Automated in-line buffer preparation from ready-made stock solutions in a mAb process step

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Buffer preparation is both time-and space-consuming and can easily become a challenge in biomanufacturing. This application note describes a lean approach to buffer preparation by implementing in-line conditioning (IC). Buffers of different formulations for a mAb chromatography capture step were prepared in an automated, consecutive manner, minimizing manual interactions. To further reduce the time and space required for buffer preparation, ready-made, highly concentrated, low-volume stock solutions were used. Feedback regulation of the final buffers, using dynamic control, ensured accurate formulations.

Introduction

Buffers are often prepared manually as concentrates to be diluted when needed. Concentrated buffers, however, can present challenges related to poor solubility of buffer constituents. Undissolved particles might precipitate or even be removed in filtration before dilution, resulting in a buffer of undesired formulation or with altered pH. Associated with manual preparation is the risk of human error. In addition, preparation and storage of the large amounts of buffers and raw materials required for biomanufacturing, is both time- and space-consuming.

In-line preparation of buffers from concentrated, low-volume, 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 IC. Table 1 gives an example of phosphate buffers that can be prepared from the same three stock solutions. In addition, automation enables just-in-time buffer production, while minimizing the error risk with manual buffer preparation, ensuring consistency between batches.

Buffer preparation by IC

Buffers in downstream processes, especially in mAb production, are typically based on phosphate, acetate, citrate, and tris formulations. Over the past years, GE Healthcare has gathered extensive experimental data. Over 100 unique buffers of five different buffer systems have been formulated in an automated manner using GE Healthcare's Inline Conditioning system (1). As listed in Table 2, buffers with a great variety of pH values, buffer concentrations, salt concentrations, and additives have been formulated, of which many are considered challenging with regards to buffer capacity. Glycerol mixtures with acetate and dilution gradients with potassium phosphate, for example, have also successfully been prepared using the Inline Conditioning system.

Table 1. Example of phosphate buffer range that can be prepared from 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

For accuracy in formulation and consistency between preparations, it is possible to select the feedback mode that best controls critical process parameters (Table 3). There are three modes of feedback control that can be used with the dynamic control functionality of the system: recipe and flow; pH and flow; and pH and conductivity.

Recipe and flow feedback: a known buffer formulation is entered in the UNICORN™ system control 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.

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.

pH and conductivity feedback: the user enters the target pH and conductivity, and the dynamic control functionality of the UNICORN 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.

Table 2. Experimental data on buffers formulated with the Inline Conditioning system from GE Healthcare

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.

While in-line dilution requires one buffer concentrate to produce one final buffer, a wide range of buffers can be prepared from a few single-component stock solutions using IC (Fig 1). Figure 2 gives an example of stock solutions required for a typical buffer preparation for a three-step mAb purification process. Considering the given example volumes, a total volume of 1428 L of eight stock solutions is required to prepare 11 different buffers of a total volume of 6664 L. Compared with offline preparation of 1× buffers (6664 L), the use of stock solutions (1428 L), in combination with in-line buffer preparation, enables a 79% reduction in stored buffer. With just-in-time preparation of buffers from stock solutions using IC, redirecting the prepared buffer directly onto the chromatography column, great time-savings can be made compared with manual preparation of the same amount of buffer.

