Pharmaceutical
Perspectives in Liquid Process Analytics

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News

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Leading Process Analytics

Beyond Spectroscopy – Benefits of "Classic" PAT Tools

"Classic" tools for the acquisition of data for process parameters like pH, dissolved oxygen, conductivity, and particularly carbon dioxide and turbidity are also powerful tools within the framework of PAT (Process Analytical Technology) for better understanding and improved control of bioprocesses.

Introduction and regulatory framework

The FDA together with the pharmaceutical and life sciences industries have recently focused on a framework known as Process Analytical Technology (PAT) set out in the FDA publication "Guidance for Industry, PAT – A framework for Innovative Pharmaceutical Manufacturing and Quality Assurance". This guidance is part of the FDA's initiative "Pharmaceutical cGMPs for the 21st Century.

The FDA defines PAT as a system for designing, analyzing, and controlling manufacturing processes through timely measurement of critical quality and performance attributes of raw and in-process materials, to ensure final product quality.

PAT significance

Pharmaceutical business models are changing and the need for pharmaceutical companies to achieve financial performance levels have impacted operations and placed a premium on effective and efficient manufacturing processes. The savings obtained from more efficient use of resources, reduced waste, faster product approvals and a lower risk of product recalls more than outweigh the cost of implementing PAT.

The aim of PAT is to encourage the industry to adopt innovative technologies to increase quality. Key components of this approach for better understanding of the product manufacturing process are data analysis, process analytical tools, process

monitoring, and continuous feedback during manufacture.

Rise in demand for PAT tools

In today's manufacturing environment understanding of the production process is limited. Knowledge about process conditions is often gained from grab samples taken from the process and analyzed off-line. However, very little valuable feedback for process control can be obtained by off-line analysis.

A recent market analysis (Fig.1) indicates a strong rise in the demand for PAT instrumentation currently led by near infrared spectrometers (NIR) followed by electrochemistry. There are well over 10,000 NIR instruments already in use in pharmaceutical applications, the bulk involved in raw material identification.

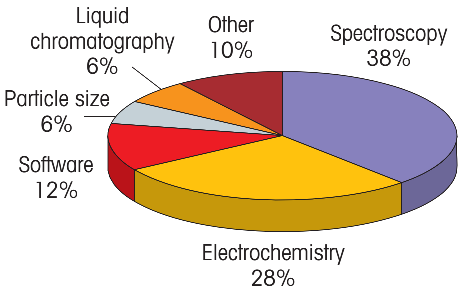


Fig.1: PAT Instrumentation demand, 2005 (Source: SDI).

Fermentation modes and turbidity control

In the drive to increase production efficiency, there is a growing shift from batch to continuous production methods in the pharma and biotech sectors, coupled with a demand for real-time process information. Primary types of continuous cultivation devices are the chemostat and the turbidostat. In both modes the benefits of inline pH and dissolved oxygen control are undisputed. Turbidity measurement has become a further important control parameter.

In the turbidostat mode, see Fig.2, cell concentration is held stable by monitoring the turbidity of the culture and controlling the nutrient feed flow rate. The culture volume is kept steady by removing an equal amount of depleted culture fluid, thereby maintaining cells at a constant physiological state and growth rate.

Function of turbidity measurement

Fiber optical backscattered light sensors are increasingly used to measure turbidity values. They have proved superior to optical density (OD) measurement systems based on light absorption due to better resolution. Measurement is based on the scattering (reflectance) of NIR light by the cells. The intensity of the backscattered light increases with fermentation time, in line with the growing accumulation of cells. The inline backscattered light system shows an excellent correlation with standard OD laboratory measurement (Fig.3). The real-time information of the cell concentration is crucial for constant product quality and enables control of the nutrient feed pump.

CO_2 inhibits cell culture processes

Animal cell culture processes are usually characterized by low cell densities and low cell specific CO_2 production rates compared to microbial cultures. High concentrations of dissolved CO_2 are known to exert a negative influence on mammalian cell cultures, inhibiting cell growth, substrate utilization, and product synthesis.

