Inline conditioning for process chromatography systems

PROCESS CHROMATOGRAPHY

Current trends in manufacturing biopharmaceuticals have put demands on securing increased buffer supplies delivered at precise times and at correct concentration and flow rates. In meeting these demands, state-of-the-art technologies such as inline conditioning (IC) have gained increased interest, not only because they reduce the need for large storage tanks, but also because IC can deliver correct solutions for a specific process need at a lowered total cost of ownership.

Beginning with the first gradient chromatography systems, IC has been a solution for researchers and process engineers having the need for mixing two different solutions before loading onto a chromatography column. Cytiva has delivered a wide range of IC systems, providing a reproducible, fully automated IC process.

Advantages of inline conditioning include:

- Timely buffer delivery
- Automated IC process increases reproducibility
- Allows for use of smaller tanks or disposable bags
- Reduces or eliminates the need for CIP and WFI demands
- Provides better process economics

Another advantage of inline conditioning is that by including the buffer preparation within the chromatography run, all relevant data for buffer characteristics and for the run itself are recorded by the control software at the same time, resulting in one batch record for both operations. The intelligent control strategy of the inline conditioning/chromatography system is designed to prepare buffers to be automatically adjusted, thereby improving the quality of the process.

Fig 1. A standard, gradient ÄKTAprocess™ system has the ability to perform the first level of inline conditioning.

System requirements

Although any gradient system can function as an IC system, a quality system design (QbD) is crucial for best mixing effects and reproducible results. Cytiva has taken > 25 years of experience in the chromatography field and our engineering experience of having delivered over 2000 chromatography systems, and combined these into a quality system for IC.

The IC system is available in hundreds of configurations and the critical process parameters can be controlled by conductivity, pH, or flow, or by a combination of these three. Automation is achieved by using our proprietary Programmable Logic Controller (PLC) within the UNICORN™ control software, independent of system configuration complexity (see UNICORN data file 18-1156-35).

The key components of an inline conditioning design include (see Fig 2):

- At least 2 pumps selected according to dilution requirements (typically a larger pump for WFI and a smaller pump for the buffer concentrates)
- Each pump has a dedicated flow meter to ensure a fast, well-controlled response at the start of a new buffer mix
- For the most common buffer systems a mixer is usually not required. A mixer can be added when needed, with type and design dependent on the properties of the fluids being mixed
- A conductivity monitor to verify the quality of the mixing (any other appropriate inline monitor can be used)

System modifications to improve system and operator safety include:

- Pressure regulating valves at pump outlets to compensate for inlet pressure variation
- The column is bypassed during initial ramp-up to the chosen set-point
- A pressure regulator is set on the column bypass to equalize column inline and bypass flowpath pressures

Applications

Buffer preparation with inline conditioning

Understanding the dependence of the buffer capacity on the buffer composition is important since the use of buffers with insufficient buffer capacity during purification can lead to low process robustness and poor yields. The buffering capacity of a particular system is sensitive to a range of factors with the so-called pKa values of the buffering substance being among the most important. The pKa value corresponds to the pH value, where half of the buffer substance is protonated and as a rule of thumb the pH will be stable in a symmetric interval around this value. However the width of the interval with good buffer capacity is dependent on the buffer concentration — the higher the buffer concentration the wider the interval. The center of the interval, the so-called pKa´ value is almost always shifted from the tabulated pKa value, which is an interpolation to the ideal case of infinite dilution.

Figure 3 is a schematic where (for the sake of simplicity) only the ideal pKa values are shown for the buffer substance, illustrating the areas of good buffer capacity. The phosphate system shown is a triprotic buffer system with three pKa values, but the arguments above are still valid. Cytiva has proprietary algorithms to calculate the correct compositions to generate buffers with good buffer capacity.

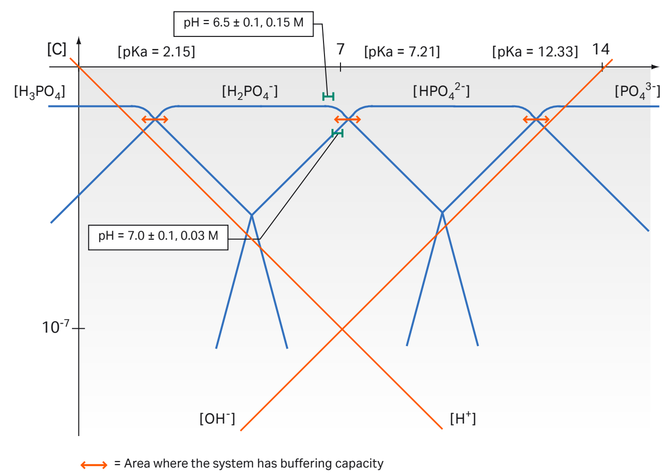

Fig 3. pH diagram of the phosphate buffer system illustrating the concept of areas of good buffer capacity.

