Pharmaceutical News 3
Perspectives in Liquid Process Analytics

Successful in-line CO_2 evaluation at Boehringer-Ingelheim in mammalian cell culture fermentation

CO_2 is a critical parameter influencing product growth during the fermentation process. Many off- or nearline measurement systems are available on the market using different technologies. However, all of them are rather expensive, cause high costs or require high maintenance. Boehringer-Ingelheim at Biberach, GER, did a thorough evaluation test on the new METTLER TOLEDO CO_2 inline system focusing on sterility and long term stability and comparing the data on a regular basis with a blood gas analyzer. The result: The system meets their requirements.

Boehringer-Ingelheim is one of the most important and fast growing pharmaceutical companies in Europe with more than 32'000 employees worldwide. At their site in Biberach they have expanded recently with further production lines and are continuously evaluating new technologies for improving their yield of products. In an internal evaluation they have recently tested the new CO_2 in-line system of METTLER TOLEDO INGOLD and have been very pleased with the result. Before this testing, Boehringer-Ingelheim had monitored the CO_2 level using a blood gas analyzer (BGA) but did not feel comfortable measuring CO_2 off-line besides having on-line Dissolved Oxygen and pH monitoring. As CO_2 is very important for the productivity and the metabolism of the cells this new probe can be used very efficiently for monitoring that important process parameter.

The CO_2 system was tested in 80 l and 2000 l fermenters by the engineering group during 3 months following a scale up fermentation process. After thorough testing over several weeks on the sterility of the 12mm sensor with N5 surface, the approval was given and practical testing started with two different INGOLD CO_2 systems. Before mounting the sensor to the fermenter the system was calibrated. After mounting it to an INGOLD socket the sterilization took place. After this step a sample calibration was made by off-line measurement

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through a Chiron blood gas analyzer (BGA) which was used as an external reference throughout the whole testing. After this initial sample calibration no further calibration had to be done during the fermentation time of up to two weeks. The fermentation was done with mammalian cells at about 0.1 bar overpressure and at about 37 °C.

"The system meets our requirements"

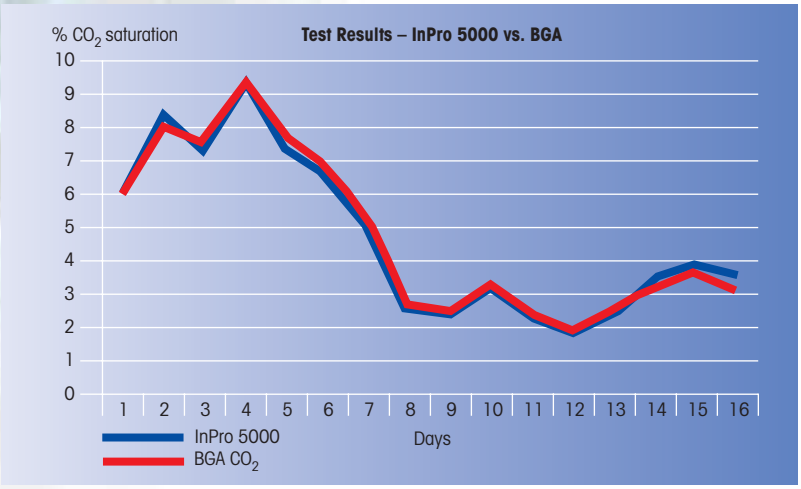
As a process calibration step the in-line system was set to the BGA value at fermentation start following a so called "sample calibration". Daily a sample was analyzed with the BGA and compared with the value of the in-line system. The curves illustrate an excellent correlation. This result was confirmed in several fermentations. As the results met the requirements Boehringer-Ingelheim is considering to use the system in more bioreactors.

About the CO_2 System:

The new CO_2 measuring systems consisting of the CO_2 sensor InPro5000 and the CO_2 transmitter 5100 e is designed for in-situ measurement in bioreactors.

What is new?

