Automated pH adjustment on an intelligent, single-use mixing platform

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Automated pH adjustment on an intelligent, single-use mixing platform

This application note describes the performance of the automated pH adjustment capability of the single-use Xcellerex™ XDUO mixing system (Fig 1). Automated pH-adjustment operations can help reduce the risks associated with manual handling of the product and vessel. Manual operations increase the risk of including operator error, microbial or virus contaminations, pH overshoot deviations, and time-to-production deviations in the process. A lack of electronic batch records and data storage and trending in manual operations introduce further complications of documentation and regulatory compliance. By automating the pH adjustment steps, the XDUO mixing system can help simplify the process and mitigate these risks.

Fig 1. The single-use XDUO mixing system.

Introduction

Low-pH virus inactivation is commonly used in monoclonal antibody purification processes and has been reliably demonstrated to inactivate > 4 $\log_{10}$ of large, enveloped viruses (e.g., X-MuLV) in several commercial purification processes. The specific conditions for maximizing virus clearance, while minimizing product activity loss, should be empirically derived. A low-pH virus clearance procedure can be applied to, for example, monoclonal antibodies that have purification process steps that include low pH and fulfill the following criteria (1):

1. pH of incubation is approximately 3.5 ± 0.1
2. Incubation time at pH 3.5 is 30 to 60 min
3. Total process time should not exceed 60 min
4. Incubation temperature is ≥ 14°C
5. Buffer system is citrate or acetate
6. Total protein concentration is < 40 mg/mL
7. Sodium chloride concentration is ≤ 500 mM
8. pI of the monoclonal antibody is between 3 and 9
9. Low-pH incubation step is performed on a cell-free harvest intermediate after the initial capture step of the recovery process
10. Product is not a retrovirus-targeted monoclonal antibody

pH adjustment steps have been routinely performed manually. A measured sample of product is removed and the pH is adjusted to the target value. The sample volume and the volume of adjustment solution are used to calculate the volume of adjustment solution required to bring the remaining bulk product solution to the desired pH. Once the adjustment solution volume is calculated, 80% of this volume is added to the bulk product. The relatively rapid addition of the solution in the absence of constant mixing can result in localized condition of pH that can be detrimental to the product. Following the addition of adjustment solution, the product is mixed manually. Next, another sample is taken for off-line pH measurement. Based on test results, additional adjustment solution is added. Again, following addition of pH adjustment solution, the product is mixed manually and more samples are taken to measure pH.

If necessary, the above steps are repeated until the target pH is reached. Deviations will occur if, at any point during adjustment, the pH target is exceeded by > 0.2 pH units. Once the target pH is reached, the viral inactivation hold time begins. Typically this is a 60 min hold followed by pH adjustment up to pH 5.0 or 7.0 following the same manual pH adjustment method, and incurring the same process risks.

Some of the risks of manual pH adjustment for virus inactivation are:

- Mechanical hazards presented by handling/manipulating or moving the product containment vessel
- Overshooting pH set points (> 0.2 pH units) because of miscalculations/operator error
- Exceeding recommended time limits for process steps and exposing product to local conditions, which could compromise quality as well as quantity of product
- Compromising product integrity during sampling and addition of pH adjustment buffers

Selecting a technology that can contain, mix, and monitor the product while automating pH adjustment steps, will simplify the viral inactivation process and mitigate these risks.

The single-use XDUO mixing system is enabled with a powerful on-board automation that is capable of controlling precise and accurate pH adjustment and capturing process data to a historian platform. Implementing the stand-alone, easy-to-use, automated XDUO mixing platform can have numerous advantages in pH adjustment operations:

- Automation: reduces risk of process deviations from operator error
- Automation: saves time and increases accuracy by eliminating manual batch records
- Closed systems with single-use bags: protects the product and operator and reduces risk of contamination
- Flexible, single-use equipment: increases plant efficiency while reducing equipment footprint requirements

To demonstrate a reduction of risks associated with manual procedures, studies of automated mixing and pH adjustment were performed in the XDUO single-use mixing system. The studies demonstrate accurate, automated pH adjustment within accepted industry process standards (1).

