Suitability of Selected Single-Use Process Monitoring and Control Technology

by Kevin J.R. Clark and Jim Furey

As disposable manufacturing technology moves beyond simple storage systems to more dynamic process unit operations, the need for single-use process monitoring and control is growing. Tubing pinch valves along with single-use dissolved oxygen (DO) sensors, pressure sensors, and flow meters offer possible alternatives to traditional technology. Points to consider with these different technologies include sterilization capabilities, process compatibility, control system integration, and performance.

Automated Tubing Pinch Valves

Automated pinch valves represent a possible alternative to automated diaphragm valves in processes where flexible tubing is used (rather than rigid piping). These valves are designed to pinch tubing closed in one position and allow liquid through in the other (Photo 1). They are typically available in "normally open" or "normally closed" configurations. Pinch valves are also manufactured in two-way valve configurations. Multiple two-way valves can be used together to replace multiway, multiport diaphragm valves.

Because there is no contact between valve and process fluid, pinch valves can be used in place of a diaphragm valve to eliminate the need for valve cleaning, disassembly for diaphragm replacement, and parts tracking. Process tubing is easily inserted into a pinch valve during set-up. If the valve is in a normally closed position, the method of insertion varies. Some models require tubing to be stretched slightly as it slides into the closed valve. Others require that the valve be opened manually to allow for tubing insertion. Either procedure takes a matter of seconds.

Valve sizes should be based on the size of tubing used. To operate properly, tubing's inner diameter (i.d.) and wall thickness should match with an appropriate valve so it both opens and closes optimally. The tubing's outer diameter may determine the required valve size, and its wall thickness may determine the default valve positions. Although a wide range of tubing types are compatible with pinch valves, compatibility requirements should be determined to ensure that tubing–valve interactions can meet process requirements.

Photo 1: Pinch valve
ACRO ASSOCIATES, INC. (WWW.ACROASSOCIATES.COM)

Silicone rubber tubing works generally well because when a valve is closed and then opened again, such tubing readily opens up. This has been the case even after thousands of open–close cycles. Our recent testing of electric pinch valves from Bio-Chem Valve, Inc. (www.bio-chemvalve.com) optimized for 0.25-in. silicone tubing showed no signs of wear after over 10,000 pinches while water was pumped through. Several types of tubing are classified as thermoplastic elastomers (TPEs). TPEs may start to deform after repeated openings and closings, so compatibility with a process should be determined. However, some processes do not require repeated valve cycling. One other consideration is that if a valve remains closed with tubing inserted for an extended period of time, that tubing should not "stick" closed when the valve is ultimately actuated to open. This may be affected by the type of process liquid involved. Most bioprocess applications do not involve such extended time frames in which tubing would stick together. If a process is extended, then valves are typically actuated more frequently than would cause a problem.

Integration of automated pinch valves into process hardware and control systems must be evaluated before a choice is made. Valves come

Table 1: Points to consider for automated pinch valves

Valve Type	Process Compatibility	Performance	Control System Integration	Sterilization Capability
Diaphragm	Wide range	Excellent for many applications	Pneumatically driven by control system solenoid	Traditionally by autoclave or steam-in-place
Electric pinch	Requires compatible tubing Low pressure Tubing size limitations	Excellent if tubing is used and qualified for application	Actuated directly by electric signal (available for voltages of 12 and 24) Appropriate mounting or housing required	Not applicable; only the tubing requires sterilization.
Pneumatic pinch	Requires compatible tubing Pressure can be higher than for electric valves Up to 1-in. o.d.	Excellent if tubing is used and qualified for application	Pneumatically driven by control system solenoid Appropriate mounting or housing required	Not applicable; only the tubing requires sterilization.

with electronic or pneumatic actuation, and the best choice depends on different factors. An electric valve has an on-board solenoid and can be wired directly; pneumatic valves require air pressure for their actuation through triggering of solenoid valves located elsewhere. For certain applications, an electric valve may be a "simpler" solution because it requires no instrument air or external solenoid valve. However, with an instrument cabinet and pneumatically actuated diaphragm valves in place, using pneumatic valves may be favorable. Both types of valves require appropriate mounting and/or housings. Also, pneumatic valves can handle larger tubing (up to 1-in. o.d.), which precludes the use of electric valves. One other consideration is process pressure. Obviously, limitations exist for all flexible tubing, but pneumatic valves generally resist opening when they are closed and exposed to higher pressures.

