Accuracy Guide

Operating Principles and Practice for Optimal Performance

Optical Dissolved Oxygen Sensors
pH Sensors

Quality Measurements for In-line Pharma Applications

Hints and Tips to Improve Accuracy

In DO and pH Measurement

METTLER TOLEDO

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1. Introduction

High quality and efficacy of products and efficiency in production are the main goals in pharmaceutical manufacturing. To achieve these aims, production processes must be stable, predictable and operate consistently at the target level of performance.

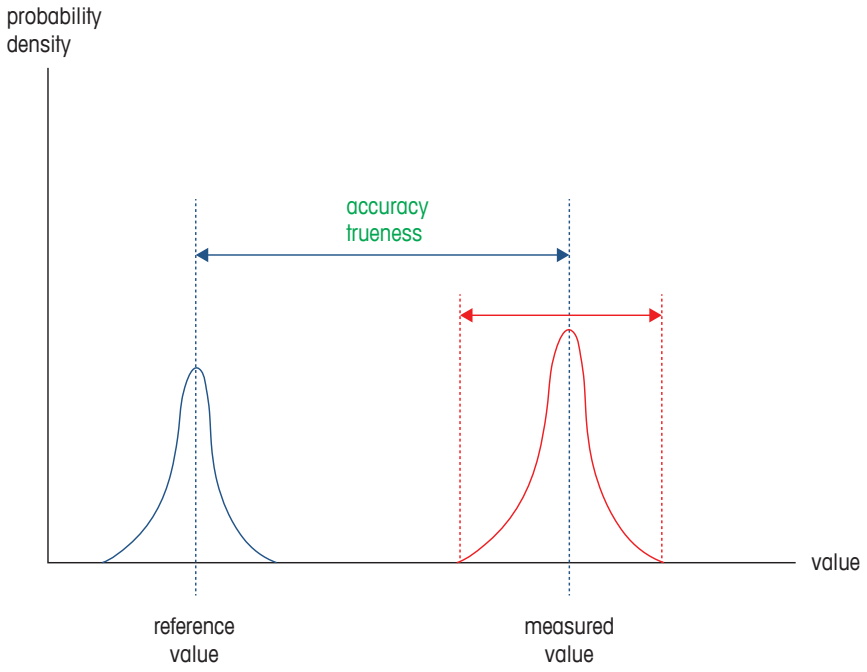
Process analytics using in-line sensors plays a major role in monitoring manufacturing to ensure the required process conditions are always being met. Obviously, reliability of the data from sensors correlates with sensor measurement accuracy.

Accuracy is not solely dependent on the use of high quality sensors. How sensor maintenance and calibration are conducted significantly influence a probe's ability to measure dependably. In fermentation or cell culturing processes, assurance that sensors will output reliable measurements throughout a batch is particularly critical.

This guide covers good operating procedures and includes advice for maintaining in-line pH and dissolved oxygen (DO) sensors to ensure high measurement reliability at all times.

2. Accuracy and precision

Whereas accuracy is the proximity of measurement results to the true value; precision is the reproducibility of the measurement.