- second generation of industrial in-line dissolved carbon dioxide sensor in the market
- patented INGOLD membrane technology to avoid interference problems
- robust industry proven technology ensures lower system and maintenance cost and less complexity than todays indirect measurement systems
- compliant with hygienic regulations (EHEDG)
- all materials used are FDA 21 CFR 117.2600 compliant
- transmitter offers the same advanced user-interface, unique sample calibration



routine and diagnostics as other METTLER TOLEDO analytical parameters (pH, DO, conductivity, turbidity)

7 good reasons for in-situ CO_2 control in fermentation processes:

- High CO_2 concentration can be toxic to mammalian cells.
- Mammalian cells require certain CO_2 levels for proper metabolic function.
- Too high CO_2 concentration prevents cell growth; too low a level leads to yield loss.
- CO_2 levels provide information on biomass concentration and substrate consumption.
- In-situ measurement enables fast and accurate CO_2 control in the reactor.
- Only in-situ measurement can indicate real-time CO_2 level in the reactor.
- Direct CO_2 measurement supports process replication and with that accelerates scale-up.

www.mtpro.com/co2

Downstream: Buffer preparation.

CO_2 sensor InPro 5000 – Technical specifications at a glance	
Measuring range	10...1000 mbar CO_2
Accuracy	<10% 10..900 mbar
Temperature range	0..60 °C/32..140 °F
Pressure range	0.2...2 bar abs.
Pressure resistance	3 bar
Temperature sensor	Pt1000 (built-in)
Wetted materials	Stainless steel in 1.4435
Surface roughness	N5 Ra < 0.4 um
O ring materials	Viton, silicone FDA compliant
Plug head	VarioPin IP68
Sterilization	in-situ or autoclave 130 °C/266 °F
Measuring principle	Severinghaus
Certificate	3.1B for steel parts, EHEDG

Pictures: Boehringer Ingelheim GmbH.
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Importance of CO_2 measurement and control in bioreactors

InPro 5000.

The oxidation of carbohydrates to CO_2 and water is the basis for aerobic forms of life. The impact of dissolved carbon dioxide in the cultivation media has, however, drawn little attention in the past. One reason has been the lack of reliable sensors and measuring methods. Off-gas measurement with an infrared gas analyzer or a mass spectrometer are two reliable, but equipment intensive methods. Another well proven technique for determining dissolved CO_2 with far less investment is potentiometric carbon dioxide electrodes (Severinghaus electrodes).

Besides pH and Dissolved Oxygen measurements, reliable monitoring and control of the CO_2 partial pressure is important for successful fermentation. METTLER TOLEDO's new CO_2 system delivers precise, real-time data so you can better manage your critical fermentation and cell culture processes. This data provides valuable insight into cellular metabolism and other changes within the bioreactor. The in-situ sensor measures exactly the same partial pressure as the cells "see."

One of the major trends in biotechnology today is the increasing use of mammalian cell lines including human, monkey, mouse and bovine cells. Various types of bioreactors are now being used to cultivate these animal cells. One of the most important requirements for optimal cell growth in a bioreactor is continuous monitoring and control of critical parameters which include pO_2, pH, CO_2 and temperature. Reliable measurement of CO_2 is essential for successful large-scale operation as the accumulation of CO_2 becomes more problematic as viable cell concentrations rise. High CO_2 concentrations can inhibit cell growth and product formation in mammalian cells and alters the glycosylation pattern of recombinant proteins. By maintaining low and constant levels, the production rate of pharmaceuticals, proteins and antibodies can be

Frequently Asked Questions

Question:

Are CO_2 measurement pressure and temperature dependent?

Answer:

The reading of the CO_2 sensor primarily depends upon the partial pressure of CO_2. If the partial pressure remains constant, the reading is not pressure dependent. The solubility of CO_2 in water depends on the partial pressure and a temperature-dependent solubility factor according to Henry's law. Generally speaking, the solubility decreases with increasing temperature.

Question:

What will happen if the sensor is sterilized at 140 °C/284 °F?

Answer:

Specified sterilization temperature is 130 °C/266 °F. As tested, we expect 10 to 15 sterilization cycles as a typical life time of the interior body (pH electrode). Sterilization at 140 °C/284 °F will lower the typical life time down to 5 to 8 cycles.

Question:

Which other substances will interfere?