Table 1 evaluates valves according to the considerations listed above.

Dissolved Oxygen Sensors

Reliable measurement of dissolved oxygen (DO) is a critical requirement for culturing industrial cell lines. Several technologies currently exist for such measurements. Traditional DO probes are galvanic or polarographic, differing only in that the polarographic probe type requires an external voltage source for operation and the galvanic type does not. A technology recently implemented for the biotech industry is based on fluorescence. For biopharmaceutical production, the traditional and fluorescent DO sensing technologies have significant differences: e.g., ease of use, response times, method and reliability of incursion into sterile bags, effects of environmental influences, sensor sterilization techniques, and compliance with CGMPs.

Process environments for biopharmaceutical production can at times pose interesting challenges in DO measurement. For example, reactor conditions can significantly affect the reliability of such measurements. Temperature affects both traditional and fluorescent probes, but salinity affects only polarographic probes. Also, the flow field the probes are subjected to can affect DO measurements.

Traditional probes rely on the diffusion of oxygen through a permeable membrane, where oxygen is reduced (and consumed) at a cathode. Although the amount of oxygen consumed by the probe is small, a continuous resupply of oxygen from the culture is needed for reliable measurements. However, oxygen is not consumed in the fluorescent method. Fluorescent DO probes rely only on the presence of oxygen in a polymer patch to quench the emissions of fluorescent molecules. Because oxygen is not consumed, DO can be measured in both static and low-flow applications.

Also of note when comparing probe technologies is the effect of environmental conditions on DO probe cables. Traditional cables can be sensitive to movement and electrical interference. They are inexpensive, however, and electrical noise interference can generally be isolated. On the other hand, many fluorescent probes use fiber-optic cables. And although fiber-optics are not susceptible to electrical interference, they are both fragile and expensive.

An ease-of-use argument can be made for both polarographic and fluorescent probes, but fluorescent probes do have an advantage in cleaning and replacement. Traditional probes require replacement or cleaning of their membranes when response times decrease. Cost is a consideration with polarographic probes; thus, simply disposing of them is generally undesirable. Traditional probes may be serviced when cleaning no longer produces acceptable results. But anecdotal evidence tends to push users away from the reconditioning of DO probes in high-value applications. Meanwhile, fluorescent probes are simple to replace with a relatively inexpensive patch and mechanical support. Interestingly, the disposable nature of fluorescent polymer patches may be the most significant selling point for such optical probes.

Disposable bioprocessing has gained significant interest in recent years, and disposable bioreactors have

Figure 1: Comparing three DO measurement methods

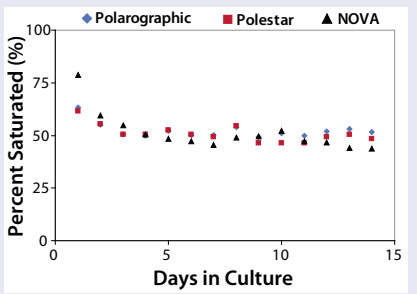
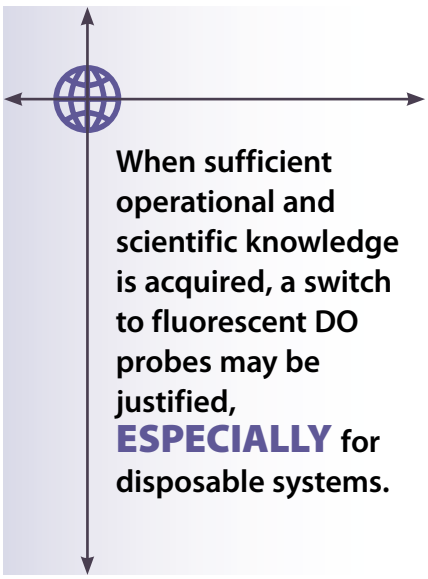


