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SENSOR SWITCH

How disposables could change your manufacturing

Electrochemical sensors have dominated the market for years, but experts from **Finesse Solutions** argue single-use sensors can save pharma and biotech companies both time and money. **BARBARA PALDUS** and **MARK SELKER** of Finesse discuss making the switch to disposables

Future Pharmaceuticals **Why are single-use sensors such a hot topic? Electrochemical sensors have been used for many years. Aren't they good enough?**

BARBARA PALDUS Much of the promise of disposable bioreactors in bio-processing can only be achieved if real-time sensors that are reliable, accurate, low cost, and capable of maintaining the sterile barrier are developed. Currently, even the baseline complement of sensors — namely oxygen, pH, temperature and pressure — struggle to meet the aforementioned performance metrics. In general, standard electrochemical sensors are being employed in disposable bioreactors owing to a lack of viable disposable options. Specifically, many organizations that utilize large disposable bioreactors such as the Hyclone SUB, Sartorius STR or the Xcellerex XDR employ traditional sensors and suffer the consequences on their effectiveness and cost.

Traditional sensors must utilize a Pall Kleenpak or equivalent interface in order to autoclave and introduce traditional sensors into the single-use bioreactor. The Kleenpak has the downside of increased per-use cost and the risk of contaminating an already sterile, gamma-irradiated bioreactor bag. Typically, at least 10 percent of Kleenpaks leak, and in some geographies, like Asia, leakage rates can be as high as 90 percent. Leakage is very disturbing to end users.

It is also well known that the calibrations of traditional pH and dissolved oxygen probes generally drift after they are autoclaved. Another issue that has been noted using traditional sensors is noise ingress. Most pH probes are very high impedance devices (hundreds of MOhms) and therefore very susceptible to electromagnetic pickup and general noise ingress. Because there is no immediate contact to ground in a plastic bag as there is in the metal head-plate of a typical glass bioreactor, or in the side ports of large stainless steel vessels, noise ingress problems in single-use systems are aggravated. Similar issues have been reported with electrochemical dissolved oxygen probes which typically only generate currents of 60-85 nA (10-9 Amps) at 100 percent saturated oxygen and 25°C.

Single-use sensors are a hot topic because they address an unmet need and are one of the clear limiting factors in the more widespread adoption of single-use technologies. Optical sensors have the advantages that they can be engineered to be non-invasive or introduced into the bioreactor before gamma sterilization occurs, generally do not have stringent grounding requirements and can often provide enhanced performance over existing chemical or electrochemical methods. For example, sensors based on fluorimetry are now beginning to provide an alternative. These sensors are based on the fact that the fluorescence lifetime of many organo-metallic dyes is reduced or quenched by the presence of analytes, such as dissolved oxygen, dissolved CO_2 or ions for pH. Through the use of telecommunications-style optics and electronics, this simple physical principle can be utilized to detect the presence of the analyte in question. Currently, single-use optical sensors based on phase fluorimetry are available that detect dissolved oxygen and pH. Single-use dissolved CO_2 optical sensors are becoming more readily available.

FP Mark, it is common industry wisdom that optical sensors – especially pH – drift, and therefore many users are averse to using them. **Can you comment on this?**

MARK SELKER Two key optical sensor technologies for single-use bioreactors allow the measurement of dissolved oxygen and pH. Both techniques apply fluorimetry and utilize a dye which has a fluorescent lifetime and concomitant amplitude that is quenched by the presence of the analyte in question. The spot must be in contact with the bioprocess inside the bioreactor bag. The excitation radiation must be delivered to the spot, and the emission radiation from the spot must be delivered to the detection system. The optical and electrical system resides outside of the bioreactor bag. In competing systems, the techniques used are either amplitude-based fluorimetry and/or are based on fiber optics. Significant drift has been observed in fiber optic pH sensors, although some of the dissolved oxygen sensors on the market seem to perform adequately. The dyes used in all fluorimetric sensors to date are subject to photo-degradation. Specifically, the dyes' fundamental properties change when exposed to a combination of light and the analyte that quenches their fluorescence; this photo-degradation manifests itself as drift in the calibration.

While there are ways to chemically stabilize the dyes, these additives are often complicated

to construct and deploy, and can be toxic to cells. We have addressed the photo-degradation issue in the TruFluor product family by using only phase fluorimetry and we have utilized a patented free space optical design, instead of fiber optics. Namely, a large area detector does not have the light collection limits imposed by fiber optics and our design can capture a much larger amount of the emitted fluorescent light, thereby allowing us to use far less excitation light, approximately 20 times less than an optimized fiber-based system.