Answer:

Volatile organic acids will not pass through the membrane. However, Hydrogen sulphide (H_2S) can transgress the membrane and may affect results. Ammonia gas (NH_3) also will change the pH of the CO_2 electrolyte but under typical fermentation conditions no problem is expected. ■

CO_2 Transmitter 5100.

Glass-free pH measurement electrodes **for the cosmetics industry**

Industry-wide campaigns to promote "pH-neutral" products for skin care are changing the public's behavior. Consumers are specifically selecting products engineered to protect the skin's acid mantle. Highly alkaline products are quickly falling out of favor, as consumers choose products with pH values closer to the skin's pH of 5.5. Therefore, maintaining tight pH limits during product processing is an important quality assurance step that extracts a return in market perception and sales.

In the manufacture of soaps, detergents, cleaning agents, fragrances and body-care products, production process requirements are similar to those in the pharmaceutical industry. In this case, pH measurement plays an important role in quality assurance and process monitoring. An hygienic process housing, the ability to maintain sterility, and good cleanability (CIP) of the equipment in use must be guaranteed. Until now, pH measurements were made mostly offline in the laboratory or on a mobile basis through grab sampling and spot checks. These procedures are costly, prone to error, and do not allow automation of the process. The use of glass pH electrodes for online measurements directly in the process was previously avoided due to the risk of potential breakage, and to protect the consumer.

Innovative, safe and hygienic

The new pH electrode InPro® 3300 is based on innovative ISFET technology, free from any glass components whatsoever. This electrode is therefore open for use in areas that until now have been closed to conventional glass electrodes. All wetted parts are made of FDA-compliant materials (PEEK, EPDM), and hygienic design permits optimal cleaning. EHEDG certification has been awarded. Pressure resistance has been tested and passed according to the Pressure Equipment Directive (PED) 97/23/EG, Art. 3, § 3.

Outstanding measuring characteristics

Due to the low surface area of the actual sensor of the ISFET electrode, response times are particularly rapid. Measurement accuracy in alkalis is extremely high, since the so-called "alkali error" often associated with glass electrodes is virtually non-existent. The high-performance reference system is guaranteed to remain incredibly stable even after several sterilization and CIP cycles. The 45° design of the tip of the electrode prevents air bubbles from covering the sensor surface, thereby assuring high signal stability.

Complete measuring systems

ISFET electrodes require special signal evaluation electronics. The new transmitters pH 2100 e allow connection of both classic pH electrodes and the InPro 3300. Process adaption is simplified through specific use of the new retractable housing InTrac® 798. ■

InPro 3300: pH electrode, glass-free.
Optimal sensor design for reliable measurement values.

Technical Specifications InPro 3300	
pH range	0…14
Temperature range	
– measurement	0…80 °C/32…176 °F
– sterilization	130 °C/266 °F
Pressure range	0-6 bar/0-87 psi (at 80 °C/176 °F)
Shaft material	PEEK (FDA-compliant)
Temperature sensor	Pt1000
Insertion length	120 mm

Low to medium turbidity measurement

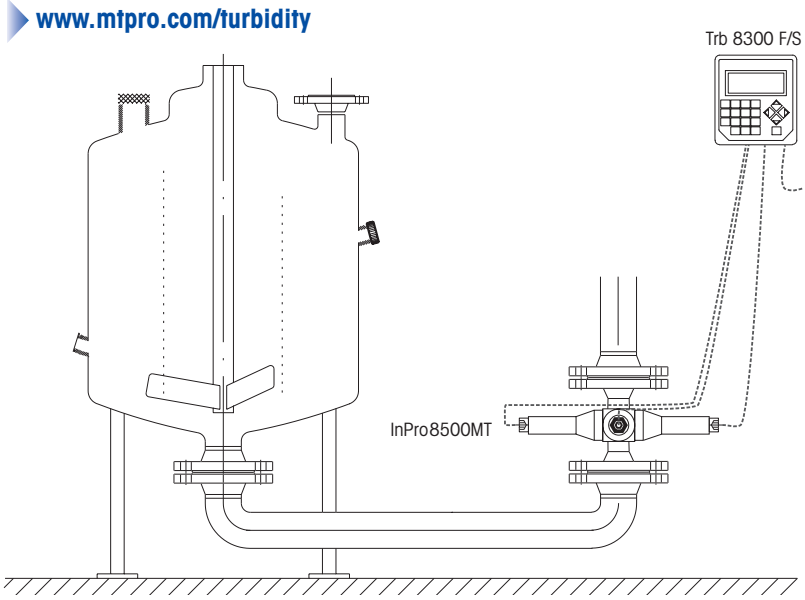
when clarity matters most in industrial process control