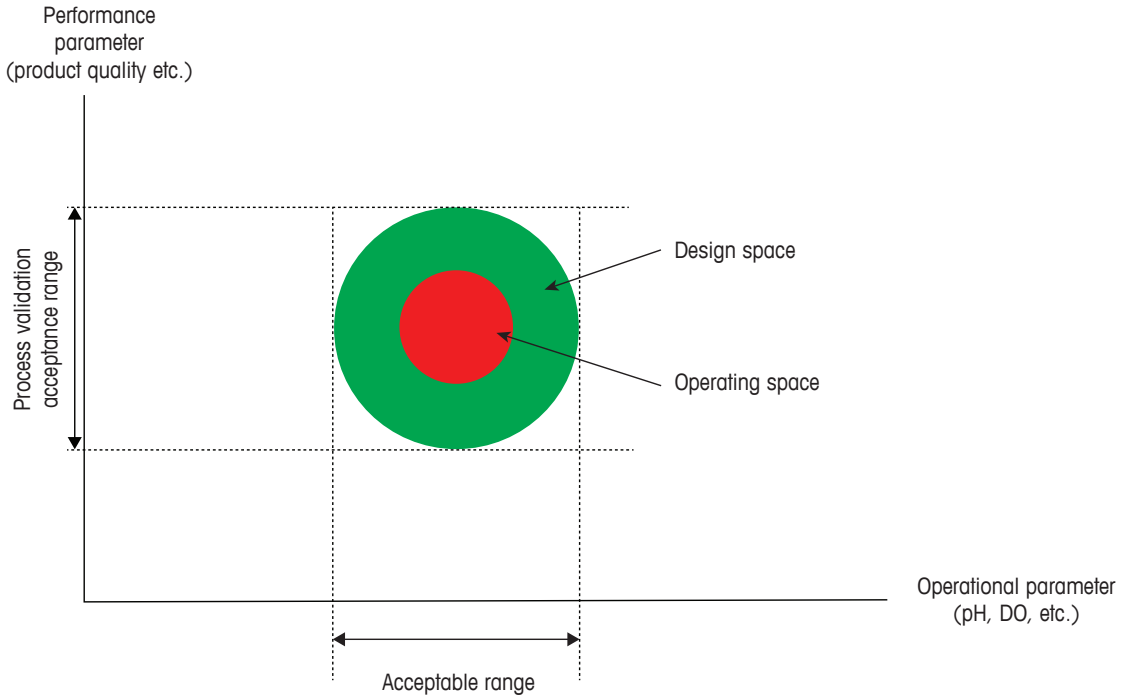


If a measurement is precise it does not mean that it is also accurate. Depending on the required precision, the opposite is also true. High accuracy can therefore be defined as a true value that is reported with great precision.

In fermentation or cell cultures often only one pH or DO sensor is installed. It is not possible to determine measurement trueness in such situations (unless an off-line measurement is taken). However, a pH reading to one decimal place is sufficiently precise for most applications. Only if two or more pH or DO sensors are installed redundantly can we have confidence in the transmitted values. Double measurement is therefore more common in demanding mammalian cell cultures.

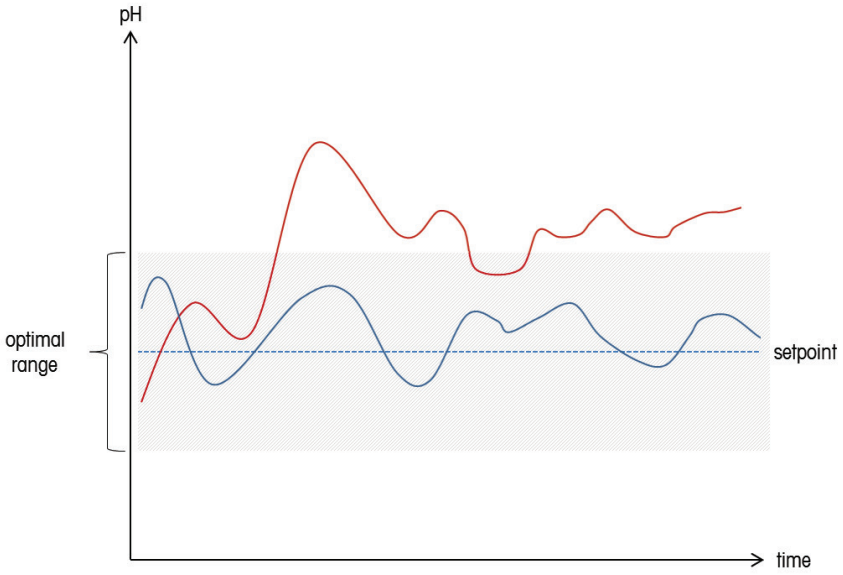
Normally in biopharma processes, reference pH or DO values are not highly precise. There is an acceptable range around these values that is determined by regulations (e.g. the USP) or the manufacturer. The concept of Quality by Design (QbD) is based on acceptable ranges rather than a single reference value. This design space philosophy enables flexibility in manufacturing.

An acceptable range suggests that sensor accuracy is not critical. However, if a sensor is reporting erroneous values, it is not possible to determine whether a measurement is within or not within the acceptable range.