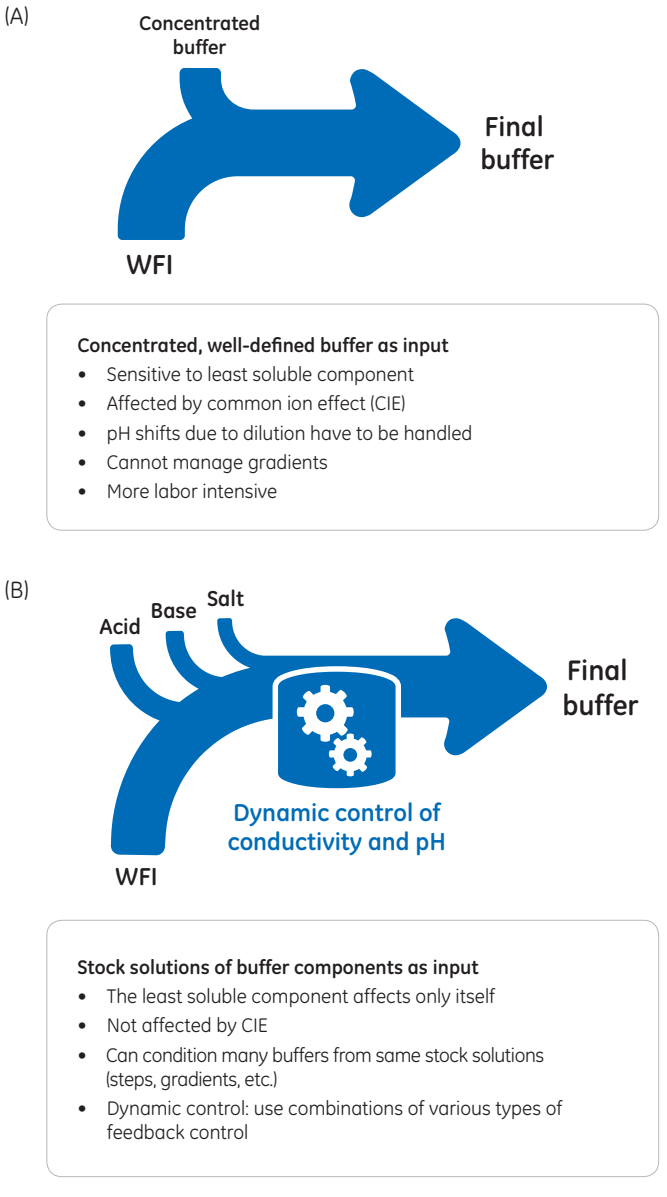


Fig 1. Two ways of preparing buffers: dilution of a buffer concentrate, in-line conditioning.

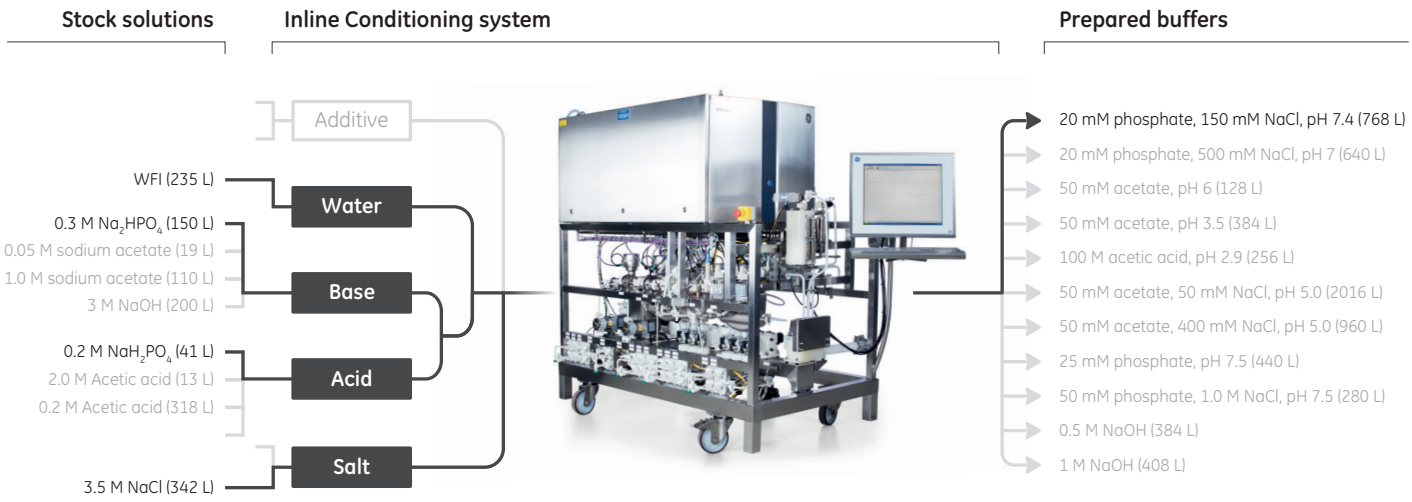


Fig 2. Highly concentrated, low-volume stock solutions required for a three-step mAb purification process from 2000 L culture feed with a mAb titer of 3 g/L. Different combination of stock solutions and water for injection (WFI) will generate the different buffers in an automated manner.