This optical system for extremely low concentrations perfectly completes the existing product offering for mid to high turbidity ranges. The system consists of the transmitter Trb 8300 F/S and the InPro 8400 and InPro 8500 sensor series. These systems are particularly well suited for applications in the BioPharm industry. METTLER TOLEDO's extensive experience and know-how in offering high quality measuring solutions in these markets has been implemented in the new system.

Different turbidity ranges require an optimum measuring technique. The forward scattered light technique of the InPro 8400 sensor series enables measurements even in liquids which seem to be clear to the human eye. The combined forward/90° scattered light technique of the InPro 8500 sensor series makes it possible to distinguish between turbidity caused by colloidal particles or larger particles. These are two requirements for reliable detection of particles in liquid streams for advanced process control and improved quality assurance measurements. Filter performance, particle concentration measurements as well as control of product purification steps are typical measuring tasks for the new InPro 8400/8500 sensors. Due to the wide variety of different process connections and line sizes, these flow-through sensors can be easily installed in main pipes or in a bypass. The rugged sensor design allows installations even in harsh process conditions and in hazardous areas (Ex-proof). The Trb 8300 F/S transmitter enables quick start-up and operation with a full text menu guide in three selectable languages. Sensor diagnostic features provide information on sensor performance and consequently reduces maintenance time. METTLER TOLEDO also provides an outstanding MaxCert™ documentation program with the system. Depending on specifications sensors are provided with application relevant certificates like, 3.1B, PED, 3A, ATEX and factory calibration certificates to satisfy safety requirements and to guarantee traceability. ■

Transmitter: Trb 8300 F/S.

www.mtpro.com/turbidity



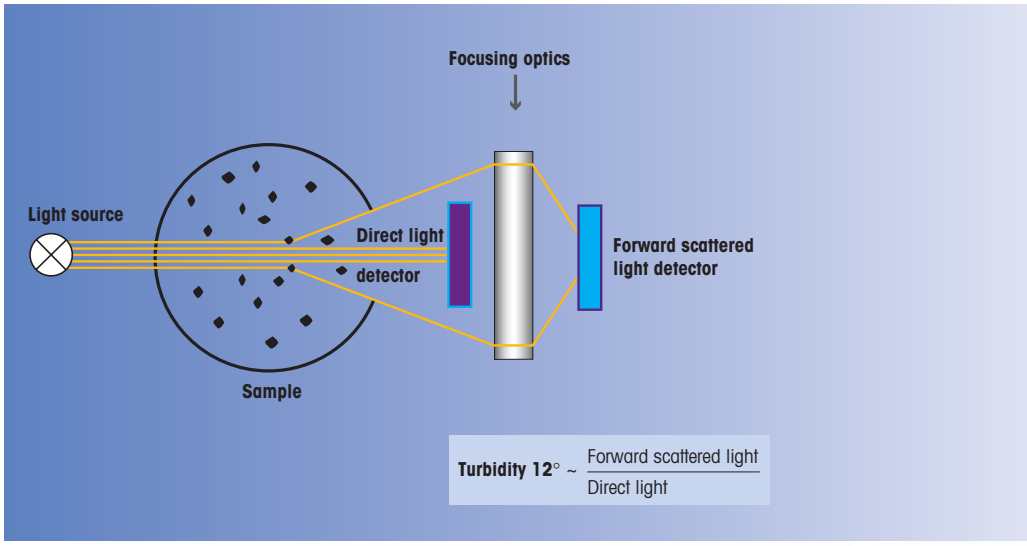
Designed for low turbidity...

InPro 8400 sensors are ideal for detection of larger particles (≥ 0.3 µm). The simultaneous measurement of forward scattered and direct light allows compensation of color changes.