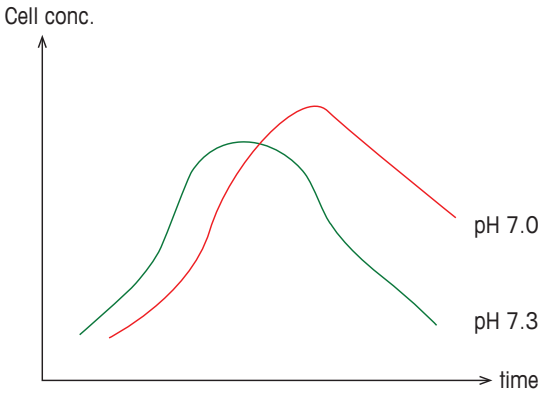


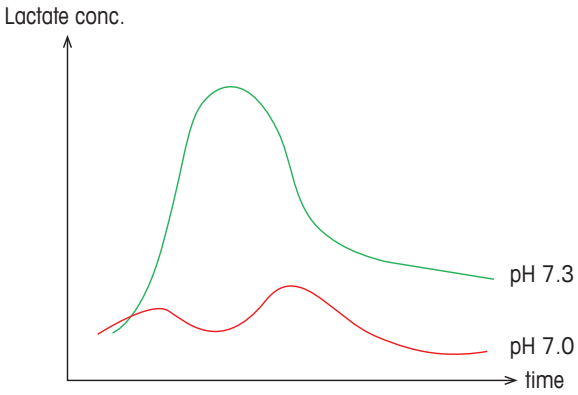
Acceptable range according to QbD

Example: pH control during fermentation



In cell cultures and fermentation processes, optimal growth conditions are necessary throughout a batch to prevent low yield, a slower batch time, or the production of unwanted byproducts.





As can be seen in the graphs, maintaining pH 7.0 increases cell concentration and reduces lactate concentration (lactic acid influences the quality of final product and make purification steps more difficult) compared with pH 7.3.

3. Calibration

Calibration is the comparison of a calibration standard of known accuracy (e.g. pH buffer) with another instrument of unknown accuracy (e.g. a pH sensor) to detect, correlate, report or correct any variation in the accuracy of the item being compared (e.g. a pH transmitter).

Benefits of calibration

pH and DO sensors have a certain accuracy associated with them as provided by the manufacturer. This accuracy can only be maintained if sensors are regularly and correctly calibrated. Further, calibration is essential in ensuring compliance with regulations such as global pharmacopeia.

Issues with sensor calibration

A common procedure with pH sensors is as follows: A calibrated pH sensor is mounted on a bioreactor. After bioreactor sterilization, filling with nutrient media and inoculation, the batch can be started. For verification of the measured pH value a sample is taken and sent to the laboratory for measurement. If the pH measured in the laboratory differs from the in-line pH measurement in the bioreactor, operators adjust the pH transmitter to perform a process calibration.

The above procedure is formulated in approved Standard Operating Procedures (SOPs). However, it is only valid if the lab pH measurement is correct. To achieve this, the sample in the laboratory must be measured at the same temperature that is in the bioreactor, and the time between sample and lab measurement must be minimal. For many pharmaceutical companies, this is counter to their actual process. In some situations the lab measurement may be conducted at ambient temperature or thermo-controlled at 25 °C. In other situations the laboratory will make use of the fact that most pH sensors contain a temperature sensor and that the connected meter or transmitter will allow temperature compensation.

However, for a proper calibration and therefore an accurate pH measurement, sample temperature should be equal to process temperature. The reason for this is that there are two independent temperature dependences in pH the measurement: temperature dependence of the chemical equilibria in the medium, and temperature dependence of the pH sensor.

Chemical equilibria are temperature dependent

Chemical equilibria are dynamic and therefore respond to changes in the conditions. Equilibria can be expressed with the equilibrium constant K, which is temperature dependent.

T (°C)	pH ± 0.02	T (°C)	pH ± 0.02
0	7.12	50	6.97
5	7.09	55	6.98
10	7.06	60	6.98
15	7.04	65	6.99
20	7.02	70	7.00
25	7.00	75	7.02
30	6.99	80	7.04
35	6.98	85	7.06
40	6.97	90	7.09
45	6.97	95	7.12

Buffer Solution pH 7.00
Temperature dependence

The temperature dependence of the media in the bioreactor is normally not known. What is well known however is the temperature dependence of buffer solutions. Each label on pH buffer bottles shows the exact value at different temperatures.

