BioProcess™ IC System

INLINE BUFFER PREPARATION

The BioProcess™ IC System (Fig 1) is a buffer management system for inline buffer preparation using concentrated single component stock solutions of acid, base, salt, and water for injection (WFI). The use of concentrates will significantly reduce buffer volumes, saving both floor space and tank volumes. In addition, many different buffers can be prepared from the same set of concentrates, streamlining buffer preparation even further. For accuracy in formulation and consistency between preparations it is possible to select different feedback modes featuring the built-in dynamic control functionality.

Intensifying large scale buffer management:

- Automated inline buffer preparation
- Quality rigor to ensure buffers meet specifications
- Integrated buffer preparation and chromatography operations

Freeing up resources

Traditional buffer preparation is a highly manual and resource-intensive activity in biomanufacturing. Large volumes of different buffers, formulated according to specific recipes, are required to produce a biopharmaceutical and due to the large quantities used, buffer management can easily become a bottleneck in production. In addition to high labor costs, there is also a risk of losing an entire buffer batch if it does not pass the release criteria.

As an extension of the concept of inline dilution (ILD), inline conditioning (IC) is a buffer management strategy that allows for buffer production on demand from concentrated single component stock solutions. Implementing IC can help to streamline the entire buffer preparation process and reduce manual handling by automating several steps, not only reducing the risk of human errors, but also making it possible to reassign personnel to other tasks.

Fig 1. BioProcess™ IC System for inline buffer preparation.

The BioProcess™ IC System has dedicated pump lines for WFI, acid, base and salt. It is also possible to include additional pumps for additives and/or sample (Fig 2). Each pump line has several inlet ports to allow different buffer families to be formulated with the same setup. This makes it possible to produce exact amounts of different buffers sequentially during a run (Fig 3). The process is controlled by a range of sensors; flow meters on each pump, and pH and conductivity sensors after each mixing point. The pH and conductivity sensors give feedback to the pumps to adjust the flow of each component. The Buffer Verification Function (BVF) in the UNICORN™ software monitors the pH and/or the conductivity of the mixed buffer in real time. The system will automatically bypass the column and redirect from outlets to waste if the detected values are outside set limits. This ensures that only the correct buffer reaches the column or collecting vessel, creating a robust and reliable production process.

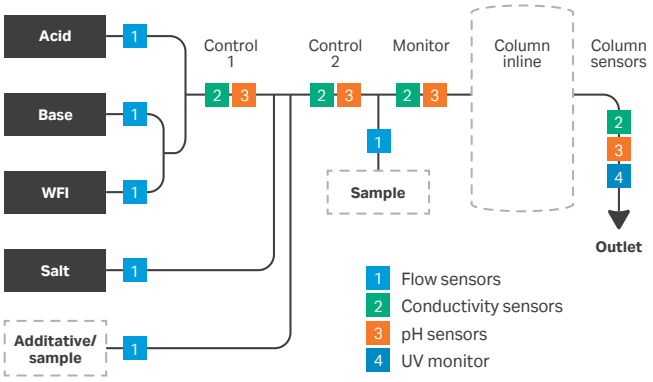


Fig 2. Schematic flow diagram of a BioProcess™ IC system.

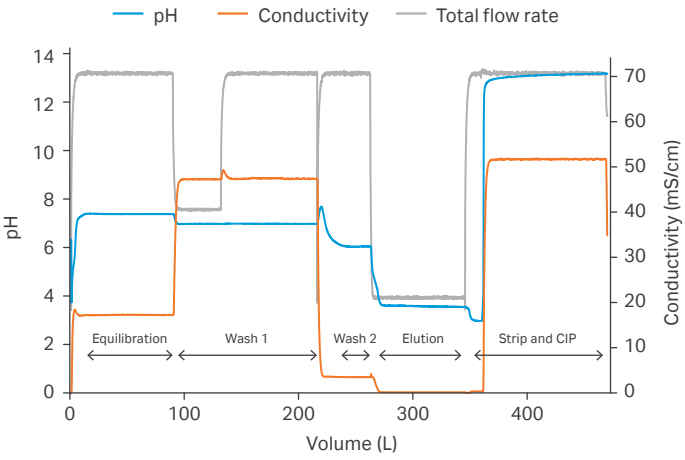


Fig 3. Preparation of buffers as well as strip and cleaning in place (CIP) solutions required for a mAb capture step. The arrows indicate preparation of formulations within specifications.

Preparing several buffers from the same stock solutions

While ILD requires one concentrate for each final buffer a wide range of buffers can be prepared from a few single component stock solutions using IC (Fig 4). This will save time and space, and drastically reduce documentation and QC testing requirements.

