

**Proposal to loan a Thermo Scientific Prima BT bench-top gas analysis mass spectrometer for mammalian cell process development**

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**Background**

Our gas analysis mass spectrometers have been used for many years with great success to monitor a wide range of microbial and bacterial fermentation processes in the production of biopharmaceuticals, biofuels and biomaterials. They combine an extremely reliable Rapid Multistream Sampler for monitoring multiple sample streams (15 in the case of the Prima BT) with a scanning magnetic sector MS analyzer for fast, precise, quantitative multi-component gas analysis. They monitor the respiratory gases being fed into and removed from the bioreactor, and have also been used to monitor volatiles such as methanol, ethanol and butanol in the off-gas.

**Interest in Mammalian Cell Processes**

We are seeing increasing interest in mammalian cell culture applications from both existing and new customers. While off-gas analysis with MS (and other) techniques is well understood and frequently applied in microbial and bacterial processes, it is not so commonly used in mammalian cell processes. Although the same respiratory gases require monitoring, there are several process differences with mammalian cell culture that offer significant challenges to the gas analysis system:-

- Mammalian cells are more fragile, so sparge gas flow rates tend to be lower.
- While sparge gas composition in microbial & bacterial fermentations tends to be constant throughout the fermentation, mammalian sparge gas composition is a frequently changing mixture of several compounds (nitrogen, oxygen and carbon dioxide) over a wide range of concentrations. This makes comparing inlet and outlet concentrations more difficult.
- Overlay gases may be used, which may complicate comparison between inlet and outlet gas compositions
- Buffers may be used to control pH; these can contribute to carbon dioxide in the bioreactor outlet.

While these challenges should not be underestimated, we believe that the high precision quantitative data provided by the magnetic sector MS offers the best hope of overcoming them. Below we detail the standard performance specification for the Prima BT:-

Measurement	Sample Concentration Range %mol	Prima BT Standard Deviation
Nitrogen (N <sub>2</sub> )	0 ~ 100	≤0.005 %mol
Oxygen (O <sub>2</sub> )	0 ~ 25 (possibly 0 ~ 100*)	≤0.005 %mol
Argon (Ar)	0 ~ 1	≤0.001 %mol
Carbon Dioxide (CO <sub>2</sub> )	0 ~ 10	≤0.1% relative or 0.0003 %mol**
Methanol (CH <sub>3</sub> OH)	0 ~ 1	≤1% relative or 0.001 %mol**
Ethanol (C <sub>2</sub> H <sub>5</sub> OH)	0 ~ 1	≤1% relative or 0.001 %mol**

\* If samples contain O<sub>2</sub> above 25%, a Fomblin rotary pump option is used to avoid an explosion risk.

\*\* Whichever is greater

- Precision is measured by periodically analyzing calibration gas over four hours.
- Analysis time including stream switching is typically 10 seconds per stream for the four air gases. This increases to around 30 seconds per stream if Methanol and Ethanol are added to the analysis.
- If both inlet and outlet sample streams are analyzed, Prima BT can calculate and report the Oxygen Uptake Rate (OUR) and Carbon Dioxide Evolution Rate (CER) in terms of percentages of inlet flow (CDC and OXC), and also the Respiratory Quotient (RQ).

#### **Current status of our involvement in mammalian cell trials**

To help develop our understanding of the mammalian cell application, we have supplied a Prima BT MS on loan to University College London's Department of Biochemical Engineering, monitoring two 5 litre bioreactors in which modified CHO cells are used to produce monoclonal antibodies. The MS analyzes inlet and outlet gases for O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub> and Ar on both bioreactors with a 10 seconds cycle time. Besides the on-line MS measurements, cell count (total and viable), lactate and glucose levels are analyzed off-line by other systems. Sparge gas is a combination of O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub> and Air. Oxygen feed is used to control dissolved oxygen and carbon dioxide to control pH.

Based on this work, UCL feel they have a much better understanding of their process and have used this understanding to improve output. The work is currently being turned into technical papers and oral presentations for conferences, including next year's IBioIC conference.

We will shortly be supplying another Prima BT unit on loan to another institute; it will be used to monitor another mammalian cell process, again at the benchtop scale.

#### **The next step**

We are now interested in collaborating on a trial with another organization working on mammalian cell processes at a larger scale, say 50 litre bioreactors or above. We will offer:-

- Prima BT gas analysis MS on loan for a mutually agreed period
- Technical support to commission the unit and support it during the trial.

We would want to share data from the trial; this data would be used to produce technical papers and oral presentations for conferences.

Potential collaborators would include biopharma companies and research institutes working to develop mammalian cell processes. We believe, based on our experience so far, that the gas analysis data provided by the Prima BT will increase significantly the collaborating company's understanding of their process.

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