A comparison of in-situ sensors and off-line measuring technologies in biotechnology applications

Introduction:

Since several years we see a clear trend towards disposable technology used in biotech applications. With the increasing demand of disposable bioreactors and increasing number of different disposable reactor systems the demand for on-line control of such disposable bioreactors increased as well.

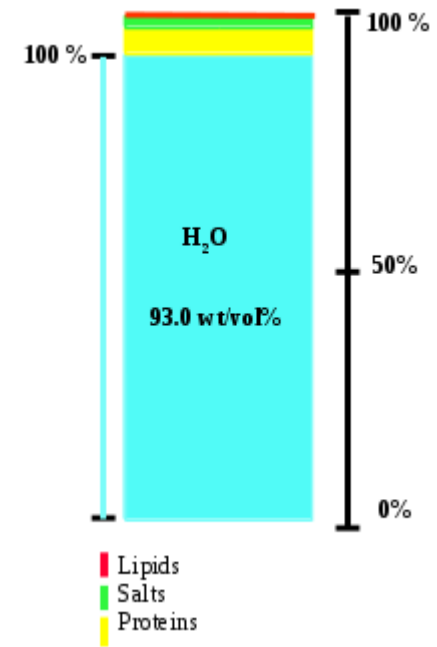
Due to the increasing demand of on-line measuring devices several technologies and new devices were released on the market for measuring not only pH and DO_2 but also glucose, lactate CO_2 and others. For the user one of the most interesting questions seems to be how precise and how comparable the methods are against off-line methods used till now. If the methods are comparable with off-line methods the necessity of re-evaluating and rewritting SOP's may be less important.

In the following report the possibility of such comparisons was investigated. It investigates how such comparissons are made today and what could be improved. At the end, it was tried to make suggestions for the best way to compare such methods.

Questions you should ask before comparing methods:

Before you start to compare methods you should consider the following questions:

- Characteristics of the method I use and I compare with. What fraction of analyte is measured?
- Which sample are we measuring under which conditions?
- What is the precision of different methods in general?
- Deviations due to sampling process and sampling preparation
- Temperature, pressure, pCO_2, pO_2
- Sampling rate and information compared to in-line sensors

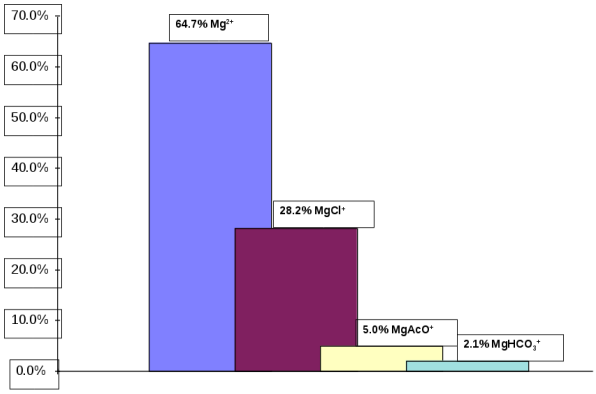


Pic. 1: Difference of total concentration and free fraction of analytes. The total concentration of an analyte is the amount of analyte solved in 1 liter solution. The activity of the analyte is the amount of analyte solved in 1 liter solvent.

What do sensors measure and what do other methods measure? Which fraction of analyte is measured

Chemical and biochemical (bio)sensors measure the free fraction of an analyte only (see picture 1). The total concentration is the concentration of analyte in 1l solution. In contrast the free fraction of the analyte is the concentration of analyte in 1l solvent.

Any analyte molecule interacting with any other compound in the sample will not be detected by the sensors and has no influence whether on-line or off-line sensors are applied. The free fraction depends on the background of the measured solution. An example of a possible difference in the free fraction and the total concentration is given in Pic. 3. If you dilute MgCl in water you will have different fractions of the analyte. Ion selective sensors do only measure the Mg^{2+} fraction. If you use now a method to compare with where sample preparation is needed you may see different results depending on pH, temperature, O_2, CO_2 and other matrix effects than measured with the sensors.

