Process mass spectrometry in pharmaceutical and cell culture processes

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By Graham Lewis

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Introduction

This article will discuss the use of gas analysis mass spectrometry (MS) in two key process applications.

Monitoring fermentation processes

Fermentation scientists have been using our gas analysis mass spectrometers since the early 1980s, to monitor reliably the composition of gas streams into and out of fermentors and bioreactors. These accurate measurements enable pre-screening for possible contamination as well as providing real-time information on culture respiration and nutrient availability.

Fermentation is used to produce a wide range of pharmaceutical products, from antibiotics and vaccines to hormone and anti-cancer therapies. There are three variations of the fermentation process – batch, fed-batch and continuous. Our mass spectrometers have been used successfully on all three process types.

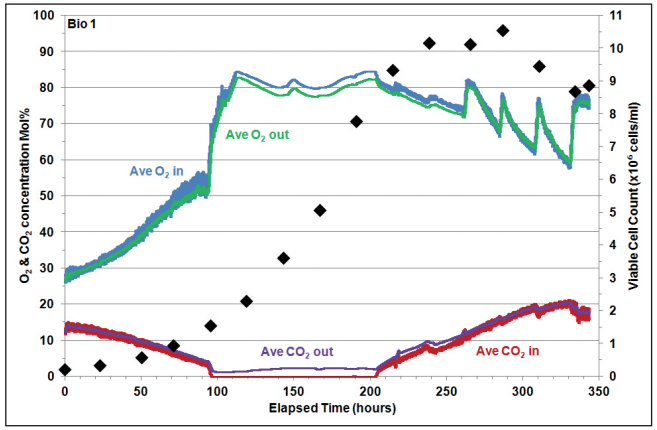
Historically microbial and bacterial micro-organisms have been used; recently mammalian cell fermentations have attracted a lot of interest because they can produce a range of extremely valuable products such as monoclonal antibodies.

Why use MS for gas analysis?

It is essential to monitor the state of the culture, since its health determines conversion rate of nutrients, formation of unwanted by-products and even onset of poisoning. Gas analysis of inlet and outlet streams is ideal for characterising the fermentation. It is non-invasive and monitors the physiological state of the fermentation, including growth kinetics and substrate consumption. It also helps determine the optimum point to halt the process for maximum yield.

Many fermentations are characterised by small changes in oxygen and carbon dioxide concentrations at critical phases of the fermentation, for example, during the lag phase. MS is fast but speed must not be at the expense of precision. It is equally important that precise data is acquired; otherwise small changes in concentration will be lost.

This is particularly the case in mammalian cell fermentations. In microbial fermentations, the feed gas composition is relatively constant – either air or air enriched with oxygen. In mammalian cell fermentations, feed gas is a frequently changing mixture of several compounds (e.g. nitrogen, oxygen and carbon dioxide). Feed gas concentrations vary dramatically – for example CO2 can vary from tens of parts per million to tens



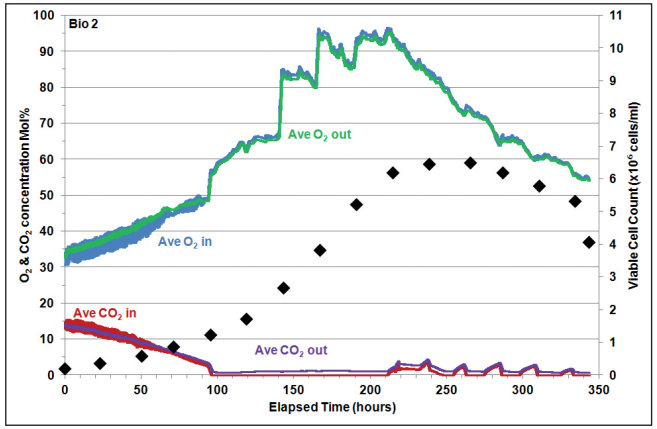


Figure 1: Online MS gas concentration data and offline VCC data from two mammalian cell bioreactors

of percent. If overlay gas is used this introduces further complications because the overall inlet gas composition is a combination of sparge gas and overlay gas.

Figure 1 shows online MS readings for O2 and CO2 for inlet and outlet streams for two bioreactors, together with offline Viable Cell Count (VCC) data. One can see there is an increase in CO2 and a decrease in O2 between the inlet and outlet for both bioreactors, but the concentration differences are greater in Bio-1 than Bio-2. The VCC is noticeably higher in Bio-1; this is consistent with the higher O2 and CO2 deltas.

Advantages of magnetic sector MS

Two types of MS have been used to monitor fermentation processes: magnetic sector, where charged particles are separated in a variable magnetic field, and quadrupole, where charged particles are separated in a variable RF field. We manufacture both quadrupole and magnetic sector mass spectrometers; over 30 years of industrial experience have shown the magnetic sector based analyser offers the best performance for fermentation off-gas analysis.

Magnetic sector MS has many inherent characteristics of benefit to the user, including high precision and accuracy, resistance to contamination and long intervals between calibrations. The latter is extremely valuable in fermentation as many processes last for days or even weeks.

Table 1 illustrates the long term stability and precision offered by our MS. A unit was configured to analyze N2, O2, Ar and CO2 in a cylinder of compressed air continuously without interruption or recalibration for 7 days; cycle time was just 5 seconds. Day-to-day mean values for N2 and O2 vary by 0.005 %mol or less, day-to-day mean values for CO2 vary by 1 ppm or less.