Most transmitters contain stored pH calibration buffer data and the chosen buffer must be selected on the transmitter for a proper calibration. Automatic temperature compensation in transmitters and lab meters has no connection with the temperature dependence of chemical equilibria.

The output of a pH sensor is temperature dependent

The internal operations of a pH sensor is based on potentiometric principles. The output of a combination pH sensor (pH and reference electrodes) is primarily the potential difference between the pH-sensitive glass and the reference electrode. This potential difference, also called cell potential, is measured in volts. The temperature dependence of this cell potential is described by the Nernst equation.

$E = E0 + 2.303 \times R \times T / F \times \log (aH+)$

or

$E = E0 - 2.303 \times R \times T / F \times pH$
the factor $2.303 \times R \times T / F$ is called the slope (V/pH)

R = gas constant 8.314 J K-1 mol-1
F = Faraday constant 96485 A s mol-1

Examples:

At 25°C (298.15 °K) slope = $2.303 \times 8.314 \times 298.15 / 96485 = 0.05917$ V/pH or 59.17 mV/pH
At 35 °C slope = 61.15 mV/pH

The calculated slope according to the Nernst equation can also be expressed as a 100% slope. An actual pH sensor shows a smaller slope. End control criteria for a new pH sensor from METTLER TOLEDO is >98% slope. After exposure in a process, slope will decrease slightly.

The temperature dependence of the slope at different temperatures is compensated for automatically in the pH transmitter or lab pH meter. This automatic temperature compensation (ATC) occurs during calibration and measurement and is driven by the built-in temperature sensor. The ATC function can also be deactivated.

The above means that temperature compensation of the slope has no correlation with the temperature dependence of the chemical equilibria of a given media.

Summary

For correct pH comparison with in-line versus off-line equipment, the measuring temperature must be identical. In some cases the temperature coefficient is quite small and correct measurement can be achieved even at different measuring temperatures.

Practical hints

Measure a sample in the lab at different temperatures with activated ATC. If you do not see any difference in the displayed pH, you can safely compare the measurement at different temperatures. This is only valid if media composition is unchanged from batch-to-batch.

Samples from a bioprocess often contain CO_2. If CO_2 escapes from the sample a pH shift can occur. Therefore, good sampling technique and quick lab pH measurement are essential.

4. How accurate is a pH measurement?

Structure of a "classical" pH measurement loop

A classical pH loop consists of a combination pH sensor, connected with a cable to a pH instrument (pH meter, pH transmitter).

A classical pH sensor comprises a pH sensitive glass electrode and a reference electrode. The gel layer build-up on the pH sensitive glass generates a pH dependent potential which can be measured behind the pH-sensitive glass (sometimes called the glass membrane) in a defined inner buffer. A lead-off element transports this potential out of the electrode.

HA465-50-S7 analog pH sensor

The reference electrode, containing reference electrolyte, is connected through a liquid junction (e.g. ceramic diaphragm) to the media or sample. Ideally, the potential of the reference electrode is pH independent.

As mentioned, the potential difference between pH glass electrode and reference electrode is the primary signal output by a combination pH sensor. This signal is of very high impedance (100-500 MOhm) and with a voltage < 1/- 800 mV. Most pH sensors are designed to generate a voltage of 0 mV at 25°C at pH 7. The pH dependence of this voltage can be described by the Nernst equation. Further, this equation explains the temperature dependence of pH sensors. This temperature dependence can be compensated for if the pH instrument is equipped with the aforementioned automatic temperature compensation (ATC), ideally based on a temperature sensor built-in the combination pH sensor. It is a common misunderstanding that ATCs do not correct for the change in solution pH with temperature.

The pH instrument must have an input resistance greater than 1 TeraOhm. With today's electronics this is a much easier task than it was 50 years ago. The pH instrument converts the mV signal to a pH value but is also used for calibration and adjustment based on measurement in pH buffer solutions. The expression "pH sensor calibration" is misleading. It is not the sensor which is calibrated, it is the attached instrument (transmitter or meter) which is adjusted to the output of the pH sensor.

Accuracy of a standard pH sensor

Every pH sensor needs to be matched to a specific instrument with buffer solutions. It is not possible to pre-calibrate a standard pH electrode, so it is not possible to have an accuracy value. To establish accuracy requires the simultaneous use of the sensor, pH instrument and buffer solutions. Only when used simultaneously can accuracy be determined. Therefore, any standard pH sensor by itself does not have a defined accuracy.