Materials and method

XDUO mixing study

A 500 L XDM Quad Plus bag was installed into the 500 L XDUO mixer and pH probes were installed into the appropriate ports. To facilitate placement of pH probes, posts were installed inside the mixing vessel through incisions made through the top of the bag. One probe was inserted through the central port in the top of the bag. To sample the pH from each part of the mixing vessel, probes were positioned in the corners as well as in the center of the vessel. pH measurements were taken using data logging software and pH readings were collected at one-second intervals from each of the probes.

The 500 L XDUO mixer was filled to 450 L with 20 mM glycine, pH 5.5. Time between each pH spike and return to uniformity was determined for each mixing speed for both clockwise (CW) and counterclockwise (CCW) directions. pH for each probe was traced following a spike with acid or base into the vessel. Volume of pH spike solution was 10 mL of 10 N sodium hydroxide (base) or 30 mL 6 N hydrochloric acid. For this test, a sample of acid or base was added through the top port of the bag in a single pour. pH readings from each probe were collected until a stable baseline returned. Variables were agitator speed (rpm) and direction of rotation, CW (downflow) vs. CCW (upflow). The pH was tracked for 120 to 160 s following each addition to measure re-equilibration time. Data were saved and imported into Microsoft® Excel®. Each probe was assignment with a number and the pH data was used to determine the time interval for return to stable baseline following each pH spike.

XDUO automated pH adjustment study

A 500 L XDM Quad Plus bag was installed into the 500 L XDUO mixer and pH probes were installed into the appropriate ports. Test volume chosen for the 500 L XDUO mixer was 125 L. Water (RO/DI) was added to the 500 L XDUO mixer with a BPG-600 pump (Levitronix LLC, Waltham, MA, USA).

From a stock of 2 M glycine, pH 4.40, 1.30 L was added through the top port of the XDM Quad Plus bag. For each run, the XDUO agitator speed was set to 100 rpm in the CW direction. To check XDUO mixer pH probe accuracy, a sample was taken from the mixer for off-line pH measurement using a calibrated pH unit. C-Flex™ tubing with 1/8" i.d. was installed into both addition pumps, pump01 (200mM HCl) and pump02 (500 mM NaOH). A pH set point of 3.0 was entered and once the set point was reached, the agitation was held at 100 rpm for several minutes to ensure a stable pH. A pH set point of 7.0 was entered. Again, once the set point was reached, vessel agitation was held at 100 rpm to ensure a stable pH. The PID settings were P = 0.30, I = 0.50, D = 0.00, dead band (DB) = 0.05. For automated pH adjustment in 125 L, 128 kg of water (RO/DI) was added to the XDUO mixer.

pH adjustment steps were performed as follows:

1. Set-point pH = 3.0, with DB= 0.05. Once the pH set point was achieved, mixer was held for 5 min.
2. Set-point pH = 7.0 with DB= 0.05. Once the pH set point was achieved, mixer was held for 5 min.

Results

Studies of automated mixing and pH adjustment were performed in XDUO mixing system. Data was captured and imported into Microsoft Excel using the data historian software.

As shown in Figure 2, a total of eight pH probes were placed throughout the vessel for the mixing experiment. Table 1 shows the average time for equilibrating all eight pH probes.

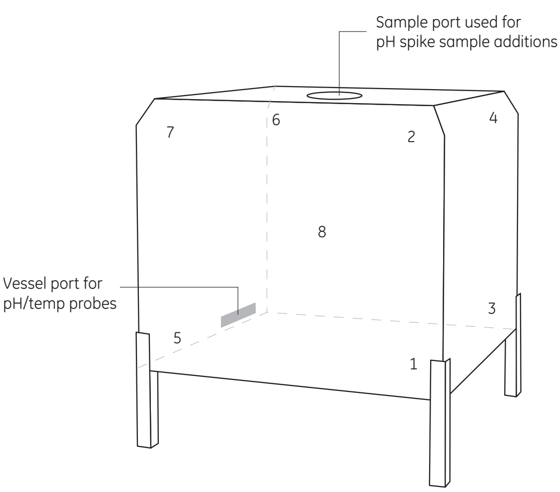

Fig 2. For the 500 L XDUO mixing experiment, eight pH probes were placed throughout the vessel. Probe 8 was located in the center of the vessel, probe 7 and 2 in the top front corners, probes 6 and 4 in the top rear corners, probes 5 and 1 in the bottom front corners, and probe 3 in the bottom rear corner. pH measurements were conducted at one-second intervals. Agitator speed was varied at intervals of 50 rpm, from 50 rpm through 200 rpm. CW (down) and CCW (up) directions were tested at each agitator (rpm) interval.

