to target, such a control strategy will be sufficient to reach the target within 1.5 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™ software 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 26 flow related curves and up to 7 totalizer* curves simultaneously.

*In UNICORN software, you can use a totalizer feature to keep track of the running total of buffer being made and the volume from each pump. This is stored in a totalizer curve in the result file; up to 7 can be saved.

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

A. Yes. In the case of using the feed pump for the product feed, it can be diluted and adjusted using one or more of the remaining pumps. This operation will be performed in ***Flow*** control mode only (pH and conductivity can be monitored but not controlled). In the case of using e.g., the additive pump for applying the feed, it can be treated as any other buffer component and pH and conductivity could be controlled.

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

A. The systems use different algorithms, but they are scalable. Buffer recipes are created on the ÄKTA avant system using the ***BufferPro function;*** the percentages of each single component solution can be mixed using a quaternary valve to produce the buffer in laboratory scale. The recipe can then be transferred to the inline conditioning system; the ***Flow*** feedback control mode can be used to produce the same formulation. In addition, the inline conditioning system can derive buffer formulations using ***pH and conductivity*** feedback control.

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 30% of our systems are used as stand-alone central buffer preparation stations to feed different processes in the facility and the rest are connected inline 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 changes 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, there are two options. The system can be assembled with both buffer and chromatography functionality on the same skid. If you prefer a central buffer prep station, the column connection and associated post-column sensors are 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 ÄKTA process™ 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.

The inline conditioning system can also be set up as connected to ÄKTA process or ÄKTA ready™ systems. The inline conditioning system then automatically prepares and delivers the requested buffers to the connected systems directly. During the run, only the pumps on the inline conditioning system are running, pushing the buffer through the pump on the ÄKTA process system or bypassing the peristaltic pump in the ÄKTA ready system (3).

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

A. If the buffer formulation needs at your facility change, an analysis of the stock concentration required for the new formulations should be conducted. The inline conditioning system offers flexibility with several inlets and pumps of different sizes, so 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 Cytiva when changing the buffer system?

A. Changing buffer system can be conducted with the help of Buffer organizer, 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.

Q. Why does the inline conditioning system have two different types of pH probes?

A. The BioProcess IC system is equipped with four pH probes: a dual pH module for controlling and another dual pH module for monitoring. Each module has the option of two different pH probes: CPS11E and CPS61E. The dual flow paths enable the system to effectively create accurate buffers where salt is present and where it's not, as certain pH sensors can suffer from salt memory when exposed to high salt conditions. This temporarily impacts the accuracy of the pH readings as the excess salt can take hours to be flushed out, resulting in inaccurate readings until the pH sensor is fully flushed. The dual pH module removes the risk of salt memory causing inaccurate buffer production (4).

For measuring buffers without salt, use the CPS11E pH probe. When salt is present, use the CPS61E pH probe, which requires some conductivity to measure correctly. The CPS61E pH probe is more resistant to sodium hydroxide and can be left in the flow path during column cleaning-in-place (CIP). The CPS11E pH probe should be removed from the flow path during column CIP.

If the system includes chromatography functionality, a fifth pH probe is also installed in the post-column position.

Q. What is the titration pump used for?

A. In combination with the column option, it is possible to add a titration pump, placed after the column connection. This pump is designed to enable pH adjustment of the product eluted from the column, using flow or pH feedback independently of the other system pumps.

