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Qualification of low drift single-use pH sensors for use in single-use bioreactor platforms

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QUALIFICATION OF LOW DRIFT SINGLE-USE PH SENSORS FOR USE IN SINGLE USE BIOREACTOR, MIXER, AND FERMENTER PLATFORMS

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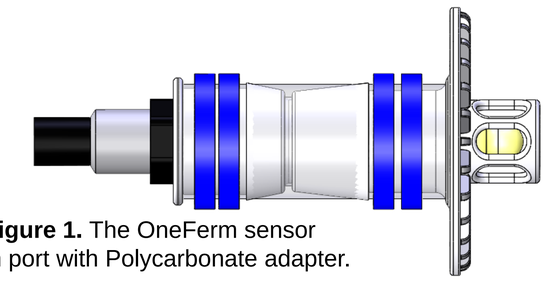

Figure 1. The OneFerm sensor in port with Polycarbonate adapter.

ABSTRACT

The biopharmaceutical industry is currently limited by access to low drift, gamma stable, easy-to-integrate single-use pH sensors for application in single-use bioreactors (S.U.B.). Hamilton Company has developed the OneFerm VP 70 sensor, a unique single-use glass electrode, which provides an additional option for customers wishing to implement reliable single-use pH sensing capabilities in the S.U.B. platform. In order to demonstrate the efficacy of these devices, a series of qualification experiments were carried out using a Thermo Fisher Scientific 50L bioprocess container (BPC) custom-fitted with Hamilton OneFerm sensors. BPCs were manufactured in a cGMP facility and allowed to age for a pre-established period (either 30 or 180 days). A 14 day fed-batch cell run was executed using an in-house CHO-S cell line (mAb producing clone) and standard operating conditions. Online pH was controlled with a Hamilton EasyFerm pH sensor; reactor pH was controlled using CO_2 without acid or base. Each BPC was built with six OneFerm sensors (containing two each from three different production lots), which were monitored using stand-alone transmitters. Offline samples were evaluated every 24 hours using an Oakton pH sensor.

INTRODUCTION

This work summarizes the results of a study evaluating the performance of the Hamilton™ OneFerm™ single-use pH sensor in the Thermo Fisher Scientific Single-Use Bioreactor (S.U.B.). Data supporting the efficacy of the OneFerm sensor is provided, as well as recommendations for the use of the sensor in single-use bioreactors, fermentors, and mixers.

The implementation of new process analytic tools is consistent with Thermo Fisher Scientific's continuous improvement process and open architecture support. Hamilton Company's OneFerm pH VP 70 sensor (part number 238999-4955) is a single-use glass electrode (Figure 2) that provides an additional option for customers wishing to implement single-use pH sensing capabilities in the S.U.B. In order to validate the functionality of these sensors, a 14-day fed-batch cell run was successfully performed using a S.U.B. BioProcess Container (BPC). The BPC contained a total of six OneFerm sensors (replicates from three different manufacturer lots), with five of the six meeting the manufacturer's published specifications. Following the cell run, the sensors were maintained in sterile cell culture media for a period of 60 days. Five of the six sensors performed according to the manufacturer's specifications after the completion of the worst-case test battery. It is recommended that critical process parameters, such as pH, always employ redundant sensors in each bag.

Figure 2. Hamilton OneFerm single-use pH sensor.

To incorporate the Hamilton OneFerm sensor within the Thermo Fisher Scientific S.U.B., a custom port adapter (SV21527.01) was selected as the most robust method for integration into the BPC. This sensor adapter is presently machined polycarbonate and protects the probe from damage during handling of the BPC. The 13.5 PG threaded pocket includes an integral o-ring sealing surface and also provides necessary fluid flow around the pH electrodes to allow for proper sensor reading of the process. The adapter inserts into a 2.54 cm (1 in.) port (SV20522.01 or SV21222.01). A section of silicone adaptor (SV21559.01) is used to connect the adapter to the port, with cable ties used to secure the connection (Figure 1). This adapter design demonstrated pressure resistance up to 1.03 bar (15 psi). Please note that the functional results presented here were performed with early adapter prototypes. The prototypes tested are deemed equivalent, but did not reflect all of the improvements captured with the final design of the probe adapter, as presented here.

Figure 3. Thermo Fisher Sientific's Hyperforma S.U.B. 250 L and Finesse G3 Lite controller.