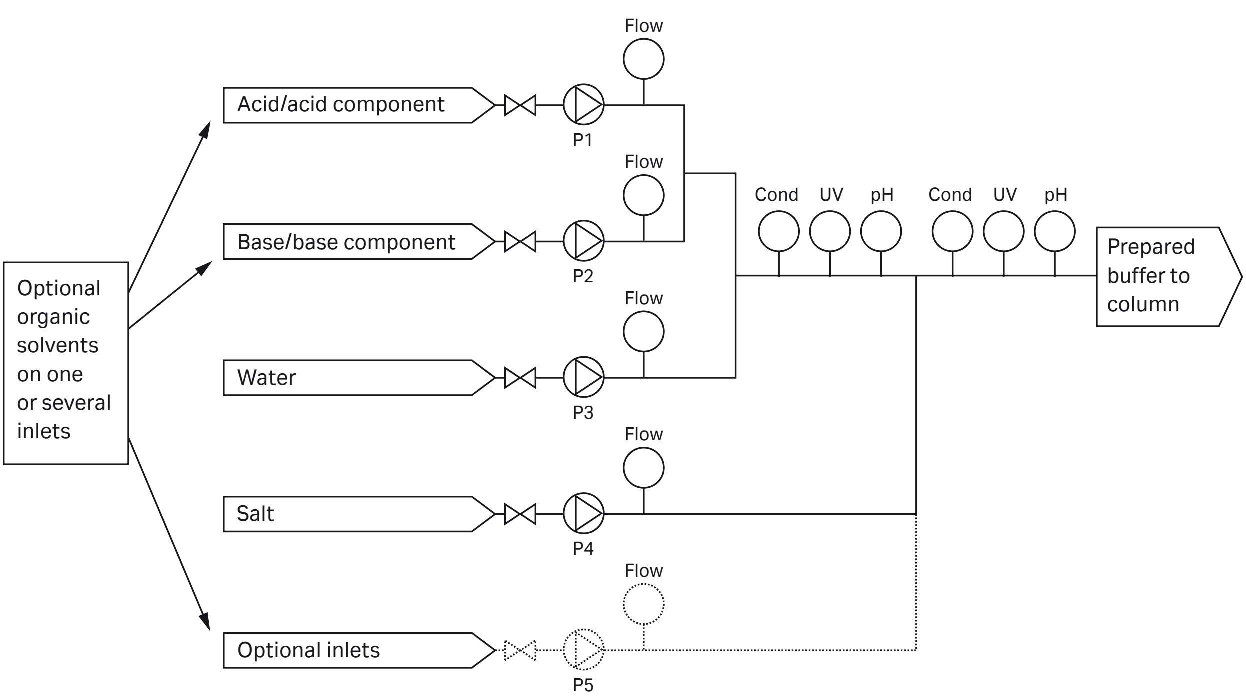

Fig 2. A flowscheme for a typical system designed for inline conditioning.

Inline conditioning

The system can be used for automatic inline conditioning from concentrated stock solutions. This was demonstrated by using the system to prepare a range of the most commonly used buffer systems in MAb protein-based biopharmaceutical production. The inline conditioning system was run at a flow of 600 L/h. In many cases a lower flow (from about 200 to about 550 L/h) has been tested as well with very similar results.

In Figure 4 an output plot from a run (mixing of 30 mM sodium phosphate buffer, pH 6.5 from 0.4 M stock solutions) in UNICORN is shown as an example.

In almost all cases, steady state was achieved in less than one minute.

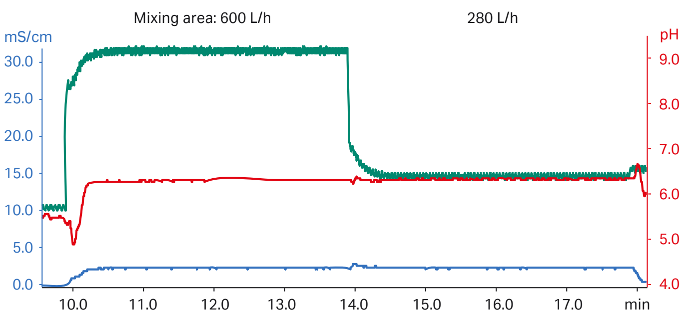

Fig 4. Example of an inline conditioning run in UNICORN. The green curve is the flow rate, with set values at 600 L/h and 280 L/h, respectively as indicated by the text above. The blue curve is conductivity and the red curve pH.