	N_2	N_2	O_2	O_2	Ar	Ar	CO_2	CO_2
	%mol	%mol	%mol	%mol	%mol	%mol	ppm	ppm
	Mean	St Dev	Mean	St Dev	Mean	St Dev	Mean	St Dev
Day 1	78.0807	0.0028	20.9459	0.0026	0.9337	0.0003	396.84	1.31
Day 2	78.0767	0.0023	20.9494	0.0023	0.9342	0.0003	397.46	1.25
Day 3	78.0761	0.0024	20.9500	0.0023	0.9342	0.0003	397.34	1.28
Day 4	78.0798	0.0023	20.9469	0.0023	0.9337	0.0003	396.31	1.31
Day 5	78.0777	0.0030	20.9487	0.0028	0.9339	0.0003	396.76	1.34
Day 6	78.0741	0.0023	20.9518	0.0022	0.9344	0.0003	397.47	1.27
Day 7	78.0750	0.0023	20.9512	0.0022	0.9342	0.0003	397.23	1.30

Table 1: Example of long term stability data

Monitoring API product drying

Our mass spectrometers are also used to monitor the removal of solvents from downstream product vessels such as vacuum, tray or rotary dryers, a process which, without online process analysis, requires offline testing to verify successful product drying.

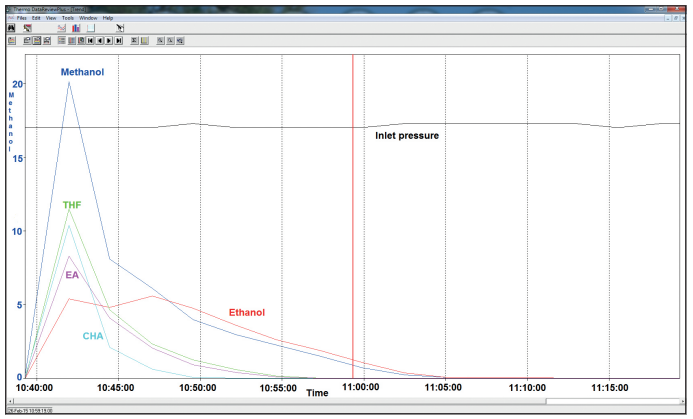
Product drying is an obvious candidate for PAT investigation. Historically the success of the process was simply measured at the end by taking a sample for laboratory analysis. If the sample failed the lab test then the drying process had to be restarted. This generated complications, particularly if the drying took place under vacuum.

Drying times tended to increase as a result, however this created additional process problems. The drying stage is often a rate-limiting process step so increased drying times had an adverse effect on lead times. In many cases the only way around this bottleneck was to increase drying capacity, at great expense. And an over-dried product could cause production problems downstream and potentially damage the final product.

Why use MS for gas analysis?

MS measures solvent concentration in the dryer headspace which relates to the bulk product and avoids problems caused by lack of product homogeneity. The MS samples at the dryer outlet and sampling is simple and straight-forward. Also the MS operates at high vacuum, typically 10-5 to 10-6 mbar, so sampling from vacuum drying processes is quite practical.

MS measures multiple species with excellent selectivity and very short cycle times (typically just a few seconds per sample point). So one MS can monitor several dryers each with completely different analytical methods.



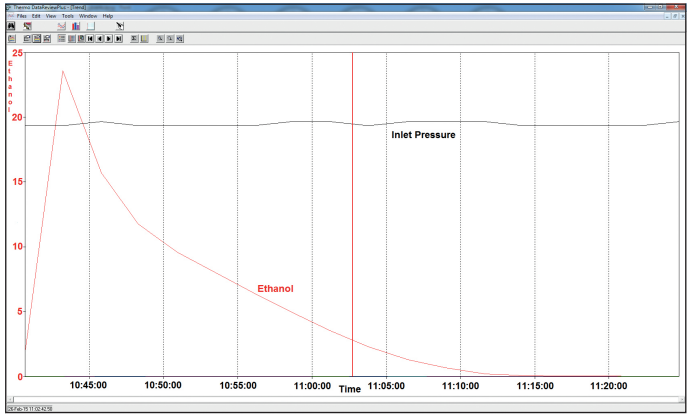


Figure 2: One dryer containing 5 solvents, the other containing just one solvent. Note that all other solvent readings remain at zero in dryer 2

Advantages of magnetic sector MS

The MS need to measure extremely high solvent levels at the start of the process so it is important that it is resistant to contamination. Magnetic sector MS has a proven track record monitoring high levels of hydrocarbons in the petrochemical industry without the need for frequent recalibration or cleaning.

Magnetic sector MS is also extremely selective; different solvents' mass spectra are measured with high precision, enabling the quantitative measurement of multi-solvent systems. Figure 2 demonstrates the selectivity of the MS - one dryer contains ethanol, methanol, tetrahydrofuran, cyclohexane and ethyl acetate while the other dryer contains only ethanol. Note that in dryer 2 all the solvent readings except ethanol remain at zero, indicating no cross interference between solvents.

Conventionally mass spectrometers are calibrated against certified gas mixtures in cylinders. Where calibration mixtures are unavailable or cost prohibitive, liquid calibration standards can be used on our mass spectrometers. This offers a simple, economical method of calibration at the high concentrations typically encountered at the start of the drying process.

Conclusion

Implementation of PAT solutions in pharmaceutical manufacturing processes is an effective means to maintain product quality while reducing operating costs. Magnetic Sector MS is ideally suited to the online monitoring and control of both fermentation processes and solvent drying processes as it provides real time, precise, quantitative measurement of the gases in the headspace of bioreactors or dryers.

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