MATERIALS AND METHODS

Table 1. Culture components and cells

Components	Description
Cell Line	CHO Cells (CHO-S 5B9)
Media Composition	Gibco™ Dynamis
Base	0.5N NaOH

Sensor Validation

In order to validate OneFerm functionality in the S.U.B., BPCs were created using Thermo Fisher Scientific production standards. Each BPC was built to include six OneFerm sensors, two each from three different manufacturer lots. The BPCs were subsequently sterilized using gamma irradiation (single dose, 25-40 kGy) and subjected to a 30-day aging period.

A 30-day aged BPC was employed in a 14-day fed-batch cell run in a 50 L S.U.B. A CHO-S cell line (mAb producing clone) was utilized in this experiment. Cells were cultured using Dynamis AGT media, and the S.U.B. was fed a constant rate of Efficient Feed C plus with concentrated glucose, as necessary$_2$. A gassing strategy of pure oxygen with secondary feeds of nitrogen and carbon dioxide was used to manage dissolved oxygen levels and control pH. No acids or bases were employed as part of the pH control strategy. A new Hamilton EasyFerm™ pH sensor (sterile autoclaved via the probe port kit) was used for online measurement and control of pH within the vessel. The readings from the single-use OneFerm sensors were recorded using standalone transmitters (Thermo Fisher AquaPro, 4 channel). Offline pH readings were performed every 24 hours using a BioProfile FLEX. Individual readings of each OneFerm sensor, as well as online and offline readings at each 24 hour time point, are shown in Figure 4.

Table 2. Bioreactor Conditions

Components	Description
Benchtop Bioreactors	2L Glass DASGIP
Single Use Bioreactor Hardware	Thermo Scientific™ HyPerforma™ 50L Enhanced S.U.B.
Singe Use Bioprocess Containers	Standard S.U.B. 50L BPC Agis 5-14 film Customized with OneFerm pH (SV21484.02, Pt100)

Culture monitoring

Samples were taken daily to measure cell density, viability, and growth rate. This was done by pulling a sample of approximately 10mL from the reactor to clear the sample line, discarding it, and then pulling a fresh 10ml sample. Of that 10ml sample, 1ml of the supernatant was run on the Nova Biomedical Bioprofile FLEX to measure nutrient/metabolite consumption and waste production, the majority of the solutions was measured for viable cell density using a ViCell, and a few ml were kept for frozen samples if later analysis was required.

RESULTS

The results from the initial 30 day aged BPC evaluation demonstrate functional activity of the OneFerm sensors over a 14 day fed-batch cell run using a TruBio DeltaV S.U.B. controller. Functional stability of these sensors was demonstrated by maintaining the devices in sterile culture conditions for a period of 60 days; five out of sixsensors met all manufacturers' specifications during this hold period.

To determine if BPC storage time contributes to loss in sensor functionality, a 14 day fed-batch cell run was repeated using a 180 day aged BPC. All six OneFerm sensors met manufacturer's specifications after the cell run with no sensor exhibiting a gross pH drift greater than 0.11 during the entire 14 day period. Additionally, all 6 sensors also met manufacturer's specifications during the 60 day hold period with no sensor exhibiting an average gross pH drift greater than 0.15 during this extended time. Furthermore, all sensors demonstrated an average response time of less than 10 seconds following the 60 day hold period.

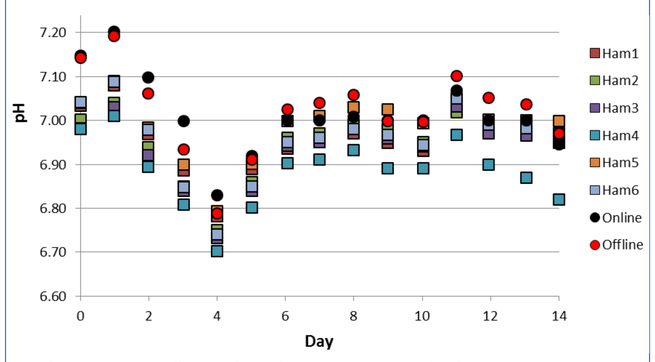

Figure 4. Readings of pH from OneFerm single-use sensors (Ham1–Ham6), the online EasyFerm reusable sensor, and the offline FLEX system over 14 days of testing.