Structure of an ISM® pH measuring loop

Intelligent Sensor Management (ISM) is METTLER TOLEDO's digital technology for in-line analytical sensors. An ISM pH loop consists of a combination pH sensor with a built-in analog-to-digital converter and memory, connected with a cable to a pH instrument which can receive the digital signal.

The analog part of the pH sensor is identical, as described earlier, to standard pH sensors. The difference is in the sensor's head which contains the analog-to-digital converter, and a microprocessor. The signal output by ISM sensors is digital. This low impedance signal is very robust. Humidity, which can affect analog signals, is not a problem and longer cables can be installed.

The ISM sensor's microprocessor has the ability to record and retain calibration data. This means that ISM sensors do not need to be calibrated at the process. Instead, they can be calibrated in any convenient location using an ISM transmitter or METTLER TOLEDO's iSense PC software. After calibration, the pH sensor can be stored until it is required.

When a pre-calibrated ISM pH sensor is installed at a process, the calibration data is automatically read by the connected ISM transmitter, which adjusts itself without any operator intervention.

The pH buffer solutions used for calibration are traceable to accepted standards (e.g. NIST) with a given accuracy and therefore ISM pH sensors can be specified as sensors with a defined accuracy.

Requirements for a high quality pH sensor

Electrode designed for bioprocesses	sterilizable, autoclavable,
pH sensitive glass	minimal zero point shift after sterilization or CIP
Liquid junction (diaphragm)	minimal clogging during fermentation, easy to clean
Reference electrolyte	free from silver ion, compatible with protein containing media

Error analysis for sensors, buffers and instruments

In error analysis the worst case in error calculations is achieved by building the sum of all possible errors in one direction, and not allowing any compensation of errors.

pH electrode

Measuring value	7.20 pH
Diffusion potential	1 mV
Error by diff. pot.	-0.02 pH
Slope 25°C	98%

pH buffers

Technical buffers	+/- 0.02 pH

pH transmitter

Uncertainty meas.	+/- 0.02 pH
Uncertainty current	+/- 0.050 mA
Scaling 4 mA	2 pH
Scaling 20 mA	12 pH
Resolution	0.63 pH/mA
	1.6000 mA/pH
Current output	12.320 mA
Uncertainty output	+/- 0.050 mA
	+/- 0.03 pA

0.01 pH

ISM

Temperature sensor

Temperature	37° C
Error temp. meas.	-2° C
Slope 25° C, practical	-57.98 mV/pH
Slope 37° C, practical	-60.31 mV/pH
Slope with temp. error	-59.92 mV/pH
Temperature error	+/- 0.00pH

0.01 pH

ISM digital communication

Worst case

Uncertainty pH electrode	+/- 0.02pH
Uncertainty buffer	+/- 0.02pH
Uncertainty temperature	+/- 0.00pH
Uncertainty transmitter	+/- 0.02pH
Uncertainty current output	+/- 0.03pH
Uncertainty total	+/- 0.09pH

+/- 0.04 with ISM

With ISM pH transmitters the uncertainty of measurement and the uncertainty of output is eliminated, and as can be seen above, the maximum error of +/- 0.09 pH can be reduced to +/- 0.04 pH.

5. Accuracy of dissolved oxygen sensors

Amperometric oxygen sensors

The measuring principle of such sensors is as follows. Oxygen diffuses through a semi-permeable membrane and is reduced at a cathode which is held at a defined potential. The reduction current is proportional to the partial pressure of oxygen in the media.

$I = K \times A \times D \times S \times 1/d \times p_{02}$

The relationship between sensor current "I" and partial pressure is only valid if membrane thickness remains constant. During sterilization, the membrane is slightly stretched, resulting in a higher sensor output. This effect reduces after each sterilization cycle. Small handling errors during membrane replacement, such as not removing electrolyte drops on the membrane module shaft, can lead to a small membrane blow-out. This can be very critical in terms of accuracy as membrane thickness has been altered.

Amperometric DO sensor with membrane module (cut view)

Linearity of sensor signal $p_{02} < 0.21$ bar +/- 1%

Stability of residual current and slope (air current) typically < 2%/week

Optical dissolved oxygen sensors

Optical oxygen technology is based on fluorescence quenching. In contrast to the amperometric sensor, which detects the reduction current of oxygen, the optical method measures energy transfer between a fluorescing chromophore (fluorophore) and oxygen.