In this application note, we show how the Inline Conditioning system can be used for fast and efficient preparation of buffers required for the capture step of a mAb purification process. Required buffers of different formulations as well as solutions for strip and cleaning-in-place (CIP) were prepared from HyClone™ concentrated stock solutions in a preprogrammed, consecutive manner. The stock solutions can be delivered in single-use bags with connectors that can readily be connected to the system.

Materials and methods

The buffer volumes and flow rates were based on purification of a mAb from cell culture feed on an AxiChrom™ 400 column (25 L, 40 cm i.d., 20 cm bed height). Before start, all HyClone stock solutions were connected to the system and the inlets were primed. As the system's inlet connections are Tri-Clamp™ connections and the single-use buffer bag has MPC connections, a small MPC-TC jumper was used between the bag and the system. The buffers were prepared at time of use, in a consecutive manner, according to the protocol outlined in Table 3.

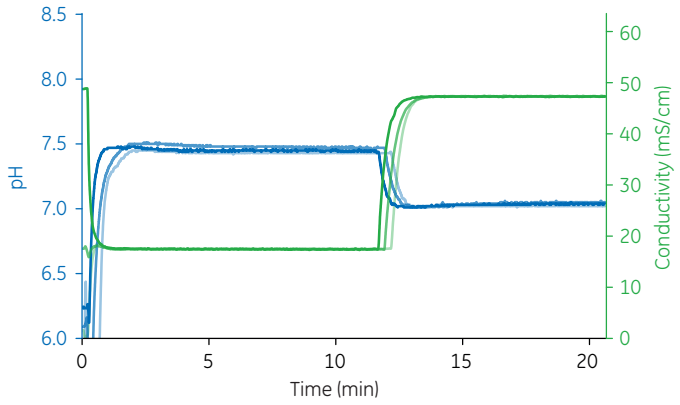
The buffer preparation method in the UNICORN system control software was set up in a way that allowed set values for pH to stabilize (about 0.5–2 min) before starting pH feedback control, where the set values were based on the molar recipes for the specific buffer. When the set pH was reached and stable, the outlet was switched from waste to the single-use buffer bag. Control of buffer formulations was performed using pH and flow feedback. For control of strip and CIP solutions, only flow feedback was used. The established baselines were used to monitor pH to ensure the value was within set specifications (high and low limits).

Table 3. Run protocol for the mAb capture step

Step	Volume (column volumes [CV])	Residence time (min)	Flow velocity (L/h)	Control mode	Buffer
Equilibration	3	3.4	443	pH and flow	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Load		6	250		Not applied
Wash 1	5	6 (1.5 CV) 3.4 (3.4 CV	250 443	pH and flow	20 mM sodium phosphate, 500 mM NaCl, pH 7
Wash 2	1	3.4	443	pH and flow	50 mM acetate, pH 6
Elution	3	12	125	pH and flow	50 mM acetate, pH 3.5
Column strip	2	3.4 (reduced to 2 min)	443	flow	100 M acetic acid, pH 2.9
Column CIP	3	5	300	flow	0.5 M NaOH

Results and discussion

In this work, three buffers as well as strip and CIP solutions required for a mAb capture step were prepared in an automated manner using GE Healthcare's Inline Conditioning system. An overlay of three preparations of two buffers with an intermediate switch between buffers demonstrates consistency between preparations (Fig 3).



Buffer	pH	Conductivity (mS/cm)
20 mM sodium phosphate, 150 mM NaCl, pH 7.4 buffer	7.41 ± 0.05 7.44 ± 0.05 7.40 ± 0.04	17.3 ± 0.3 17.3 ± 0.2 17.4 ± 0.2
20 mM sodium phosphate 500 mM NaCl, pH 7 buffer	7.00 ± 0.03 7.01 ± 0.04 6.99 ± 0.02	47.2 ± 0.2 47.3 ± 0.3 47.3 ± 0.3

Fig 3. Overlay of triplicate preparations of 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 buffer, followed by preparation of 20 mM sodium phosphate 500 mM NaCl, pH 7 buffer, showing reproducibility of buffer formulation. The change between the buffers is very similar each time and takes about two minutes.