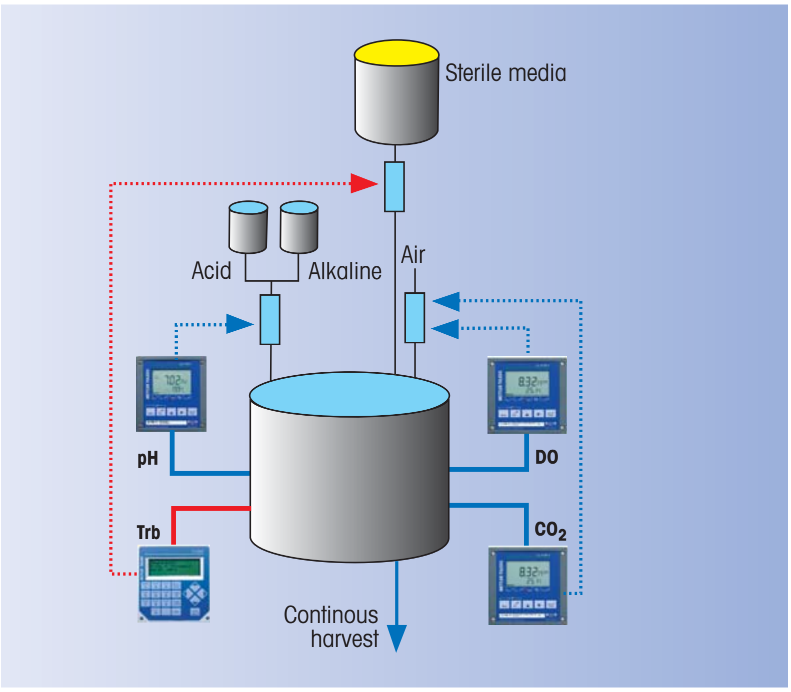


Fig.2: Turbidostat.

Publisher / Production
Mettler-Toledo AG
Process Analytics
Im Hackacker 15
CH-8902 Urdorf
Switzerland

Illustrations
Archive MarCom
CH-8902 Urdorf
Switzerland
Archive Roche

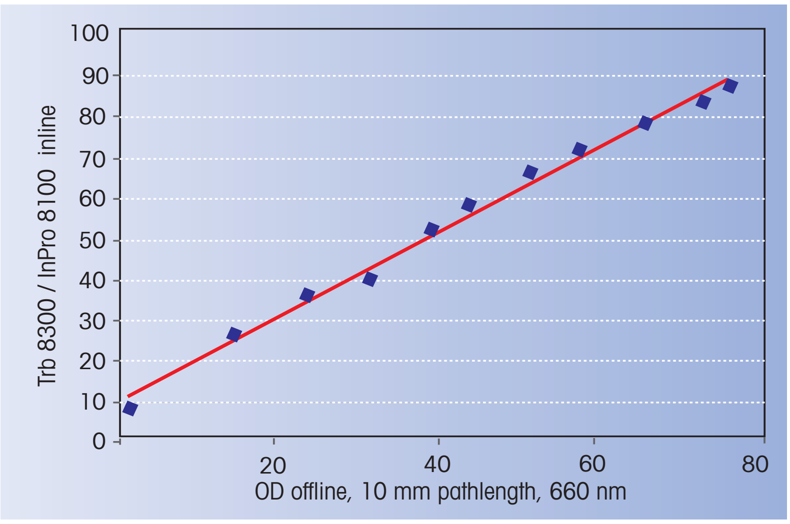


Fig.3: Turbidity/optical density correlation.

The measurement and control of dissolved CO_2 partial pressure (concentration) as well as the CO_2 production rate in mammalian cell suspension culture are both attracting greater attention, since the effects on major cell properties, such as cell growth rate, product quality/production rate and intracellular pH have been firmly established.

Optimized CO_2 control

In fed-batch cultures (Fig.4), nutrients are fed into the process continuously or semi-continuously, while effluent is removed discontinuously.

Inline CO_2 measurement is used to control the (preferentially intermittent) feed of nutrient solution containing glucose. The addition of glucose will increase the production of CO_2 by the cells. In-situ measurement avoids overdosing of glucose and creation of an inhibitory level of CO_2. The prosperity of the cell culture becomes optimized as demonstrated in an increased yield. The sensor acts indirectly as an in-situ glucose sensor showing an excellent response time and therefore optimizing the growth of the cell culture.