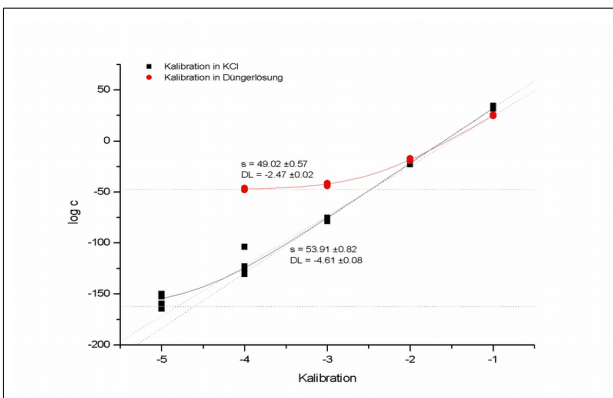


Pic. 2: Different fractions of Magnesiumchlorid when disolved in water. Ion selective sensors do measure only the Mg2+ fraction while AAS (Atomic Absorbtion Spectroscopy) measures the totel concentration. In this example the difference in the result will be around 30%

Examples are:

- pO2 (and not all oxygen in the sample)
- Ion activity or free ion concentration (not all ions of a specific one)
- ß – D – Glucose (not total glucose); and of ß – D – Glucose, only the free fraction.

The effect of such matrix effects on the measuring result and the calibration curve is shown in Pic. 3. If a sensor is now calibrated in a pure salt solution and used then in a sample solution with complex matrix the results will be wrong.



Pic. 3: Different calibration behavior for Magnesium sensor in different solutions with different ion background. The effect on different free fractions of Magnesium

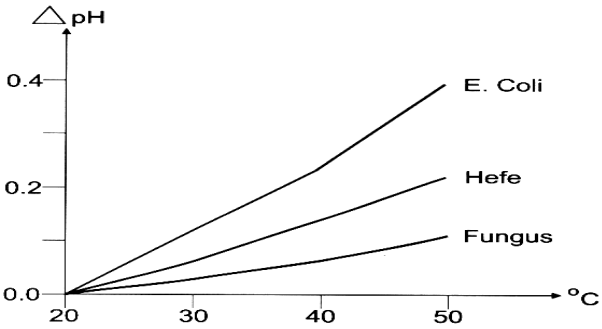
The free fraction of a certain analyte is depending on temperature, pH, O_2 and CO_2 and the different compounds in a sample. Depending on sample preparations, off-line methods can measure total concentration of a certain analyte. What sensors measure, is strongly depending on the background of the sample.

Which samples are measured under which conditions (some examples we came accross)?

If we know what each method really measures, we have to figure out under which conditions we are measuring. Are the conditions of the off-line and in-situ technique identical? What is differente if there are differencies, and what effects the sample, the matrix and the analyte?

Let's have a look on the comparison of pH measurements. Today many pH sensors do exist on the market. Optical pH sensors as well as elecrochemical sensors (ISFET as well as standard potentiometric pH sensors). All the sensors can be found in different shapes and for different applications. They all have their benfits as well as their drawbacks. Now very often those sensors are compared among eache other. Sometimes optical sensors against electrochemical sensors or in-situ sensors to off-line sensors. Due to the fact that all pH measuring methods do claim a temperature compensation very often it is thought that at least temperature differencies don't have an effect on the pH value. This is entirely wrong and even though a temperature compensation is realized, temperature effects cannot be entirely compensated.

In Pic. 4 you can see a comparison of the behavior of a pH sensor to temperature changes in different cell cultures. While the pH changes only by 0.1 pH unit for 30°C higher temperature, the same pH sensor changes by 0.4 pH units in a E.Coli culture. This means if you calibrate your pH sensor in pure buffer solution at 25°C and you use it then in a E.Coli cell culture at 36°C, you probably have no clue what the effect on the pH will be. The pH measurements may have an error of above 0.5pH units. Or, in other words, if you now compare an optical in-situ sensor working at 37°C with an off-line methode measuring at 25°C calibrated in a water solution rather than in the cell culture media, you may have dramatic pH shifts only because of the different environmental conditions and the calibration procedure rather than due to the method itself.