Table 2: Points to consider with DO sensors for cell culture monitoring

Sensor Type	Process Compatibility	Performance	Control System Integration	Sterilization Capability
Traditional DO probe	Well-established compatibility Requires port for probe insertion	Currently used in most processes	Easy integration with control systems	Traditionally by moist heat in autoclave or SIP
Fluorescent DO probes	For CGMP use, leachable profiles and toxicology evaluation must be evaluated Can be attached to inner surface of disposable container where measurement is desired (reading is made externally)	May provide performance advantages Areas to be further explored include accuracy, frequency of recalibration, and response times	Easy integration to control systems from monitor	Compatible with gamma sterilization Moist-heat sterilization degrades probe (six to eight cycles only)



made significant inroads with the biopharmaceutical industry. A significant technical hurdle to their implementation is the need for breaking sterile boundaries for adequate mixing and probes for critical process parameters such as DO, pH, and temperature. It is here that the fluorescent DO measurement technology has a distinct advantage. The polymer patch in fluorescent probes can withstand gamma radiation, so they can be inserted into disposable process containers and sterilized along with such vessels. Traditional polarographic probes must be polarized, autoclaved, calibrated, and inserted into a gamma-irradiated bag. Certainly traditional probes are feasible for use in bags with either pump-around manifolds or technologies such as Pall Kleenpak connectors. However, the risk of microbial or other contamination from frayed silicone tubing is a strong deterrent. So embedding fluorescent polymer patch technology inside a bag and measuring fluorescence through it is an alternative worth consideration.

Fluorescent DO probes present a unique opportunity, but there are still significant obstacles preventing their widespread use in development and manufacturing organizations. New technologies break into the GMP arena slowly. Manufacturing organizations often only grudgingly accept new technologies — and justifiably so, in the case of fluorescent DO technology. Leachable and extractable data on such probes has yet to become widely available. But the technology could help eliminate the requirement for an autoclave (with its associated capital and ongoing cost) from a cell culture production facility without compromising the quality of process data.

Finally, it will be of paramount importance to determine the comparability of the new optical technology with polarographic probes and the widely used BioProfile chemistry analyzer by Nova Biomedical Corporation (www.novabiomedical.com). Unreliable or inaccurate probes will be inappropriate for use in GMP-compliant cell culture. Figure 1 compares a traditional polarographic DO probe, an optical DO monitor probe from Polestar Technologies, Inc. (www.polestartech.com), and an off-line BioProfile analyzer over a period of two weeks, with no probe adjustments.

Agreement between the two in-situ technologies is reasonably good, averaging 9% difference over the life of a single culture. So when sufficient operational and scientific knowledge is acquired, a switch to fluorescent DO probes may be justified, especially in disposable systems.

Table 2 evaluates DO probes according to the considerations listed at the beginning of this article.

Pressure Sensors

Knowledge of process pressure is a valuable piece of information for a wide range of bioprocess unit operations. For automated pressure measurements in fluid streams or vessels, a stainless steel pressure measurement device with an integral transmitter is typically used. Depending on the process application, the product contact surface of the device requires either sanitization or moist heat sterilization. The device is connected to the process most frequently by an integral sanitary fitting. Some devices are compatible with steam-in-place (SIP), in which only the product contact surface is exposed to steam and even to devices that can be placed in an autoclave where the entire device is exposed to steam. However, if sterilization is required, many single-use process components are not compatible with moist heat sterilization temperatures, so there may be a requirement for separate sterilization of the stainless steel pressure transmitter device and possibly nonoptimal connection to a presterilized disposable assembly. Even if a process is only sanitary (not sterile) and uses tubing with a small

Photo 2: Disposable pressure sensor
UTAH MEDICAL PRODUCTS, INC. (WWW.UTAHMED.COM)

Figure 2: Deltran 1 disposable pressure sensor test data with 5% full scale (37 psi) error bars