A sterilizable pCO_2 sensor with semi-permeable membrane is used. Dissolved CO_2 passes through the membrane and reacts with the electrolyte, forming hydrogen carbonate and hydrogen ions. The change in pH value of the electrolyte is in relation to the partial pressure of CO_2.

Powerful PAT tool

"Classic" tools such as used for acquisition of process parameter data (pH, dissolved oxygen, conductivity, and in particular carbon dioxide and turbidity) are also powerful PAT tools for the control of bioprocesses. Critical material and process attributes relating to product quality are identified and measured in real-time. Signals from the process analyzer can be directly used for process control through a feedback loop, resulting in improved batch-to-batch consistency – a great benefit offered by inline measurement.

www.mtpro.com/turbidity

www.mtpro.com/CO2

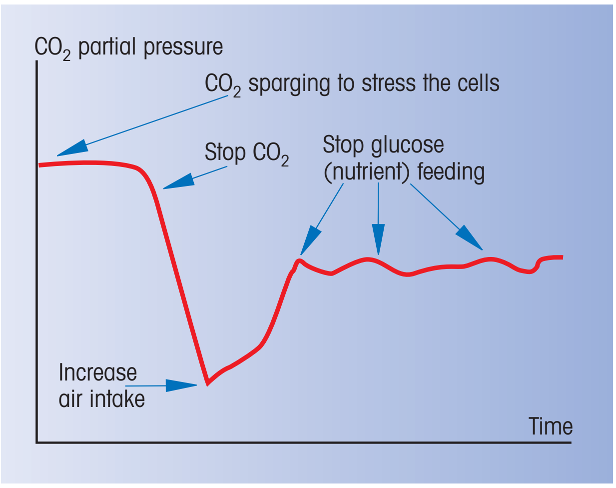


Fig. 4: CO_2 partial pressure development in fed-batch cultures.

Modeling with CO_2 Measurement Allows
Faster Scale-up of Fermentation Processes

The InPro 5000 sensor from METTLER TOLEDO enables precise and reliable inline measurement of dissolved CO_2 throughout an entire cultivation period at variable gas transfer from liquid to gas phase.

Kluyver Center, Delft, Netherlands

The Kluyver Center for Genomics of Industrial Fermentation in Delft, Holland, is a consortium of several different Universities and Research Centres. The center works closely together with Elscolab, a company which has represented METTLER TOLEDO Ingold in the Benelux countries in the field of process analytical measurement systems for more than 20 years.

Microbial genomics

The Kluyver Center applies microbial genomics to improve the performance of microorganisms in industrial fermentation processes. Fermentation is used in the production of renewable feedstocks for food products and ingredients, beverages, pharmaceutical compounds, nutraceuticals, through to fine and bulk chemicals.

Scope of possible application

In connection with research programs covering yeast fermentation, fungal fermentation, lactic acid fermentation, bio catalysis and genomic tools including bioinformatics, the center is always on the lookout for the latest developments in in-line measurement technology.

In this regard, Martin Hoogedoorn, Product Specialist at Elscolab Nederland B.V., introduced METTLER TOLEDO's new CO_2 measuring system to Sjaak Lispet, head of instrumentation at the center.