Over 100 unique buffers of five different buffer systems have been formulated in an automated manner using the BioProcess™ IC System from Cytiva. As listed in Table 1 and 2, buffers with a great variety of pH values, buffer concentrations, salt concentrations, and additives have been formulated. Glycerol mixtures with acetate and dilution gradients with potassium phosphate have also been successfully prepared using the BioProcess™ IC system. Salt gradients are also accurately created by mixing the relevant stock solutions (Fig 5).

Monitoring real time data for full process control

The UNICORN™ control software is used to plan, control and analyze each process run. All buffer characteristics are recorded, allowing operators to monitor the trending of those properties by looking at chromatography results in real-time.

There is also a possibility to integrate into a DeltaV™ automation system or to use a standard for open platform communication (OPC) for compatibility with other third-party system control softwares. This allows for real-time data to be shared effectively, for run data to be stored within the planned automation system, and for appropriate start and stop signals to be properly sequenced.

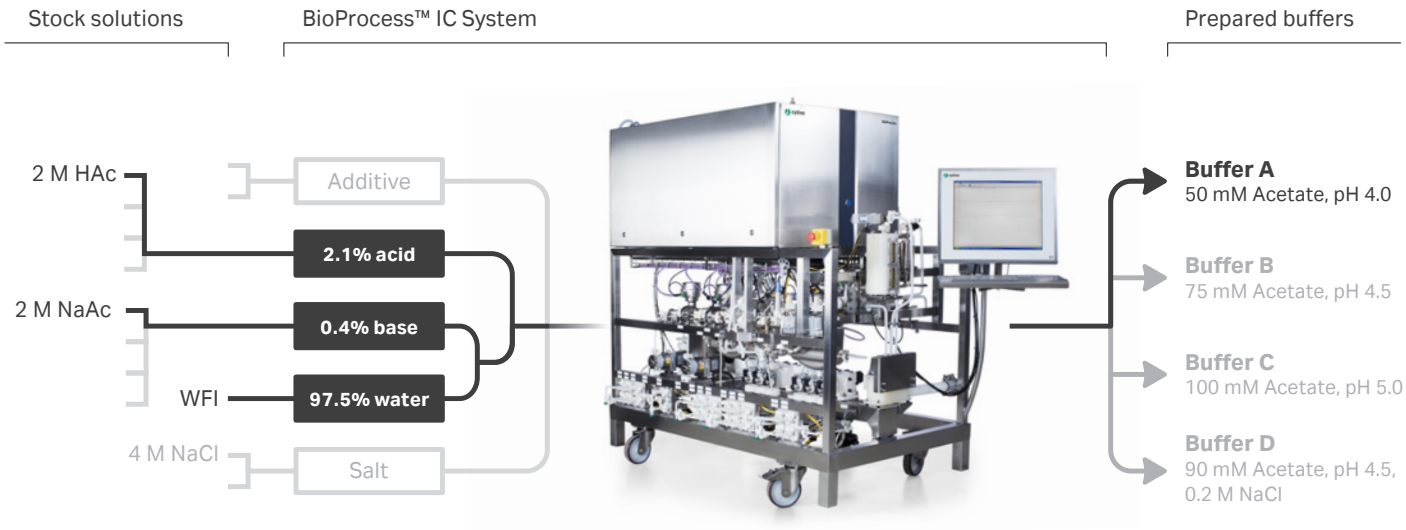


Fig 4. Different combinations of stock solutions and WFI generates different buffers in an automated manner.

Table 1. Example of phosphate buffer range that can be prepared using only three stock solutions

Input	Output		
Stock solutions	**Buffer conc. (mM)**	**Salt conc. range (mM)**	**pH range**
0.3 M NaH_2PO_4	20	0–500	6.8–7.3
0.3 M Na_2HPO_4	30	0–500	6.8–7.4
3.5 M NaCl	50	0–500	6.8–7.4

Table 2. Experimental data on buffers formulated with the BioProcess™ IC System from Cytiva