The inline conditioning system gave reliable, accurate, and robust pH results independent of the flow rate and was also capable of mixing buffers at large scale as well as or better than manually at lab scale. The results show an agreement with the *a priori* set target pH values with an r. m. s. d. of less than 0.05 pH units or within 0.1 pH units as measured by the pH measurements offline in samples taken directly from the system. This illustrates the robustness and accuracy of inline conditioning for pH control as the system is free from process bias as compared to other systems relying on the inline pH values for feedback control.

Using only one or two buffer systems in manufacturing can lead to significant savings in raw material and equipment costs, as well as a reduced need for storage space. However, a balance has to be struck between such savings and the potential impact on process robustness, yield vs contamination profile, etc. This approach will require a well-documented design space and PAT tools to enable adjustments of the process since the feed composition will vary and this variation can have a potentially greater impact (since the buffer system will not act as a buffer system in many cases, and therefore be more sensitive for changes in the feed stream).

Case study

Cytiva was invited to help identify solutions to a biopharmaceutical manufacturer's facility space constraints problem. The use of our inline dilution system resulted in the elimination of two large buffer hold tanks (25 000 L each). These vessels were replaced with two 5000 L concentrate hold tanks. The most obvious benefits were reduced capital costs (capital savings of > $300 000) and floor space savings (200 ft^2 of floor space). However, other less visible benefits included:

- Reduced buffer preparation time due to the significantly reduced volumes of concentrate to prepare
- Reduced maintenance time on the larger buffer hold tanks
- Reduced CIP volumes required for the smaller concentrate buffer hold tanks

Gradient inline conditioning

A study was performed to evaluate if a gradient inline conditioning system can match the performance of an isocratic inline dilution system. A ternary pump system was used to mix and dilute acetone-water solutions with only flow meter feedback control, as conductivity feedback was disabled. This was done in order to demonstrate the high level of linearity in the mixing of the buffer concentrates.

Table 1 shows that the mixing set points were reached and maintained to within ± 1% error, just as it was for the isocratic system. The results verify that truly linear blending is accomplished when just flow meters alone are used to control the process. This is especially important for solvent blending steps.

Table 1. Results of a gradient inline conditioning performance study. Acetone solution was used with a dilution factor of 6×, with a total flow rate of 1000 L/h

Gradient setpoint (%)	Calculated value	Read value	Equivalent (%)	Error (%)
5	0.0211	0.0214	5.06	0.06
10	0.0423	0.0424	10.03	0.03
25	0.1057	0.1066	25.22	0.22
50	0.2114	0.2123	50.22	0.22
75	0.3170	0.3176	75.14	0.14
90	0.3804	0.3809	90.11	0.11
95	0.4016	0.4014	94.96	-0.04
100	0.4227	0.4227	100.00	0.00

Summary

Cytiva's inline conditioning technology provides a number of advantages to biopharmaceutical producers. The primary benefits include:

- Secured mixing of different solutions by a single system
- Easy method set-up and IC control with UNICORN
- Reproducible results
- PAT-based IC process technology
- Access to an IC system test rig for pre-purchase mixing studies

Technical specifications

System characteristics and IC accuracy

UV wavelength range	Single (280 nm) or multiple wavelengths
pH range	0-14
pH accuracy*	0.2 pH units
Conductivity range	1 mS/cm to 200 mS/cm
Conductivity accuracy*	± 2% or 0.5 mS
Process temperature	4°C to 80°C (stainless steel systems)
	4°C to 60°C (polypropylene systems)
Electrical standards	UL 508A, EN 61010-1

* Accuracy values are minimum – much higher accuracy can be reached under most conditions.

System flow rates

6 mm i.d. PP	4–180 L/h
3/8" o.d. (7.7 mm i.d.) SS	4–180 L/h
10 mm i.d. PP	15–600 L/h
½" o.d. (9.4 mm i.d.) SS	15–600 L/h
1" o.d. (20.4 mm i.d.) PP	45–2000 l/h
1" o.d. (22.1 mm i.d.) SS	45–2000 l/h
1½" o.d. (34.8 mm i.d.) SS	500–5000 L/h
2" o.d. (47.5 mm i.d.) SS	1000–10 000 L/h

System requirements

System	BioProcess™ or ÄKTAprocess liquid chromatography
Software	UNICORN control software, v5.2 or later*
pH	≥ 1 pH monitor
Conductivity	≥ 2 monitors
Flow	≥ 2 meters
Pumps	≥ 2

* Recommended.

Ordering information

For ordering information and further technical details, please contact your regional Cytiva sales office.

cytiva.com/cbs

CY14430-24Sep20-DF