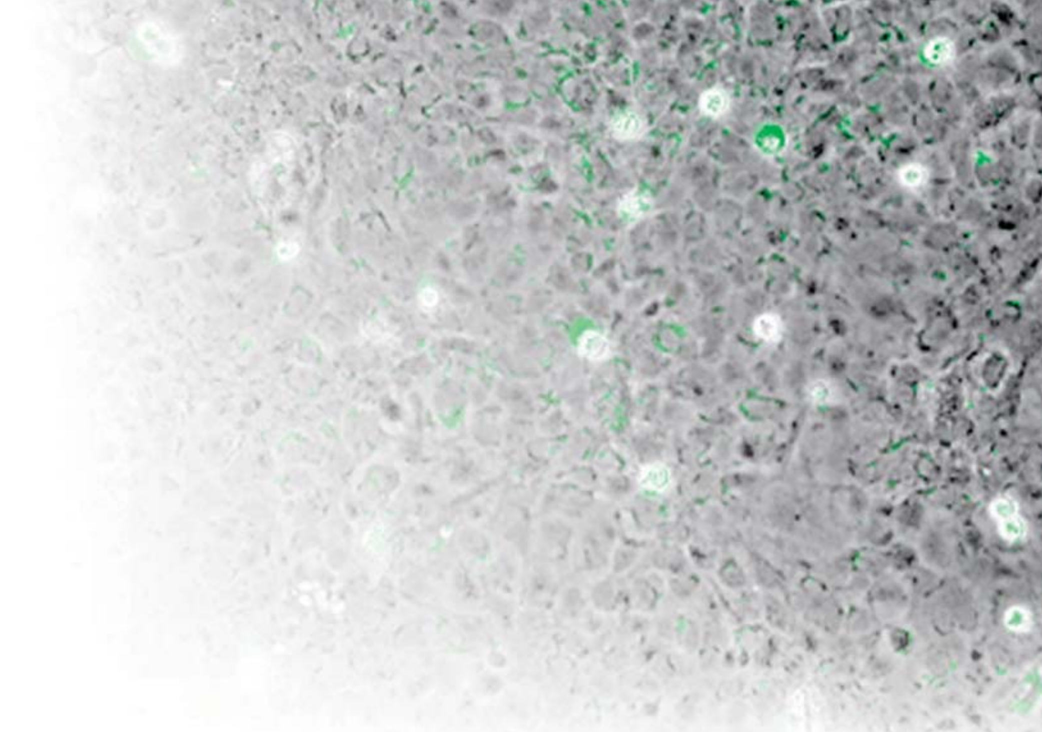
Importance of CO_2 measurement

Sjaak Lispet stated that "dissolved CO_2 is, next to pH and dissolved oxygen, the most important measurement parameter for us". Frederik Aboka, a PhD student in Bioprocess Engineering explained the reason why it is so important to be able to measure dissolved CO_2 directly inline: "Many cell cultures are stimulated or inhibited by dissolved carbon dioxide. It is therefore very important to measure carbon dioxide content in the fermentation broth".

Faster scale-up

An in-situ CO_2 sensor delivers information on the liquid phase of the bioreactor. It has to be considered that the liquid phase is generally never in a thermo-

Fig. 1: 4.0 Liter laboratory fermenter with side-mounted CO_2 sensor.



dynamic equilibrium with the gas phase during the process. A measurement in the liquid phase is dedicated to actual condition values of the volumetric coefficient of transfer. This coefficient is an important design and scale-up parameter for bioreactors. In all processes where dissolved CO_2 plays a key role in the metabolism, in-situ CO_2 measurement allows a faster scale-up during process development and finally a faster time to market for new products.

Use of the CO_2 Sensor InPro 5000

Asked about the use of the CO_2 measurement system, Frederik Aboka explained: "In our lab, the sterilizable sensor InPro 5000 was employed in aerobic yeast fermentation in two ways. In the first instance, side-mounted in a fixed position in a 4.0L laboratory fermenter during the whole period of the cultivation (see Fig. 1). In the second instance, the sensor was installed as shown in the diagram (Fig. 2). In this configuration, the sensor was placed in a small measurement cell of our own design. Culture broth is drawn off from the bioreactor and fed to the measurement cell by means of a pump.

Reliable and stable measurement

In respect of results, Frederik Aboka stated: "We are satisfied with the steady state measurement of dissolved CO_2 using the METTLER TOLEDO InPro 5000 sensor. Currently the data is being evaluated and will be made available to a wider public in a scientific journal".

We would like to thank Frederick Aboka for providing us with this initial insight into his research project, and are pleased that we were able to supply him with the CO_2 sensor InPro 5000.