Using simple mathematical and fundamental physical arguments, we can show that the decrease in excitation light is proportional to a decrease in the photo-degradation rate. We have shown that with a very stringent test — about 10 days at 37.5 °C in 21 percent oxygen gas, as opposed to dissolved oxygen and liquid, and sampling every two seconds — there is less than a 0.5 percent change in the TruFluor dissolved oxygen reading. Therefore, a 30-day run should have the same drift when sampling every five seconds (a sampling rate that is more than adequate to capture any change occurring in the growth run). Similarly, we have shown less than 0.05 pH units of drift when sampling every second for six days, which is equivalent to five-second sampling in a 30-day run. Thus, we are confident that both of our dissolved oxygen and pH optical sensors have a performance that is comparable to, if not better than electrochemical sensors in their specified range of use.

FP **Are there differences in calibration between traditional electrochemical sensors and single-use optical sensors? Do single-use sensors have any advantages here?**

MS There are several complications that occur during the calibration of electrochemical sensors which affect their measurement efficacy. Most importantly, autoclaving electrochemical dissolved oxygen (dissolved oxygen) and pH probes leads to drift in the calibration.

The dissolved oxygen probe's Teflon membrane diffusivity can be changed by autoclaving, and its anode cathode pair can be aged. Additionally, not all membranes are identical, not even from the same vendor. Typical transmitters use temperature compensation based on the manufacturers' expected mean membrane diffusivity. In practice, the actual membrane's temperature response may be quite different from this assumed mean value. This fact is often hidden by the calibration process, which is performed at a static temperature; the user rarely has a reason to change the temperature while maintaining the dissolved oxygen level using another sensor as the standard. For a dissolved oxygen probe, if the zero point has drifted significantly after sterilization, only a full two point calibration will correct the reading. Typically, one point standardization is performed. The practice of disconnecting the probe from the cable to perform the zero point calibration is also common, but can be a dangerous in that it is possible to introduce gross error.

Electrochemical pH sensor calibrations change as the measuring and reference electrodes age. If the pH electrodes are not properly handled, incorrect cleaning or wet storage procedures for example, the chemical composition of the membrane glass and/or gel layer can change or the diaphragm of the reference electrode can become clogged. This pH electrode aging results in an increasing membrane resistance, a decrease in slope response and/or zero point drift, meaning a shift of the asymmetry potential. Because pH electrodes can drift during a process, users typically perform one-point standardization against an off-line reference such as a blood gas analyzer throughout a batch run. Typically, users will perform a two-point calibration on a pH electrode prior to a run, and use this calibration to determine if the slope response is sufficient to continue using the electrode.

The design intent of Finesse single-use probes was to minimize the need for user intervention and probe specific knowledge. The probes are therefore designed to need minimal attention from the engineer or operator in order to use and calibrate. Specifically, the goal is to maintain a United States National Institute of Standards and Technology (NIST) traceable calibration that can be validated to a cGMP standard.

The TruFluor dissolved oxygen and pH products have two components that affect the calibration accuracy and precision; the reader/transmitter pair and the sheath. The reader and transmitter are the fixed equipment while the sheath is the single-use component. The reader and transmitters have been characterized in great detail and every effort is made in production to ensure that they are interchangeable to a level of accuracy and precision that makes the change unnoticeable to the end user.

The sheaths register to the reader very tightly in all axes, and therefore the sheath to sheath repeatability is well controlled. There is some variation in the dye from spot to spot and from lot of dye to lot of dye. This is accounted for in our calibration process in which a minimum set of the sheaths is fully calibrated, but in which every single sheath is tested for performance and consistency.

The readers are all normalized to read the same value within a very small uncertainty so that they are interchangeable. The disposable sheaths are pre-calibrated in a computer controller station. The calibration is entered on a gamma radiation-resistant Smartchip that is embedded in each sheath. This system allows the calibration to be read in without user intervention and eliminates any confusion. If the Smartchip fails, Finesse can provide a backup RFID tag that is simply held to the transmitter and the system is calibrated. The Smartchip reader can also be disabled and the RFID reader can be used to read in special calibrations. Finally, the sheath has a 316L electro-polished stainless steel plate in the front which serves as a thermal window. This window and the reader provide a temperature measurement good to better than about a 0.25°C over the entire range of operation and good to better than 0.15°C if measuring within 15°C of ambient temperature. The temperature measurement can be further corrected as a one point offset or a full two point calibration if accuracy beyond this level is deemed necessary. This temperature measurement is used internally in the transmitter to correct for temperature dependence of the dye, but is also available as a process variable from the transmitter and obviates the need for a separate RTD.