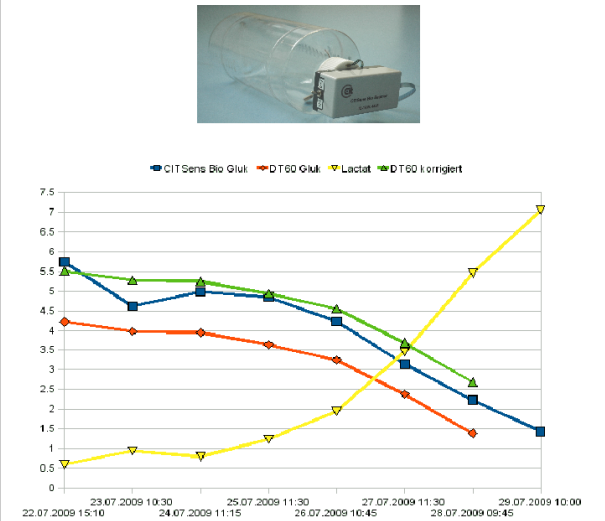
Pic. 4: Different behavior of pH values according the temperatur in different cell cultures. This matrix effect of pH / Temperatur dependencies can not be adusted by temperatur control.

Some examples of wrong measuring methods used and the effect on the final result of the analytical procedure (cell culture):

- A medical device (Glucometer) was used to measure glucose in a cell culture media: Whole blood is not the same as cell culture media. The off-line (Glucometer) method showed 10 g/l glucose; according to the declaration of the cell culture media, it contains 6 g/l glucose. Though the device measured 40% higher values than the cell culture media really contained.

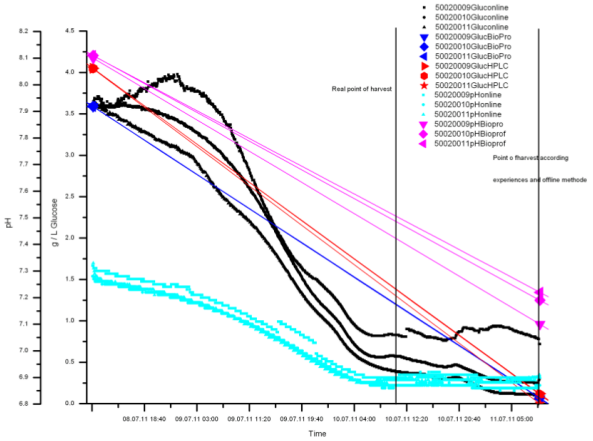
- Medical device (off-line analyzer) to measure glucose in a cell culture media: The off-line method shows 4.2 mMol/L glucose; according to the producer of the cell culture media, it contains 5.5 mMol/L glucose (see Pic.5). The in-situ sensors did measure the right values proved by HPLC.

- Off-line method to measure pH at 0.03% CO_2 concentration and 21% oxygen at 25°C. This is not the same environment as in an incubator with 5% CO_2 and saturation of media with oxygen at 37°C. pH off-line methods very often show higher values than in-line measurements in an incubator process (see Pic.6). Sometimes the difference is up to 1 pH unit. This is not because one of the pH sensor is wrong and the other is correct but only because of the environment measured in. Very often the cell culture media in an incubator is buffered by HCO_3- in the cell culture. HCO_3- is in an



Pic. 5: Results of glucose measurements in a CHO cell culture with an off-line medical device (red) and with on-line sensors (green). The samples were measured with HPLC too. The off-line methode showed a offset which was than corredted by the HPLC results (green line). After this the medical device showed correct data too. Yellow line is lactate measurement. This experiment shows that the background of the calibration solutions has to be addapted to the real sample. If not the results can change dramatically. A medical device is optimized to full blood measurement and not for cell culture media used in biotechnology.