Table 1. Average time to pH equilibration

Agitator direction	Agitator speed (rpm)	Average time to equilibrate all 8 pH probes (s)
CW	50	71
CCW	50	70
CW	100	58
CCW	100	60
CW	150	51
CCW	150	46
CW	200	43
CCW	200	40

The study of automated pH adjustment was performed in 20 mM glycine, pH 4.40 with the starting volume 125 L. Inputs, entered remotely, were: first pH set-point = 3.0, DB = 0.05, second pH set-point = 7.0, DB = 0.05.

For pH 3.0 adjustment, 2.69 L of 200 mM HCl was added over 31 min to a final pH of 3.03. For the pH 7.0 set-point, 1.70 L of 500 mM sodium hydroxide was added over 19.5 min to reach the final pH 6.98.Table 2 summarizes the process parameters and the result of a fast and accurate pH adjustment. The automated pH adjustment process is displayed in Figure 3.

Table 2. Summary of automated pH adjustment of 125 L 20 mM glycine, pH 4.40 performed using the XDUO mixer

Start: 125 L 20 mM glycine	
Total pH adjust time	0:50
Adjust time to pH 3.0	0:30
Adjust time to pH 7.0	0:19
Initial pH, obtained value	4.40
pH 3.0, obtained value	3.03
Final pH, obtained value	6.98
Initial volume (L)	129
pH 3.0 volume (L)	2.69
pH 3.0 to 7.0 adjust volume (L)	1.70
Final volume (L)	133
Total volume (L)	4.39
Tubing size used for pH adjustment (i.d.)	1/8”
Pump01 (200mM HCl) Average flow rate to pH 3.0 (L/min)	0.09
Pump02 (500mM NaOH) Average. flow rate to pH 7.0 (L/min)	0.09

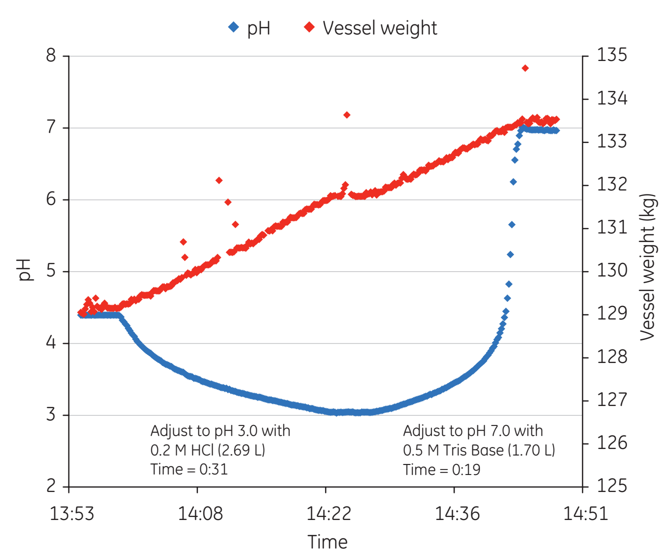

Fig 3. Automated pH adjustment of 125 L of 20 mM glycine, pH 4.40 using XDUO mixing system.

Conclusions

The XDUO mixing system integrates a robust, single-use mixer with an on-board automation platform enabling process excellence. XDUO is capable of fast, robust liquid-liquid mixing for applications such as pH adjustment.

Manual execution of pH adjustment can jeopardize product integrity and ultimately process success because of risks from pH deviations, repeated sampling, protracted process time, and product loss due to accidents during manipulation. Accurate, automated pH adjustment in the XDUO mixer can eliminate these risks and simplify viral inactivation by containing, mixing, and monitoring the product while automating the pH adjustment steps.

Reference

1. Shukla, A.A. *et al*. Eds., Process Scale Bioseparations for the Biopharmaceutical Industry. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA (2007)

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