Reliability and robustness

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

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

- With the buffer verification function, pH and conductivity limits can be defined so that if the buffer is outside this interval, the system is programmed, to bypass the column and/or direct the buffer to waste. When within range again, the buffer will be redirected to the column. If the system does not recover within a defined time, for example, the buffer is out of range because one or 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 again, 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 status 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 ***Flow*** feedback, a known buffer formulation is entered into 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. Inline conductivity and pH measurements can be used for monitoring the buffer properties and for release.
2. In ***pH and conductivity*** feedback, you enter 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, you enter 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, you will need 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 generally robust and reliable, and are widely used for conductivity feedback control of buffer gradients in e.g., ÄKTA process systems. For manual quality control and adjustment of buffer formulations, pH measurements are used to ensure the buffers meet the required acceptance criteria. 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 dual pH probe modules, one for control and one for monitoring. In both modules, you use one probe for salt containing buffers and one for low-salt buffers to avoid the salt memory effect.
2. An alarm can be set to control the flow rates of acid and base.
3. The pH probes should be regularly calibrated; for example, before each campaign and replaced as part of annual service maintenance visits, or more frequently based on your usage and protocols.
4. 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. Contact us for information on other possible system sizes.

Table 1. Flow rate ranges for inline conditioning systems from Cytiva

BioProcess inline conditioning system	Flow rate range (L/h)
10 mm PP, (½ in.)	60 to 600
20.4 mm PP (1 in.)	500 to 2000
32.6 mm PP (1½ in.)	1000 to 5000

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 module includes a temperature sensor which can provide feedback control to ensure the right conductivity target value is achieved. When selecting conductivity control, the flow rates will be adjusted to meet the conductivity target value. This is performed based on the readings of the controlling sensor by adjusting the flow rate of the components. 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 ÄKTA process system and can be found in the system data files. These tolerances are also valid for stability over time.

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 with a manual valve that was half closed, no impact of the pressure increase on the pH and conductivity control was observed (Fig 1).

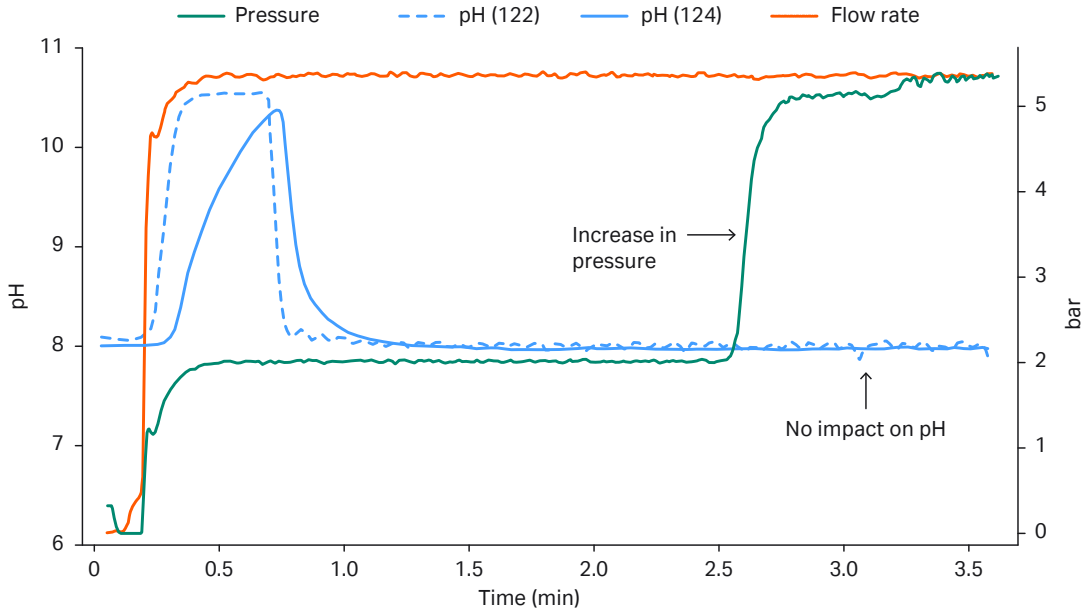
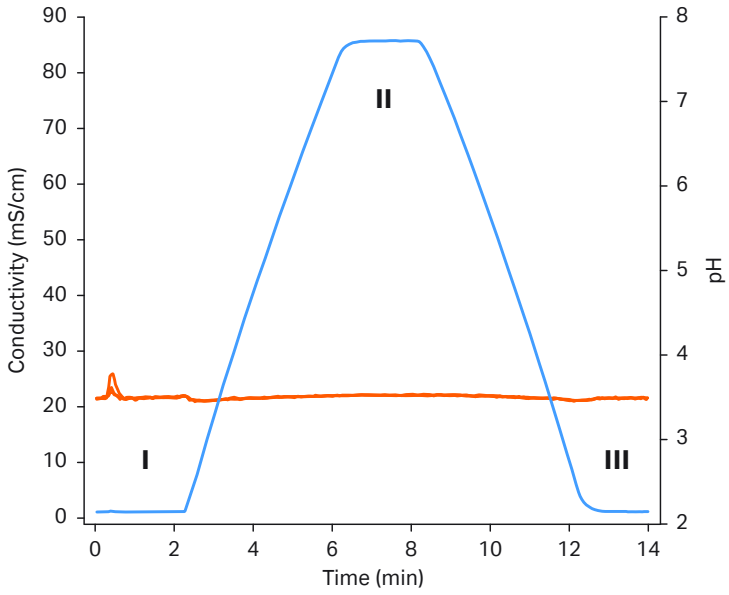