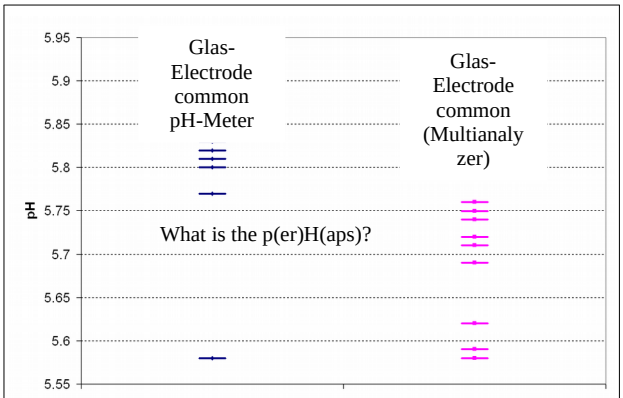
equilibrium with CO_2 in media which again is in equilibrium with CO_2 in the gas phase of the incubator. If the CO_2 concentration changes to environmental concentration (drop from 5% to 0.03%) the pH will change quickly to higher values and will be buffered at the new pH value. The buffering effect between CO_2 and HCO_3- can also be observed if a cell culture medium is stored in an open bottle under room conditions. If it is kept sterile you will see a change of color to purple which indicates a higher pH value.



Pic. 6: Comparison of an in-line and off-line pH analysis during the cultivation of CHO cells in shaker flasks in the incubator

Next question you should ask is: What is the precision of the compared methods and what precision is really needed?

Very often in SOP's precision is defined for a certain analysis method (e.g 0.01 pH unit). Normally the precision of the analysis method is chosen based on the ability. You should better choose a certain precision which fits to your application. The data from supplier and producer of a device are based on optimal laboratory condition. And the measurements performed for the evaluation of the precision and detection limits are done automatically at the lowest level of possible errors.



Pic. 7: One sample measured by 22 students on two different pH measuring devices. The difference is up to 0.3 pH units

If you measure real samples in the lab by different staff using the devices many times the precision is not as good as it would be in automated processes. In Pic. 7 measurements of the same pH buffer measured by 22 students using 2 different devices. The difference between highest and lowest measured value is 0.3 pH units. Allways consider and reconsider the needed precision. If you have SOPs, try to change SOPs rather than trying to get analytical results which are hardly accessible by the method used under the given circumstances.

Statistics, analytical rules, sampling process, time of comparison, sample preparation

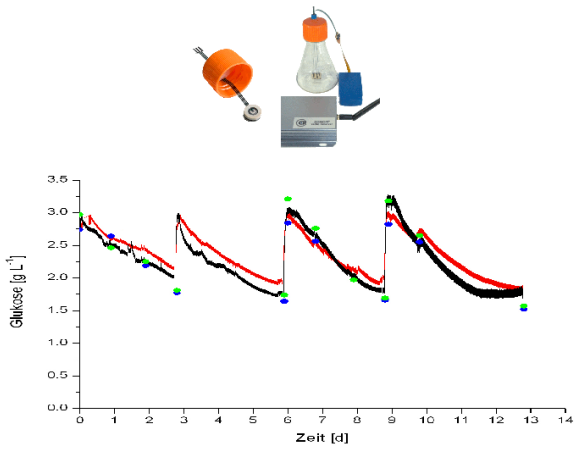
In cell culture technology, very often only one sample is taken and analysed. Good Analytical practice means that at least three samples are measured, take the average and calculate the Stdv. If you compare two methods do measure the same sample several times and take the average.