These are key features typically required in the pharmaceutical industry for:

- filtrate quality measurements and filter breakthrough detection
- centrifuge/separator control
- particle concentration measurements in liquid streams

Forward scattered light sensors: Ratio measurement for color compensation



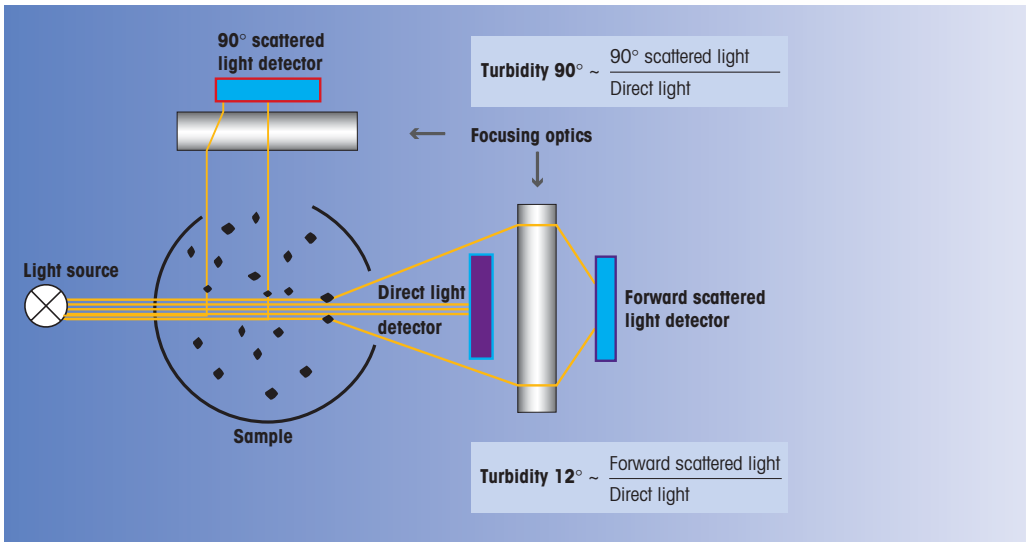
...and trend monitoring of particle size distribution

InPro 8500 sensors provide additional trend information on the size composition of undissolved particles. The magnitude of the 90° and 12° ratio turbidity indicates if more colloidal or larger particles are present.

These sensors are specifically designed for:

- filter performance measurements
- product quality assurance measurements after purification processes ■

Forward / 90° scattered light sensors: Ratio measurement for color compensation



Regular maintenance increases safety and avoids production failures

O-rings have the important task of sealing moving parts such as sensors and housings in order to prevent the escape of medium. Mechanical and chemical stress effects in everyday production are so great that O-rings should be replaced at regular intervals. Furthermore, strict requirements of the industry, more specifically the pharmaceutical industry dealing with FDA compliant compounds, have increased the attention given to regular maintenance.

Mechanical stress intensifies in relation to the increase in solids content. The degree of chemical resistance depends upon the material composition of the O-ring, and is well understood for many chemicals. Therefore, it is essential for the pharmaceutical industry to select O-ring materials not only for chemical compatibility, but also to meet the demands of the FDA for compliant compounds. The most important specifications of the materials of the O-rings supplied by METTLER TOLEDO can be seen in the table below.

Due to the chemical and mechanical strain placed on O-rings in the industry, METTLER TOLEDO recommends that the O-rings are replaced at least once a year. Depending on the application a higher rate of exchange is advisable to assure a high level of process safety. O-rings can be replaced on site or at METTLER TOLEDO Service Center. Qualified service technicians carry out replacement according to the manufacturers' instructions and test and verify the operation of each retractable housing. If O-ring replacement is performed at a METTLER TOLEDO Service Center, the retractable housing is also subjected to a pressure test. ■

O-Ring-Material	Characteristics
Viton®-FDA	Well suited for use with many chemical liquids, particularly combustible products; not suitable for use in hot CIP alkaline solutions; high thermal stability
EPDM-FDA	Well suited for CIP operations in acids and leaches; moderate thermal stability
Silicone-FDA	Well suited for fats and oils, low thermal stability
Kalrez®-FDA	Chemical resistance, rubber-like elasticity, high thermal stability

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