Fig 1. Pressure and pH readings during preparation of 16.0 mM Tris, 13 mM NaCl, pH 8.0 at 600 L/h.

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).

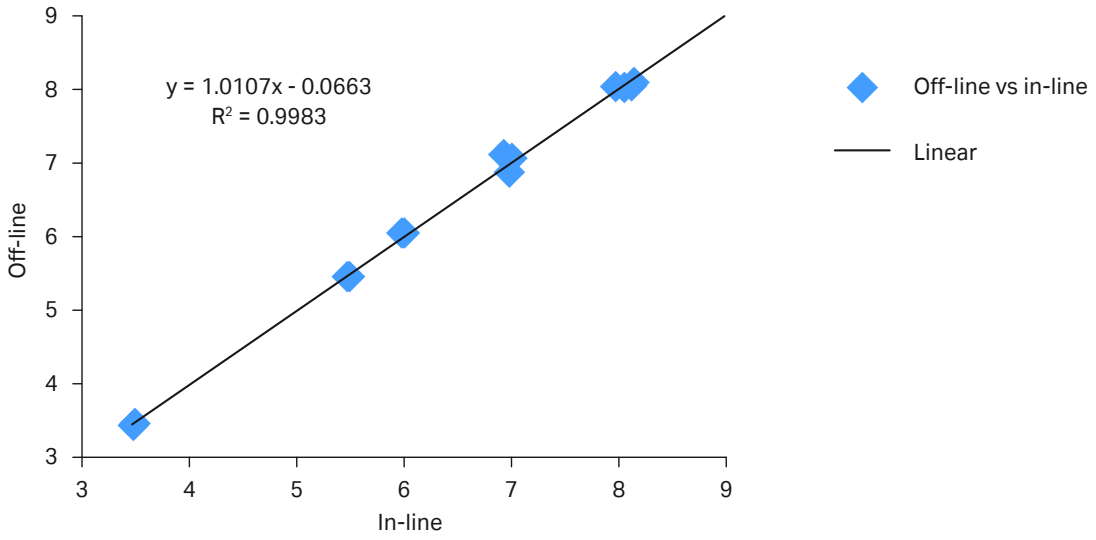


Fig 3. Correlation accuracy of in-line and off-line buffer mixing.

Q. What about 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) using pH and conductivity control.

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

A. To know if inline conditioning is suitable for a specific application, you 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 inline conditioning. In general, buffers containing components of good solubility (e.g., imidazole) can be prepared by inline 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.