FP There's been a lot of concern about leachables, extractables, and general USP certification of single-use sensors. **How are you addressing this?**

MS Finesse offers the TruFluor line of disposable optical sensors for detection of dissolved oxygen and pH with full USP Class VI testing for leachables and extractables documented. These sensors come completely pre-calibrated, as mentioned before, with NIST traceable standards.

The sensor dyes used have undergone full USP Class VI testing including heavy metals, cytotoxity, and implantation testing. The Smartchip that contains the calibration parameters also contains the sheath serial number and information on the material lot, dye lot and fabrication date information so that a lot can be traced by its serial number. For each sheath, all USP certificates can be downloaded from the

"IT IS OVERALL **LESS EXPENSIVE TO OPERATE SINGLE-USE SENSORS** BECAUSE THERE IS A LARGE AMOUNT OF OVERHEAD IN USING ELECTROCHEMICAL PROBES IN SINGLE-USE BIOREACTORS. ADDITIONALLY, AS OVERALL VOLUMES GO UP, THE SINGLE-USE SOLUTIONS WILL BECOME LESS EXPENSIVE."

Finesse Web site using the sheath serial number. This certificate package can be attached to the batch record. Thus, full traceability of the process is ensured for the single-use sensors.

FP **Barbara, can you comment on whether or not there are cost benefits to employing single-use sensors?**

BP Single-use sensors can provide significant cost savings for upstream bio-processing. Specifically, they are pre-inserted into the single-use bioreactor bag and gamma irradiated with the bag, so that the entire system arrives sterile and the time from unpacking the bag to inoculation is minimized. "Smart" sensors, such as Finesse TruFluor and TruTorr sensors, arrive in the bioreactor pre-calibrated and thereby minimize operator time during process setup.

It is interesting to look into the economics of the disposable element in a little more detail. In a basic cost comparison between the uses of a TruFluor dissolved oxygen optical probe and an electrochemical probe in a single use bioreactor, the total parts cost was $200 versus $187, respectively, in favor of the electrochemical sensor. The total time value of the single-use sensor was $0 versus $229 for the electrochemical sensor. Therefore, the total cost per batch was $416 for the electrochemical versus $200 for the TruFluor sensor.

The assumptions of our model were as follows: Initial capital outlay of "loop" equipment is similar, that is transfer cable and more was similar; traditional dissolved oxygen and temperature sensors are used about 25 times over their lifetime, about a year; the traditional sensor membrane is replaced every 10 runs; the value of time was estimated to be $23 per hour for an operator; and the value of a batch for a 250L reactor was about $150,000 for a three-week run, at 1 g/L titer.

Basically, the disposable solution has a parts cost that is 7 percent greater, but it saved the time required for handling the Kleenpak and sensor assembly, thereby resulting in overall cost savings of 52 percent per batch. An analysis for pH sensors yields similar economic results. Thus, it is overall less expensive to operate single-use sensors, because there is a large amount of overhead in using electrochemical probes in single-use bioreactors. Additionally, as overall volumes go up, the single-use solutions will become less expensive.

Additionally, the disposable sensors provide significant intangible benefits such as minimizing sterility breaches which can result in the complete loss of a batch. To reiterate what was stated previously, we have found that even trained users experience about 10 percent of their Kleenpaks leaking, resulting in contamination, whereas untrained users can have as many as 90 percent of their Kleenpaks leak. For a 1,000L bioreactor, where a batch can be worth up to $1 million, the cost-to-benefit ratio of single-use sensors can be substantial. **FP**

BARBARA PALDUS, PH.D, is the CEO of Finesse Solutions. Prior to Finesse, Ms. Paldus served as the Chief Technical Officer of Picarro, a company she founded in 1998 that developed a solid-state Cyan laser product in 2003 and cavity ring-down spectroscopy products in 2004. She is also currently a partner at Skymoon Ventures. Ms. Paldus received both her Ph.D. and MSEE degrees in electrical engineering from Stanford University.

MARK SELKER, PH.D, is the Chief Technical Officer of Finesse Solutions. He has worked in various areas within the optics industry, including analog and digital optical communication, near field optics/plasmonics and bio-optical systems. Mr. Selker has held positions at NASA, Coherent Laser Group, Harmonic, and Stanford University. He received his Sc.B. and Ph.D. in electrical engineering from Brown University.

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