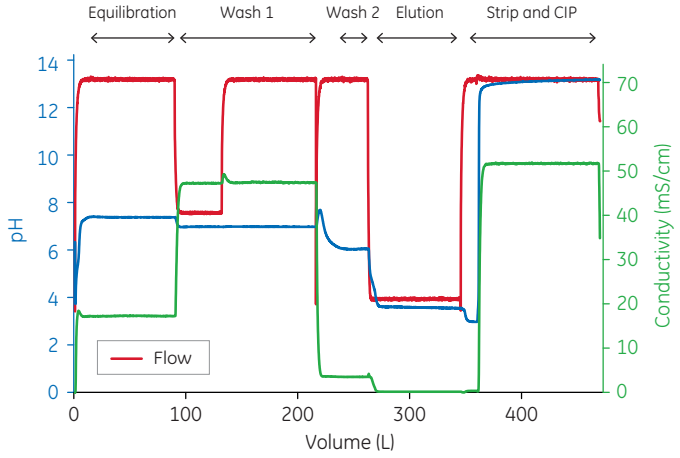


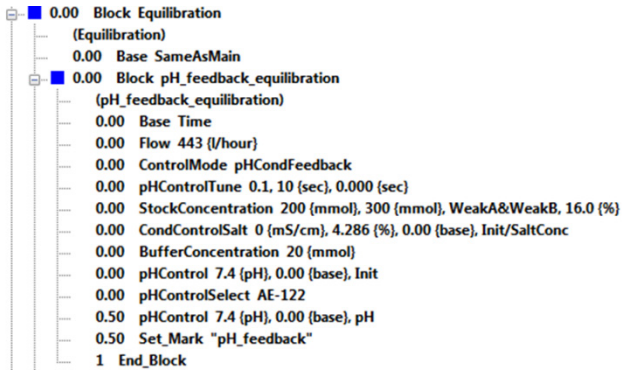
Fig 4. Preparation of buffers as well as strip and CIP solutions required for a mAb capture step. The arrows indicate preparation of formulations within specifications.

At flow rates between 125 and 445 L/h, the switch between buffers takes 0.5 to 2 min (depending on the use of the same or different stock solutions as previous formulation), during which the mixture is directed to waste. As soon as set pH is reached and stable, the outlet is redirected to the single-use buffer bag (or directly to the chromatography column if buffers are prepared in-line). Experimental evidence shows that the change in back pressure, occurring when redirecting the outlet back to the buffer bag (or column), has no effect on pH or conductivity. The complete buffer preparation process, with transitions between formulations, is shown in Figure 4.

Compared with preparing buffers as a separate operation, significant time-savings can be made with buffer preparation integrated with the chromatography step. Here, buffer preparation time is determined by the time required for the different parts of the chromatography method. As an alternative, the Inline Conditioning system can be used as a stand-alone unit operation for buffer preparation only. In such a scenario, maximum feed rates can be used to shorten the overall buffer preparation time. In addition to contributing to further time-savings compared with preparing buffers manually, the use of ready-made, highly concentrated, low-volume stock solutions also saves space in storage of raw materials.

The preparation method for the formulations, defined in the buffer configurator of the Inline Conditioning system, were conveniently created in the UNICORN software (Fig 5). Feedback control of the final buffers ensured accurate formulations. In addition, the established baselines were used to secure that final buffer formulations were within set specifications. Should the buffer formulation fall out of specification, the system can be programmed to direct the mixture to waste until set conditions are reached again. During the buffer preparation process, data is recorded and can be accessed from the UNICORN result file or sent, for example, to a distributed control system (DCS). Tables, containing data such as minimum, maximum, and average pH, can be exported for later evaluation.

(A)



(B)