Very often we see customers transferring 10 – 20 flasks to the flow bench. The sampling process needs roughly 20 – 40 minutes. During this time, CO_2, O2 and temperature change. Simultaneously all chemical interactions and compounds change (different oxidation degree, different pH etc.) Centrifugation, filtration and any other sample preparation step change the background of the sample and will most probably change the result as well. So direct comparison of in-line and off-

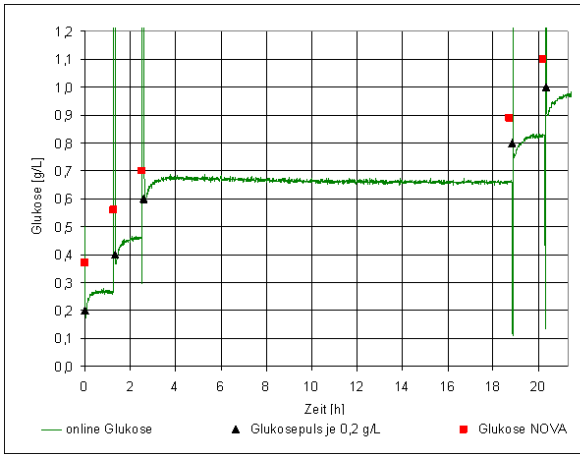
line methods are very difficult to perform and one has to consider several circumstances.
Nevertheless if the right things are considered and the measurements have been well done and the expectations are not higher than necessary, it is possible to compare methods.

List of things you should consider when comparing methods

- **The device, the method:** Is the device, the method and the protocol I use optimized for the application?
- **Temperature changes:** Even you have a temperature compensation it can only correct the behavior of the sensors and the temperature effect on the sample. If you compare, always compare at same temperature
- **Changes in gas concentrations:** CO_2 has an effect on pH. Especially in CO2/HCO3 buffered solutions which is often the case in cell culture media used in incubators. O2 may have an effect on the amount of oxidised molecules and ions.
- **pH changes:** Even you have buffered media, the pH may change if the environment changes (CO_2 buffered solutions)
- **Sample preparation:** Filtering, centrifugation, cracking cells and so on they may all have an effect on results. E.g if you crack cells the glucose which is in the cell will be released to the media. If you have an in-line sensor, this glucose will not be measured. The off-line method used after cracking the cells will measure this glucose. The higher the cell concentration is, the bigger this effect will be. Consider also the time between sampling and measuring. The longer the time is, the more reactions may occur in the sample.

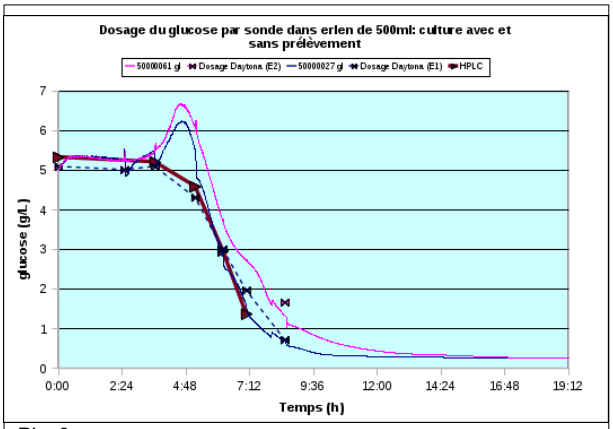


Pic. 7: Comparison of HPLC, off-line analysis and in-situ sensors in a real cell cultures. All methods are optimized for biotechnology applications. All three methods do vary a little from each other but you can't see a clear tendencie to a certain method which is more precise than the others. If you compare three methods you cannot expect higher precission than <10%. In most applications this is far good enough to work with.



Pic. 8: Pulse feed of glucose to a cell culture media. Controlled by in-line glucose sensors and off-line analysis method. In the graph you see the calculated pulse (blue) and the off-line method (red) and the sensor (green) as controlle methods. Both methods do show little higher values than calculated. But still in-line sensor does show slightly better results (error less than 10%) because of the calibration in the sample. Due to this you can make sure that background effects will be considered during calibration.

How you should compare



Pic. 9:

- Make sure you compare under same conditions
  - pH
  - Temperature
  - Gasconcentrations
- If you can't compare under same conditions be aware of the differencies and the eventually effect on the analyte
- Make sure the method, device, standard protocol you use are optimized for your application
- Due to the fact that in-line sensors do measure continously you may have more information about the process than with the off-line method and some times you will see things you have not bee aware of it yet.
- You have doubts on the results of the comparison contact the provider of the device.

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