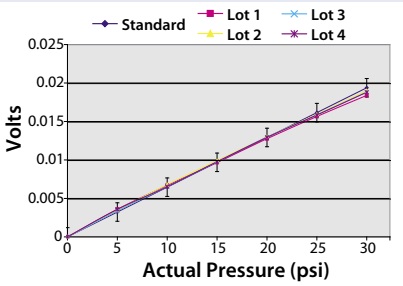


Table 3: Results of pressure cycling testing of pressure sensor

Time	Atmospheric Reading	Reading at 25 psi Applied Pressure[1]
Start	0.0 psi[2]	24.6 psi
8.3 h[3]	0.0 psi	24.6 psi

[1] set on calibrated gauge
[2] calibrated to 0.0 psi at start of test
[3] 1000 cycles

Table 4: Results of static pressure test at 30 psi pressure sensor reading

Time	Atmospheric Reading	Reading at 30 psi Applied Pressure[1]
Start	0.0 psi[2]	28.8 psi
13 h	0.0 psi	28.8 psi

[1] set on calibrated gauge
[2] calibrated to 0.0 psi at start of test

i.d., it can be cumbersome to connect a pressure measurement device with a sanitary fitting to a process stream.

Disposable pressure sensors sterilized by gamma irradiation or ethylene oxide (ETO) have been available for many years in medical and critical-care applications such as on-line blood pressure monitoring. Some companies have adapted them for use in process development and small-scale operations of the bioprocessing industry. We tested Deltran I pressure sensors (Photo 2) from Utah Medical Products, Inc. (www.utahmed.com) to evaluate their compatibility for use in applications outside the medical field, for which they are designed.

Deltran sensors use a silicone diaphragm in a Wheatstone bridge circuit placed in the fluid path of a medical device. Applied voltage to the circuit gives a voltage output directly proportional to pressure. The device has an integral 12-inch cable that connects to a second, reusable cable, which in turn connects to a pressure-monitoring system. For medical applications, the manufacturer suggests an operating range of –0.97 psi to 5.8 psi, with a specified sensitivity of ±2% (typically <1%). The sensors are specified to be overpressure-protected from –7.7 psi to 77 psi. To establish a suitable range for use in other applications, we tested sensors from four different lots at different pressures to determine their accuracy above the typical operating range but below the overprotection limits. Testing was performed with regulated static air pressure and factory span calibration. Figure 2 shows the results.

Above 30 psi, drift increases between the actual pressure and the sensor reading with rising pressures. Error bars indicate ±5% of a 37-psi full-scale range (using a range between –7 psi and 30 psi). A two-point calibration (rather than the factory calibration) could be used to give better accuracy at pressures closer to 30 psi. With the four lots tested, we demonstrated repeatability of performance.

To test the response of the pressure sensors over time at pressures higher than their typical operating range, we conducted two more tests. One test cycled air pressure to 25 psi for 15 seconds, then removed the applied pressure for 15 seconds, and was repeated for 1000 cycles over a period of 8.3 hours. Resulting data appear in Table 3, showing no apparent impact on performance of cycling the sensor repeatedly to a pressure above its typical high-end operating-range pressure of 5.8 psi. Using the same sensor, we applied a static pressure of 30 psi for 13 hours in a second test. The results in Table 4 indicate no change in readings stability (including the zero reading shown when pressure is removed) after a period of pressurization outside the manufacturer's typical high-end operating range pressure of 5.8 psi.

The sensors are currently offered both in a nonsterile format and sterilized by ETO. However, when they are used as part of a disposable assembly in a bioprocess application, the preferred method of sterilization is gamma irradiation. The polycarbonate used in the product contact surfaces is gamma stable, so we did some initial performance testing of these pressure sensors after exposure to a gamma radiation dose of 25–40 kGy typically used for sterilization of disposable assemblies in bioprocess applications. Three sensors were irradiated, with no performance difference detected afterward (data were similar to those in Figure 2).