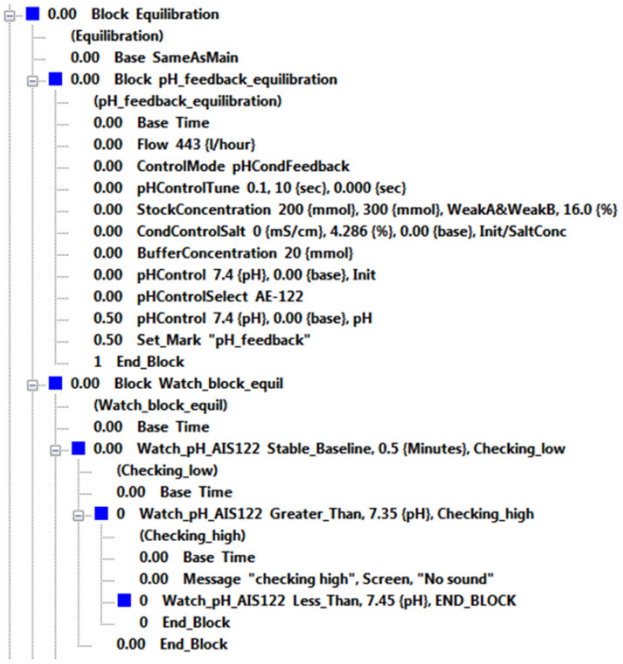


Fig 5. Screenshot of the UNICORN programing view, showing (A) input parameters and (B) acceptance criteria for control of correct and stable pH.

Accuracy in pH and conductivity control

The accuracy of each pH sensor in the system is ± 0.1 pH units. When using pH feedback, the system uses two sensors, one for controlling and one for monitoring. To define the accuracy of the overall operation, the variance of the two sensors are taken into account, resulting in a buffer accuracy of ± 0.15 pH units.

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), and it usually takes some time before the equilibrium is established and the pH measurement is again free of bias.

Several pH sensors are used by the system to ensure reliable pH measurement, for example, to avoid a pH measurement bias caused by the salt memory effect. To overcome this effect, the system offers the possibility to choose the pH sensor to be used for pH control. The general recommendation is to use the pH sensor downstream salt addition junction for all buffers including salt, whereas the pH sensor upstream the salt addition junction can be used for buffers without salt. It is recommended that the pH sensors are regularly calibrated, for example, between production campaigns and that they are replaced at every service of the system.

The accuracy of the conductivity monitors is ± 0.5 mS/cm in the 0.1–100 mS/cm range and ± 1 mS/cm in the 100–300 mS/cm range, the same as for an ÄKTAprocess™ system.

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 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.

Accuracy in flow control

The accuracy of the flow rate for each pump is ± 1% of the pump range or ± 2% of the reading (whichever is greater), meaning 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.

Buffer preparation strategies

Using IC, the goal is to prepare buffers that meet the specifications of critical process parameters, including, not only pH and conductivity, but also concentrations of other components such as additives and detergents.

When choosing buffer preparation strategy, buffer components and their concentrations need to be considered. Buffers can be prepared by mixing corresponding acid and base (weak/weak) or by using the acid or base of the buffering component and adjusting the pH with a strong acid or base (weak/strong). Inline Conditioning takes into consideration these parameters to ensure target values such as pH, buffer concentration, and/or conductivity are reached.

With IC, buffer is produced from its building blocks: concentrated single component stock solutions. Solubility of chemicals and buffering capacity of any given buffer, are factors that will determine the concentrations that can be achieved for each stock solution.

When producing a buffer with low buffering capacity, small variations in flow can generate large variations in pH. For this type of formulations, in order to have stable pH readings the recomendation is to lower the concentration factor of, for example, the strong base. This strategy can be used to avoid large fluctuations in pH readings during the production run.

Selection of control modes is another strategy that can be adapted to the needs of the buffer formulation process. For example, in cases where there are fluctuations in the water loop or ambient temperature, it is possible to select pH and conductivity feedback control to formulate temperature-sensitive buffers such as tris and piperazine. When temperature or pH shifts are not a concern, buffer preparation using flow feedback control can be a good strategy.

The strategies described here for buffer preparation using IC help ensure that high concentration factors for the stock solutions can be used, and that critical process parameters such as pH, conductivity, and buffer concentration are met on the final buffer formulation.

Conclusion

Using the Inline Conditioning system, buffers for a mAb capture step could be formulated in an automated, consecutive manner. Compared with preparation of buffers as a separate operation, integrating buffer preparation with the chromatography step both saves time and reduces facility footprint. Contributing to further time-savings, the use of ready-made, highly concentrated, low-volume stock solutions also saves space in buffer preparation and minimizes the need for raw material qualification. Feedback control of the preparation process ensures accurate formulation of the final buffer, and automation enables high consistency between batches. Compared with traditional buffer preparation, IC can help increase efficiency in buffer production for biomanufacturing applications.

Reference

1. Application note: Overcoming buffer challenges with in-line conditioning. GE Healthcare, 29209677, Edition AA (2016).

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