Buffer conc. (mM)	pH	NaCl conc. range (mM)
Phosphate buffers		
10*	6–8	0–6
20†	6.5–7.4	0–500
25‡	6–8	0–1000
30	6–7	0–1000
35	7.2	50
50§	6–7.4	0–150
200	6.8	-
Sodium acetate buffers		
1.8	3.6	0–100
10	3.5	0–107
20	5.2	100
25	3.5–5.5	0–500
30	3.6	100
38	5.1	100
42.3	5.3	-
50	3.5–7.5	0–500
150	4	0–500
Sodium citrate buffers		
10¶	4.5–5	0–300
25	3.2–4.2	0
100	3.5–5	-
Formic acid buffers		
390	2	2000
15	3.5	50–250
Tris buffers		
10	8.2	-
16	8	13
16.2	9	0–1000
20	7.5	20–500
25	7–9	0–1000
50**	8–9	50–1000
80	8	-
100††	8	-

* Prepared with 0–2 M urea and 0–3 mM MES.
† Prepared with 0–3 M urea and 0%–0.04% Tween™.
‡ Prepared with 0–0.1 M $(NH_4)_2SO_4$.
§ Prepared with 0–1 M $(NH_4)_2SO_4$ and 0%–0.1% Tween™.
¶ Prepared with 0–7 M urea.
** Prepared with 0–0.1 M $(NH_4)_2SO_4$.
†† Prepared with 0.1% Tween™.

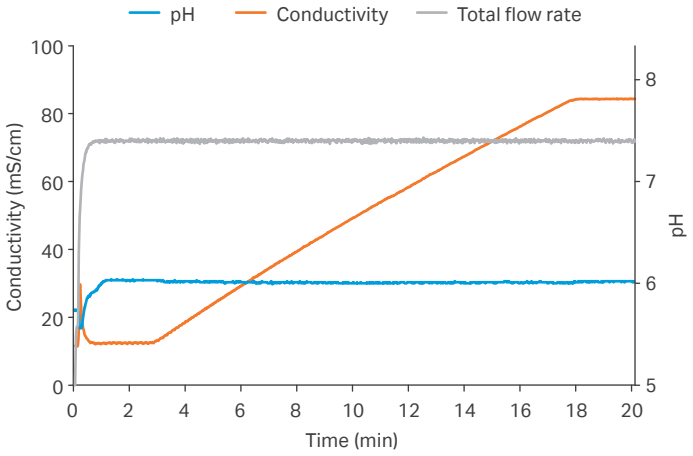


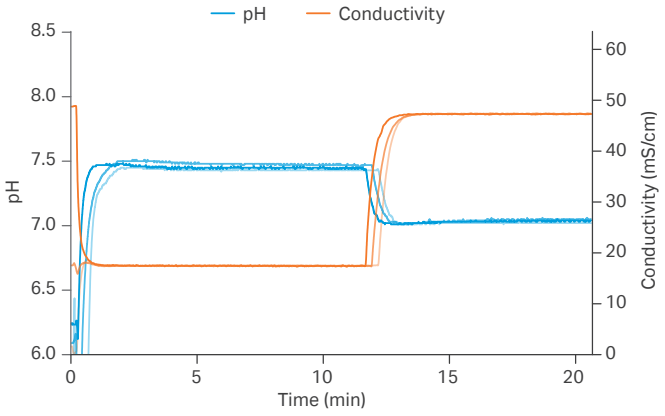
Fig 5. Gradient. Sodium phosphate buffer with NaCl–conductivity increases while total flow and pH remains unchanged. Acid and base are adjusted to maintain pH at the set point, while conductivity changes during the gradient run (Sodium phosphate pH flow with gradient 30 mM Sodium phosphate pH 6). Stock concentrations: 500 mM NaH_2PO_4, 350 mM Na_2HPO_4. Stock solution NaCl: 3.5M = gradient 0.1 to 1 M.).

Fulfilling quality rigor

Because buffers are used to maintain purification conditions, as well as to stabilize the product and preserve its functional characteristics, correct buffer formulation is crucial for success in bioproduction. The BioProcess™ IC System features built-in dynamic control to ensure accuracy in buffer formulation. Three modes of dynamic feedback control can be used: recipe and flow; pH and flow; and pH and conductivity.

- **Recipe and flow feedback:** A known buffer formulation is entered in the system control software. The software adjusts the flow rates of the specified stock solutions to achieve the desired formulation. This control mode is useful when the temperature is constant, and the stock solutions are accurate.
- **pH and flow feedback:** The method specifies target pH and the software adjusts the flow rates of the acid and base stock solutions to achieve the desired pH and buffer concentration in the final formulation.
- **pH and conductivity feedback:** The method specifies the target pH and conductivity, and the dynamic control functionality of the software uses feedback from flow, conductivity, and pH sensors to adjust flow rates of the stock solutions to achieve the desired conductivity and pH. In this control mode both the temperature and the concentrations of the stock solution can vary without affecting the pH and conductivity accuracy of the final buffer formulation.