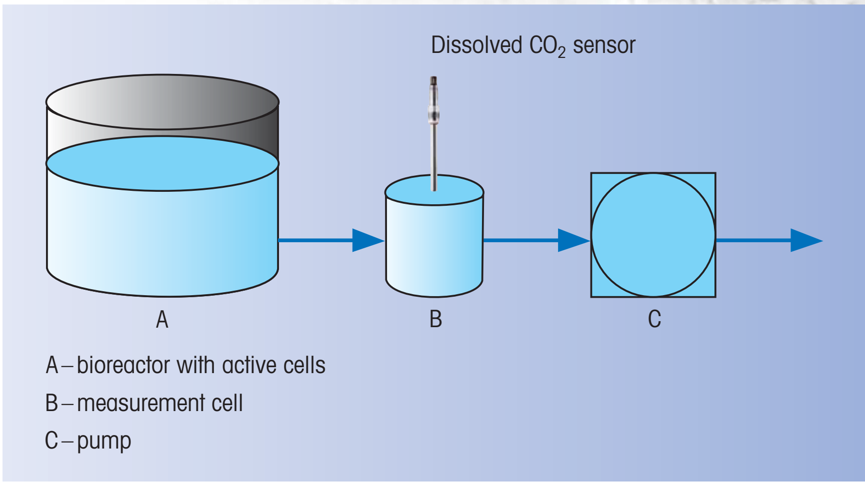
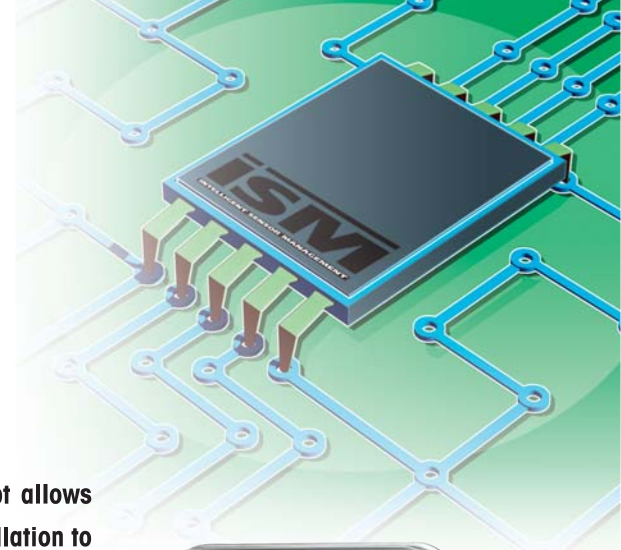


Fig. 2: Experimental setup for dissolved CO_2 measurement outside the bioreactor.

www.mtpro.com/CO2



With ISM® into the Future of Process Analytics

METTLER TOLEDO's "Intelligent Sensor Management"® concept allows easier to operate process measuring systems from initial installation to maintenance right through to sensor exchange which enables you to lower maintenance costs and reduces the risk of sensor failure.

Quick and easy installation thanks to "Plug and Measure"

- Sensorss are immediately recognized during installation.
- Sensor-specific data is stored in the transmitter.
- Operational availability of a measuring point within seconds.

Pre-calibration of pH-electrodes and oxygen sensors in the lab

- Sensors can be pre-calibrated in the laboratory.
- Calibration at point of measurement is no longer necessary.
- The sensor can be replaced and is operational within seconds, saving time as well as increasing operational availability.

Ideal tool for sensor documentation

- ISM-enabled measuring systems recognize manufacturer, type, serial number, order number and date of last sensor calibration.
- Previous calibration datasets available for trend analysis.

Optimized maintenance with sensor wear indicator and adaptive calibration timer

- Measurement data are continuously monitored in order to obtain information on sensor wear.
- Operating hours are recorded for each individual sensor.
- The adaptive calibration timer takes the process conditions into account and reduces the present calibration interval accordingly.

ISM – an open, data management

- METTLER TOLEDO ISM sensors are compatible with the Communications Standard IEEE 1451.4
- ISM sensors with a VarioPin connector can be connected to transmitters of other types and brands without problem, and vice-versa.
- Measurement of the main parameter and temperature continue to function without any restrictions.

www.mtpro.com/pH

www.mtpro.com/DO

Optimization of Bromination through Online ORP Control

In an API manufacturing process, a redox reaction stage to reduce excess bromine was optimized via online ORP monitoring. This exemplifies the benefits offered by modern process analytical technology (PAT).

Redox reaction monitoring

The progress of oxidation / reduction reactions, which involve the transfer of electrons, can be monitored by measuring the difference in potential throughout the process.