Our test results are positive indications of reliability in performance of these sensors at pressures in the ranges tested. This testing represents part of the fitness-of-use testing that would need to be

Table 5: Points to consider for disposable pressure sensors

Device	Process Compatibility	Performance	Control System Integration	Sterilization
Stainless steel pressure transducer	Wide range but can be difficult with small flow paths May be issues with sterile connection to disposable process	Excellent; wide range of devices available	Wide range of applied voltages possible with standard output signals (e.g., 0–5 V or 4–20 milliamps)	Traditionally by autoclave or SIP
Disposable pressure sensor	Good for measuring static pressures For use in line, currently requires adaptation to larger i.d. flow paths Meets relevant standard for biocompatibility for medical use applications	Testing indicates some positive results at pressures below 30 psi More testing required for bioprocess applications, must be qualified for application	Requires narrow range of applied voltage Nonstandard field output signal (device-to-control system integration may be required)	Compatible with ETO sterilization and initial test results with gamma radiation sterilization are positive

Table 6: Points to consider for disposable flow meters

Device	Process Compatibility	Performance	Control System Integration	Sterilization
Nondisposable flow meters	Wide range	Excellent wide range of devices available	Easy integration to control systems	Traditionally by autoclave or SIP
Disposable rotary-vane flow meter	Flow meter output affected by shifts in liquid viscosity Applications with high solids, high viscosity, or opaque liquids must be tested Materials meet USP Class VI	Claimed <±1% with proper calibration	Requires standard voltage input (5V) to electronic assembly and a digital pulse accumulator to count pulses	Compatible with autoclave or SIP Materials are compatible with gamma radiation

Photo 3: Disposable rotary blade flow meter
DIGIFLOW SYSTEMS (WWW.DIGIFLOWSYS.COM)

conducted by a user to evaluate product suitability for a particular application.

Table 5 evaluates pressure sensors according to the considerations listed at the beginning of this article.

Liquid Flow Meters

A wide variety of flow meters use different measurement principles to measure liquid flow rate. Some are based on mass flow and some on volumetric flow. One flow meter (typically called a turbine or rotary blade flow meter) has a rotating blade assembly placed in a flow path to rotate in direct proportion to the liquid flow rate. Digiflow Systems (www.digiflowsys.com) offers a disposable version in two different i.d. sizes for a flow-rate range of 70 mL/min to 25 L/min (Photo 3).

The liquid-contact part of this flow meter resembles a hose barb connector, and an electronic assembly attaches to it for use. The externally mounted electronic assembly counts blade rotations by interruption of a light beam, and the results are directly proportional to flow rate across a specified linear range. The manufacturer claims an accuracy with water of <1%. Each flow meter is calibrated with water defined in pulses/L. The meters can be calibrated for other liquids at varying temperatures within a broad operating range of –15 to +85 °C (5–185 °F). They are manufactured from polyvinylidenefluoride (PVDF), which is material typically used in bioprocessing that is indicated as being stable after exposure to gamma radiation (**1**).

One disadvantage of this type of flow meter is that if liquid viscosity changes during a process (by either a shift in temperature or a changing liquid composition), the calibration of pulses/L will change. So a flow-rate change could be indicated at the same actual volumetric flow. One other concern is that liquid streams with a large quantity of solids or very high viscosities can occlude the use of these meters (at viscosity ranges typically not seen in bioprocessing). Opaque liquids can interfere with measurement of blade rotations, but some adjustments may be possible for use with consistently opaque liquid streams.

Disposable rotary blade flow meters must be tested if there will be temperature shifts or changing liquid composition in a process. Constituents of the liquid stream must not be allowed to interfere with the performance of the device. If a change in liquid composition or temperature does not cause flow-rate shift beyond a desired percent variance, then the same calibration can be used. If the resulting shift is outside that desired percent variance, then a new calibration is necessary. A control system could be used to adjust the calibration constant of pulses/L at different points in a process if known liquids will be used and if the temperature is steady (or its impact on the constant is known).

Table 6 evaluates liquid flow meters according to the considerations listed at the beginning of this article.

Great Potential

Currently the technologies described here are at different levels of readiness for integration into biopharmaceutical processes. All will require application qualification as the industry accumulates more experience and knowledge of their limits of operation. As these single-use monitoring technologies progress, they should be able to perform in disposable manufacturing processes — perhaps more efficiently than their reuseable counterparts — without sacrificing the quality of process control capability or process data.

Reference

1 Radiation Sterilization Working Group (AAMI Sterilization Standards Committee). *Radiation Sterilization — Material Qualification*. Technical Information Report No. 17–1997. Association for the Advancement of Medical Instrumentation: Arlington, VA, 1998.

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