Feedback control minimizes buffer variability and compensates for variability in the stock solutions, and thus improves process robustness and quality of products (Figs 6 and 7).



Buffer	pH	Conductivity (mS/cm)
20 mM sodium	7.41 ± 0.05	17.3 ± 0.3
phosphate, 150 mM	7.44 ± 0.05	17.3 ± 0.2
NaCl, pH 7.4 buffer	7.40 ± 0.04	17.3 ± 0.2
20 mM sodium	7.00 ± 0.03	47.2 ± 0.2
phosphate 500 mM	7.01 ± 0.04	47.3 ± 0.3
NaCl, pH 7 buffer	6.99 ± 0.02	47.3 ± 0.3

Fig 6. Robustness, reliability and reproducibility: The image shows three runs of preparation 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 followed by preparation of 20 mM sodium phosphate, 500 mM NaCl, pH 7. The switch between the two buffers takes 2 minutes. Source: Application note Automated inline buffer preparation from readymade stock solutions in a mAb process step. Cytiva, 29260552, Edition AA (2017).

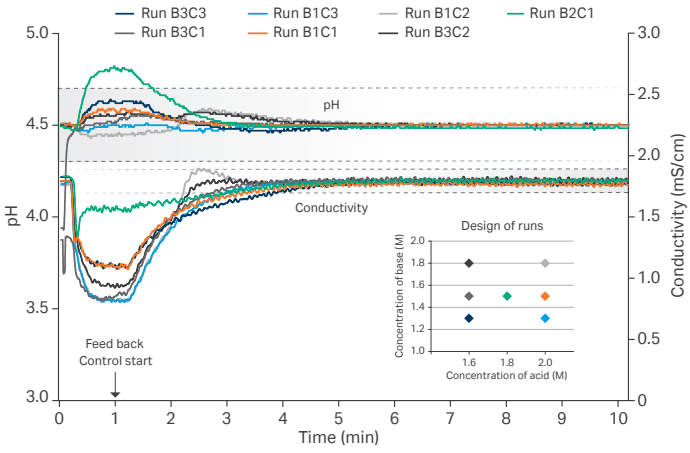


Fig 7. Starting point: Three different concentrations of base and acid stock solutions and seven different combinations were tested. All combinations reached target values of pH and conductivity.

Several sensors throughout the flow path

When using pH feedback the system uses two sensors, one for controlling and one for monitoring. The accuracy of each pH sensor in the system is ± 0.10 pH units, which is also the accuracy of the buffer. By adding the variances, the monitoring pH can at the most be ± 0.15 pH units from the target pH.

The accuracy of the conductivity monitors is ± 2% of reading or 0.5 mS/cm in the 0.1–200 mS/cm range.

When selecting pH and conductivity feedback control, the flow rates will be adjusted to meet the conductivity target value.

The accuracy of the flow rate for each pump is ± 1% of the pump range or ± 2% of the reading (whichever is greater), meaning that a smaller pump has a smaller error. Hence, to optimize flow accuracy the highly concentrated acid and base stock solutions are connected to the smaller pumps.

Integrating buffer production and chromatography

The BioProcess™ IC System has the functionality required to operate as a chromatography unit, allowing direct connection to a chromatography column to make it possible to deliver buffers directly on to the column without the need for storage in bags or tanks (Fig 8).

Since the BioProcess™ IC System can be directly connected to a column, the buffer mixing and the chromatography run can be done in an integrated manner. With all steps controlled by the same software, all relevant data for buffer characteristics and for the run itself are recorded at the same time, resulting in one batch record for both operations, simplifying documentation.

