Dealing with Disparity in On-line and Off-line pH Measurements

Genentech found pH drift in its on-line measurements for a cell culture process, and continues to investigate its cause.

By Heather Evans and Tina Larson, Genentech, Inc.

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Measuring and controlling pH is one of the most common and robust process analytical technologies. However, even with such an established technology, there are still challenges in controlling pH for large scale cell culture processes. pH is often a critical parameter in cell culture process. Very small variations in pH can have significant effects on cell metabolism and product quality. The need for robust pH control makes it imperative that biochemical manufacturers have a solid understanding of all factors involved in pH control.

Several factors need to be considered in understanding the on-line pH measurement of cell culture, such as the sterilization of the probe, the single point calibration method used to calibrate a probe used in an in-process culture, and the off-line pH measurement of samples taken throughout the culture duration. Certain factors can cause a disparity between the on-line and off-line pH measurement including a drift in the on-line probe, sample handling, and the instrument used to measure off-line pH. This paper will discuss a recent case study involving pH control in a large-scale cell culture process.

Background

pH is measured and controlled in the manufacturing setting using a pH probe, a transmitter, a controller, and acid and base. The probe measures a signal in mV, which is sent to the transmitter, which is then converted to an appropriate value and sent to the controller. The controller then determines the response and sends the appropriate signal to control pH. Acid or base is added to the culture as a result.

Comparison of the on- and off-line pH values for the cell culture process for Product A has demonstrated a divergence in value towards the end of the run (Figure 1, below). The reason for this divergence is unknown but is consistent with other cell culture processes run in this facility, although not all processes run in this facility demonstrate this same phenomenon.

pH is often a critical process parameter in cell culture because it can affect growth, productivity, and key product quality attributes. It has been shown that controlling the pH to even within ±0.20 pH units can still have an impact on culture metabolism and productivity, which may be of a concern if the pH control system cannot maintain less than a 0.20 pH unit difference between the on-line and off-line measurements (1).
Controlling pH to within ±0.10 pH units may be more appropriate to ensure more consistent and robust process performance in terms of both productivity and product quality.

Materials and Methods

The BioProfile 200 (Nova Biomedical, Waltham, MA) is used to measure off-line pH in the manufacturing facility. A SevenMulti bench top pH meter (Mettler-Toledo, Columbus, OH) was used for an additional comparison to the BioProfile pH measurement and the on-line pH measurement. The bench top pH meter was calibrated using pH buffer 7.0 and 10.0 prior to each use. The on-line pH probe used is Model F-615-B130-DH pH FermProbes (Broadley-James, Irvine, CA). Samples were taken at the beginning of each run, prior to the first manipulation, and at the start and end of the pH drift for three runs.

pH Disparity

A comparison of the on-line and off-line pH showed a divergence starting on day 8 of the production culture for Product A. The same sample used to measure the off-line pH via the BioProfile 200 was used to measure the pH via a bench top meter, which confirmed that the downward pH drift of the sample is real (see Figure 1 above). This data implicates either sample handling as the cause of the drift or the pH of the culture actually drifts in the vessel but this drift is not observed with the current on-line pH measurement methods. The time and mixing effects of sample handling were ruled out as the cause of the pH drift in the off-line sample both early in the culture and later in the culture (see Figure 2 below).
Figure 2: Samples were taken from the tank (Product A) and the pH was measured every minute for 10 minutes using the bench top meter located at the sample valve. The final data points are the pH measurements of the sample after it was inverted 3 times and >10min after it was pulled from the bioreactor. This was determined by moving the bench top pH meter near the sample valve to minimize the time between pulling the sample and measuring the off-line pH. The pH was measured every minute for 10 minutes immediately after the sample was pulled, and measured again after the sample was inverted three times to simulate expected mixing of the sample.

Figure 3: Comparison of the On-Line and Off-Line pH for Product B before and after pH cable change-out. The dashed lines represent trends prior to the pH cable change out.
It is likely that the drift is due to compromising the integrity of the on-line probe during the duration of the culture. This drift is sometimes seen with other processes with identical pH control strategies using the same on-line probes, which supports the idea that the drift is related to the probe. There is also data showing that the cable connecting the pH probe to the transmitter can impact the degree of deviation between the on- and off-line pH measurements (see Figure 3, above).

Conclusions

• There is a real disparity between the on-line and off-line pH measurements that cannot currently be explained.

• The off-line downward trends have been confirmed using two different types of pH measurement methods.

• The true pH drift is in the on-line measurement across products, although it is unclear what causes the drift.

Future Work

• Continue to investigate the validity of the on-line pH drift.

• Determine preventative maintenance schedule for cable swap out to eliminate impact of degradation of cable.

• Determine consistency of pH drift behavior between tanks for several products using the same pH control strategy.

• Investigate other on/off-line pH measurement methods.

• Investigate other possible mechanisms of pH drift including the effects buffering capacity of the medium may have on the culture pH measured and pH control.

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References

1. pH: Reducing Error Comparing In-line versus Off-line Techniques, *Mettler-Toledo publication*