Excess bromine must then be reduced to a point where a slight excess is still present, needed to protect the intermediate from losing a bromine and resulting in the undesirable monobromo molecule impurity. The action of quenching the excess bromine with sodium bisulfite ($NaHSO_3$) is secondary to the formation of the intermediate product, but critical to downstream processing and product purity. The secondary redox reaction involves sequentially charging a set amount of sodium bisulfite to the reaction as a quench followed by a series of off-line in-process control tests to determine if the correct amount of excess bromine remains. Overcharges of sodium bisulfite are particularly problematic as this could necessitate a repetition of the bromine charge and quench.

New ORP sensors for online measurement

The Redox reaction constituents described above have electronic potentials or oxidation states: bromine is 1087 mV E_H, and sodium bisulfite is -1120 mV E_H. Cell potentials and balanced equations can be obtained experimentally. The difference in electronic potential can easily be measured online with the new generation of rugged, oxidation/reduction electrodes.

METTLER TOLEDO solution

In production, an InPro 3250 SG was installed in a recycle line of the reaction vessel. This electrode allows simultaneous measurement of redox and pH .The gel-free reference electrolyte is prepressurized giving a very stable reference potential. The InPro 3250 SG electrode was connected to a M 700 transmitter with two pH modules, one of the modules configured for mV reading of the platinum and then to the Distributed Control System (DCS) via an analog signal for real-time data utilization. By charting the mV response during the reducing agent addition, the progress of the reduction reaction can easily be followed and stopped at the optimal endpoint when a slight excess of bromine is still present. The redox endpoint data was gathered simultaneously (using physical samples) with the ORP/pH electrode and offline testing in the laboratory. The results proved relatively simple to interpret; for when the mV response from the redox probe was approx. 500 mV, the UV/vis absorbance was less than 0.100, indicating that a slight excess of bromine remained and the desired endpoint reached.

Benefit of applied technique

Optimization of the sodium bisulfite charge using on-line redox monitoring eliminated reprocessing due to overcharging the reducing agent, eliminated hazardous sampling and operator exposure, and eliminated laboratory test time. Issues around reprocessing and overcharge of bisulfite were reduced by more than 10-fold after the implementation of this application.

PAT applications improve process understanding

The underlying benefit of inline redox measurement is away from strict cost-savings arguments towards understanding the process. The greatest advantages of such a "PAT" application are process knowledge and understanding, and robustness and consistency of product.

www.mtpro.com/pH

InPro 3250 SG for redox and pH measurements.

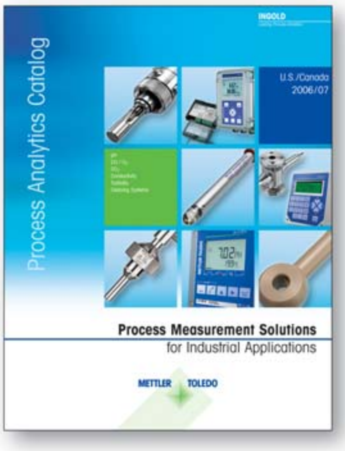
Process Analytics Product
Catalog and SpecBook

The Process Analytics Catalog and the SpecBook of METTLER TOLEDO are proven, frequently consulted reference books either to get an overview of all products on offer or to provide plant engineers with the relevant information to design their plant architecture.

Process Analytics Catalog

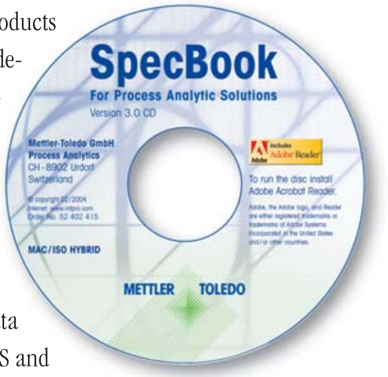
The catalog offers comprehensive overview on Process Analytics Measurement Solutions key products features, customer benefits, recommended application areas, order numbers and much more. The catalog comprises complete measuring solutions for the following parameter

- pH
- Dissolved oxygen and O_2 in gases
- CO_2
- Conductivity
- Turbidity



SpecBook

The SpecBook allows engineers to easily define and select the right products for their process during the design stage of a plant and during preparation of the relevant technical specification. The SpecBook technical CD version 3.0 also provides comfortable navigation aids, product manuals, technical data sheets, application notes, MSDS and others.



Please contact your METTLER TOLEDO representative to obtain these documents.

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