For further information, download our "Good Operating Procedures for Optical Dissolved Oxygen Sensors" www.mt.com/Optical-GoP-Guide

Optical sensors do not contain any electrolyte or replaceable membrane. In METTLER TOLEDO's InPro 6860i, the lifetime of the oxygen-sensing element, the OptoCap, is dependent on the quantity of light it is exposed to. The OptoCap is the only part of the sensor which must be replaced periodically. Typically, in fermentation applications a lifetime exceeding 6 months can be expected.

InPro 6860i optical DO sensor with OptoCap relaceable oxygen-sensing element

InPro 6860i optical DO sensor

Due to its measurement technology, handling errors such as seen with amperometric sensors are avoided with optical sensors. Further, the maintenance requirement is significantly lower than with amperometric sensors.

Another substantial advantage of the design of the InPro 6860i is very low measurement drift: four to five times lower than typical amperometric sensors (< 0.5%/week).

Stability control for optical DO sensors

The degradation of the fluorophore is very linear over the lifetime of an OptoCap. Occasional calibration corrects for measurement drift observed in normal sensor ageing. The InPro 6860i utilizes a proprietary alogorithm that monitors the sample rate, oxygen level and process temperature to accurately compensate for shifts in Phi values caused by fluorophore degradation. This so-called stability control stabilizes the oxygen reading and significantly reduces the need for frequent calibration.

The stability control algorithm is able to learn process specific sensor ageing. By performing a one-point calibration in air after a few batches, the sensor compares calculated Phi shift values with calibration Phi shift to accurately compensate for future fluorophore degradation. As a result, sensor drift is minimal. This represents a major benefit for long duration batches with cell cultures.

Calibration

The most common calibration can be easily performed by holding the sensor in air. In fermentation or cell cultures, calibration is done after sterilization in the air saturated media. This can be applied to amperometric or optical sensors.

2-point calibration

Many pharmaceutical companies dictate a 2-point calibration in their SOPs. Additionally to the high oxygen calibration (air saturation) a zero point calibration must be performed.

For optical DO sensors a 2-point calibration is required with every OptoCap exchange. For correct calibration, nitrogen gas or other oxygen-free medium with a purity level of at least 99.99% should be used to achieve the Phi 0 point, followed by exposure to air for the Phi 100 point. 2-point calibrations are performed on METTLER TOLEDO transmitters or on a computer running iSense software. The resulting calibration curve is stored on the sensor and is continuously referenced as the sensor makes its measurements.

For normal use a zero point calibration is not necessary. It should be noted that a faulty zero calibration with a non-defined, oxygen-free medium will have a negative effect on accuracy. Some operators perform a zero-point calibration during sterilization with the assumption that the bioreactor is oxygen free. Such a procedure is not recommended as the measuring temperature is very different to the specified range. This will result in poor measurement accuracy.

1-point calibration

A 1-point calibration establishes the slope (for amperometric sensors) or a new Phi 100 value (for optical sensors). 1-point calibrations can calibrate sensors in air with measurement settings at 100% and local atmospheric pressure.

Process calibration

Process calibrations differ from 1-point calibration in that the former are conducted with a sensor in situ in a reactor. For optical sensors, process calibration establishes a new Phi100 value against the stored calibration curve to create a new curve. Because calibration curves for optical sensors are not linear, 1–point process calibrations in vessels with head-space must accurately account for system pressures or risk jeopardizing the accuracy of the entire curve. Because of this, we recommend using 1-point process scaling instead of process calibration for the vast majority of post-SIP applications.

Process scaling

Unlike 1-point process calibrations, process scaling sets the measurement value to a desired level without making any adjustments to the calibration curve. With this method the real process pressure and the solubility factor for oxygen can be ignored, resulting in improved accuracy.

6. Conclusions

In bioprocess applications, maintaining a culture in an acceptable range around the optimal setpoints is key for efficient and consistent production and minimal byproduct production.