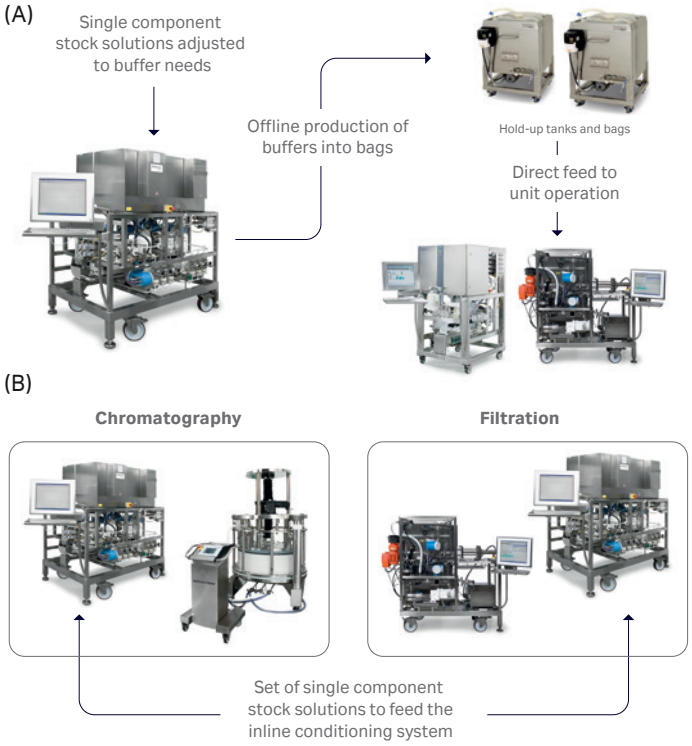


Fig 8. Inline conditioning system can be used as (A) central buffer preparation station or (B) as an integral part of a chromatography or filtration unit operation.

Technical specifications

System specifications are listed in Table 3.

Table 3. BioProcess™ IC System specifications

	BioProcess™ IC 10 mm	BioProcess™ IC 1 inch	BioProcess™ IC 1½ inch
Flow rate range	60–600 L/h	200–2000 L/h	1000–5000 L/h
Dimensions W × D × H	1349 × 2639 × 1926 mm	1608 × 2952 × 2196 mm	1858 × 3424 × 2241
Operating max. pressure	6 bar g (max 3 bar g at 40°C–60°C)	6 bar g (max 3 bar g at 40°C–60°C)	5 bar g (max 3 bar g at 40°C–60°C)
Pressure sensor			
Range	0.1–6 bar g	0.1–6 bar g	0.1–5 bar g
Accuracy	± 0.12 bar g	± 0.12 bar g	± 0.12 bar g
Temperature sensor			
Range	2°C–60°C		
Accuracy	± 2°C		
Conductivity sensor			
Range	0.1–200 mS/cm		
Accuracy	± 2% of reading or 0.5 mS/cm		
UV sensor - fixed or variabel/ multiple wavelength			
Range	0–2 AU		
Accuracy	linearity ± 5%		
pH sensor			
Range	2–12		
Accuracy within IC application range*	± 0.10 pH units		
Flow accuracy	± 2% or 0.5 L/h	± 2% or 2 L/h	± 2% or 4 L/h
Wetted parts			
Main piping size	10 mm	1 inch	1½ inch
Piping	Polypropylene (PP)	PP	PP pre-air trap and stainless steel (SS) post-air trap
Flange type	Hygienic tube clamp connection (TC)		
Gaskets	EPDM		
Flow meter	UNS N06022 (Hastelloy™)		
Air trap	Ethylene propylene rubber (EPDM), polyamid (PA), PP	EPDM, PA, PP	EPDM, PA, SS
Air sensor	PP	PP	PP pre-air trap and SS post-air trap
pH sensor	Glass, Polytetrafluoroethylene (PTFE)		
Conductivity sensor	Polyetheretherketone (PEEK)		
Temperature sensor			
Integrated in conductivity sensor	PEEK		
Safety	UNS N06022		
UV sensor	PEEK, EPDM, quartz, titanium		
Pressure sensor	UNS N06022		
Pump chamber	PP, PTFE, PEEK, oxide ceramics, EPDM	PP, PTFE, PEEK, oxide ceramics, EPDM	ASTM 316L (1.4435), EPDM, PP, Santoprene™
Material requirements for wetted parts	USP class VI, 21 CFR 177, animal origin free or in compliance with EMA/410/01		
Wetted and pressure retaining materials	PP, 316L (EN 1.4435), glass, quartz glass, EPDM, PEEK, FPM, FKM, PTFE, oxide ceramics, titanium grade 2, Santoprene™		
AxiChrom™ column compatibility			
AxiChrom™ column range (mm)	200–600	600–1200	1000–1600
System control			
Software	UNICORN™ 6.3.2 or higher		

* Buffer capacity of at least 5 mmol NaOH per pH unit for the controlling pH electrode.

Ordering info

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

The system can be optimized to fit the process and facility by either integrating it into the chromatography unit operation or by using it as a standalone buffer preparing unit. To further intensify the buffer preparation process, ready-made stock solutions can be used, eliminating all raw material handling and manual preparation of stock solutions. Advantages of using single-component stock solutions as opposed to concentrated buffers for dilution, are that the common ion effect will not limit the maximum concentration to the same extent and there is no need to handle pH and conductivity shifts due subsequent dilution.

For more information, please visit: cytiva.com/buffer-management

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