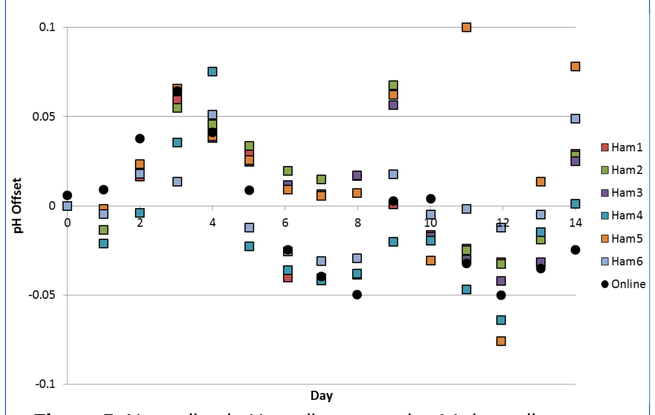

Figure 5. Normalized pH readings over the 14 day cell run. Readings are from OneFerm single-use sensors (Ham1–Ham6), the online EasyFerm reusable sensor.

Table 3. pH data for average observed variance in comparison to suppler specification documents through 60 days of continuous monitoring.

Parameter	Supplier specification	Observation (best/worst)
Precision vs. online	+/- 0.10 pH	+/- 0.00 pH / 0.17 pH
Precision vs. offline	+/- 0.10 pH	+/- 0.00 pH / 0.15 pH
Raw drift (7 day)	+/- 0.10 pH	+/- 0.02 pH / 0.07 pH
Raw drift (14 day)	+/- 0.20 pH	+/- 0.01 pH / 0.15 pH
Raw drift (23 day)	+/- 0.30 pH	+/- 0.07 pH / 0.28 pH
Raw drift (52 day)	+/- 0.70 pH	+/- 0.09 pH / 0.20 pH
Raw drift (60 day)	+/- 0.80 pH	+/- 0.13 pH / 0.33 pH

This cell run confirmed functionality of the OneFerm sensors during a challenging 14-day cell culture S.U.B. batch, which was then maintained in sterile media for a total period of 60 days. This coincides with the manufacturer's recommended sensor application life. The S.U.B. was subjected to adjustments in temperature over the course of this period, with subsequent titrations performed to evaluate sensor accuracy. At the end of the 60-day period, sensors were removed from the S.U.B. and evaluated for accuracy and response time using pH standards.

Comparison of sensor performance life post-cell culture process resulted in all six sensors meeting specification at day 52, and five of the six sensors maintaining functionality at day 60. Furthermore, the sensors that maintained their function also demonstrated a less than 10 second response time to changes in pH.

Recommendations and product specifications

- The manufacturer has qualified the pH sensor for 18 months of dry storage post-irradiation. Until the actual test qualification has been completed using S.U.B. BPCs, a 2X safety factor is prescribed, with a 9-month shelf life recommendation.
- During long-term storage (more than two weeks), the gel in the reference electrode can pull away from the bulb due to gravity. For this reason, it is important that the BPCs be stored according to packaging labels to ensure proper performance.
- Operators and quality personnel should be aware that these sensors will inherently leach small amounts of KCl salt into the BPC. This particulate is a non-toxic salt originating from the reference electrode that may appear to be a foreign particulate.
- The BPC must be stored at the recommended temperature to ensure expected performance (4–40C).

Table 4. Product specifications and results

Requirement	Desired specification	As-tested results
Gamma stable	Tolerate > 25 kGy	30 kGy (+/- 3 kGy)
Biocompatible	USP class 6	Pass, internals also non cytotoxic
Packaging/transport	ISTA 2A	Pass
BPC dry storage	Up to 18 months	9 months (release with 2X SF)
BPC integration	1 in. port (SV21222.01)	Pass, also 1 in. EJ port
pH range	3-10 pH	3-10 pH
Temperature range	5-37˚ C	2-50˚ C
Pressure range	> 5 psi	> 15 psi
Pre-calibration accuracy	0.1 pH	0.1 pH (0.05 pH w/ one point)
Low drift	< 0.05 per 7 days	Confirmed (14-day fed-batch)
Temperature comp.	Integrated	PT-1000
Cable connector type	Keyed quick connect	VP-6 (twist lock)

CONCLUSIONS

The results of these experiments demonstrate the effectiveness of Hamilton OneFerm sensors in the Thermo Fisher Scientific HyPerforma S.U.B. platform. Thermo Fisher Scientific has since developed a custom polycarbonate probe port adapter to robustly integrate the OneFerm sensor in a S.U.B. BPC. Future work will continue to evaluate the performance of sensors aged in BPCs for 24 to 36 months. We are excited to share this growing body of data with the bioprocess industry as probe drift, ionic strength sensitivity, and shelf life have greatly limited implementation of single use pH over the past decade. These results appear to indicate a viable technology is now available and is suitable for cGMP bio manufacturing.

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TRADEMARKS/LICENSING