To achieve the best possible accuracy from in-line pH and DO sensors, the following points are important:

1. Choose sensors which are designed for bioprocessing and SIP/CIP conditions. For pH, select sensors with A41 membrane glass and pre-pressurized liquid reference electrolyte. For dissolved oxygen sensors we recommend optical sensors.
2. Sensor must be cleaned prior to calibration.
3. Calibration is essential for ensuring measurement accuracy. Consistently follow approved calibration procedures. For pH sensors always use fresh buffers. For DO sensors perform zero calibration only with defined non-oxygen media.
4. Measure pH in the lab at the same temperature as in the process for process calibration.
5. Errors due to handling or faulty buffers directly lead to low accuracy.

Intelligent Sensor Management (ISM) can improve sensor accuracy

1. ISM's digital signal is very robust and many errors due to analog signal transmission and can be eliminated.
2. Calibration without handling errors can be performed off-line with iSense software in any convenient location rather than in the production area.
3. ISM optical dissolved oxygen sensors feature very low maintenance. Less handling will improve accuracy.
4. Pre-batch diagnostics ensure use of sensors that will operate reliably throughout a batch.

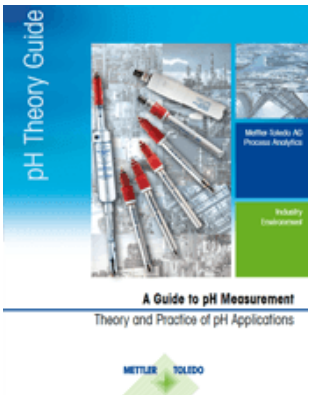
Best Practice Guides

Maintaining product quality while improving process safety and controlling operating costs is a constant challenge in the process industries.

METTLER TOLEDO Process Analytics has created several Best Practice Guides for different industries with examples of how advanced process analytical systems can help operators increase product quality and yield, while at the same time reduce operating costs.

Find the appropriate guide for your specific needs and download your free copy today!
Get access here: www.mt.com/pro-guides

pH and DO in Theory and Practice

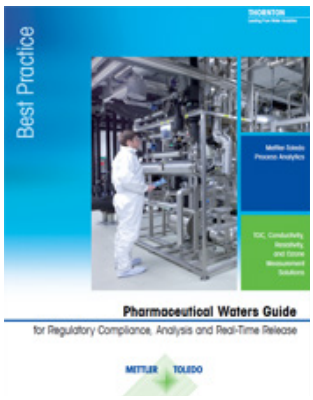


pH Theory Guide: A guide to pH Measurement – the Theory and Practice of pH Applications

DO Guide: Good Operating Procedures for Optical Dissolved Oxygen Sensors

Pharmaceutical Industry

Pharma Guide "Achieving the Highest Level of Performance in Bioreactor Process Control"



Pharmaceutical Waters Guide for Regulatory Compliance, Analysis and Real-time Release

In-line Analytics Website

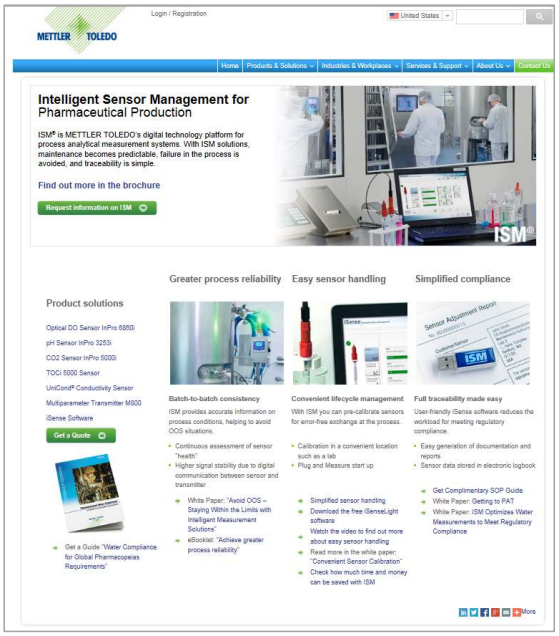
Dedicated to the Pharmaceutical Industry

The METTLER TOLEDO Process Analytics website for the pharmaceutical industry is packed with information on how our in-line measurement solutions improve process reliability, increase production yield and reduce operating costs.

Visit our website to:

- Discover our extensive portfolio of sensors and transmitters.
- Download white papers and application notes, watch videos and webinars.
- Find out how intelligent measurement solutions can prevent batch losses and simplify sensor documentation.

www.mt.com/ism-pharma



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