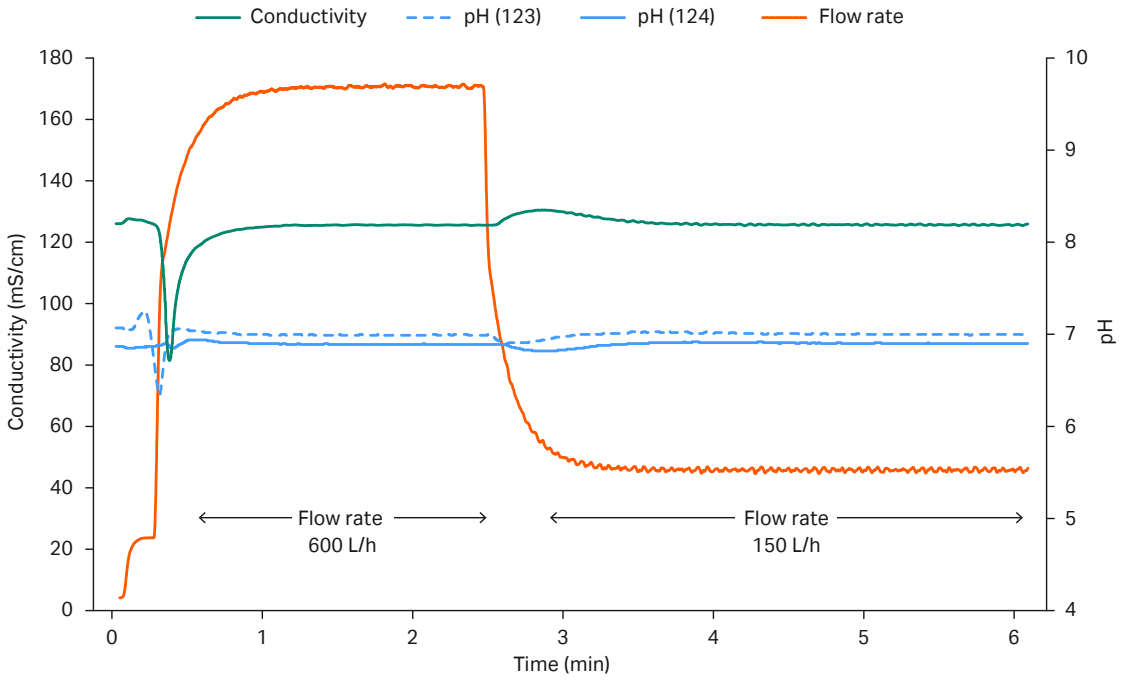


Fig 4. Preparation of 10 mM Tris buffer, 1 M (NH4)2SO4, pH 7.0 from the stock solutions 0.1 M Tris-HCl, 0.01 M Tris base, and 3 M (NH4)2SO4 at 600 and 150 L/h using pH-flow feedback. Preparation time was 1.0 min for both flow rates.

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 of 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 concentration higher than 1.25 M can be difficult to use due to viscosity.

Maintenance

Q. How is the system sanitized?

A. The inline conditioning system can be sanitized in the same automated manner as the ÄKTA process system, using the built-in CIP valve block.

Q. Can I keep the pH probes inline during column CIP?

A. The CPS61E pH probe is more resistant to sodium hydroxide and can be left in the flow path during column CIP. The CPS11E pH probe should be removed from the flow path during column CIP. However, when doing system CIP, both pH probes should be removed from the flow path.

Reference

1. Application note: Automated in-line buffer preparation from ready-made stock solutions in a mAb process step. Cytiva, CY13953, Edition AA, (2017).
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3. Article: Connected inline conditioning for better process efficiency. Cytiva, CY31006 (2022).
4. Busson, K.; Sheehy, J.; Anderson, L.; Westman, T.; Carredano, E. Maximizing performance of pH measurements for inline conditioning buffer preparation for chromatography. *BioProcessing Journal* **2026**, 25. https://bioprocessingjournal.com/maximizing-performance-of-ph-measurements-for-inline-conditioning-buffer-preparation-for-chromatography/

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