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The use of the miniaturised Microsaic 4500 MiD® mass spectrometer as a point-of-need analyser for Process Analytical Technology (PAT) in automated bioprocessing

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INTRODUCTION

Mass Spectrometry (MS) is key to the development and manufacture of modern biologic medical products and has helped the rise in approved biologic licence applications (BLAs) in recent years [1,2]. With the drive by the pharmaceutical industry and regulatory bodies towards Quality by Design (QbD) and Process Analytical Technologies (PAT), the information rich data provided by MS is ideally suited to facilitate this move [3]. However, the use of conventional mass spectrometry for real-time process monitoring in biologics manufacturing has been limited to centralised analytical laboratories due to size, cost and complexity, despite its potential to improve process safety, efficiency and economics.

Here we describe the use of the Microsaic Metabolite Monitoring PAT system, consisting of a Microsaic 4500 MiD® compact Mass Spectrometer coupled with a Hydrophobic Interaction Chromatography (HILIC) workflow, in the upstream manufacturing of a biotherapeutic. This system was integrated with the Cogent Datahub, Seg-Flow automated sampling system (Flownamics) and PharmaMV software (Perceptive Engineering) to work automatically in an upstream bioprocessing workflow. This allowed monitoring of metabolites and control of glucose in a large 10 L upstream cell culture close to real-time and at high frequency, 24/7.

METHODOLOGY

The Microsaic Metabolite Monitoring PAT system consists of a miniaturised microflow electrospray ionisation (ESI) source MS coupled to a HILIC Chromatography workflow. Microsaic's 4500 MiD® miniaturised point-of-need MS, equipped with a chip-based single quadrupole, was used as the MS detector in this workflow. This 4500 MiD® system measures 55 x 35 x 25 cm and contains all the vacuum pumps and control PC integrated into a single unit. The HILIC workflow and sample delivery was undertaken by a modified Microsaic MiDas™. This modified MiDas™ consisted of a 2-position 10 port switching valve, ceramic pump head for biocompatibility and additional column oven. The modified MiDas™ allowed receipt of samples from the Seg-Flow automated sampling system and control of an isocratic HILIC separation. The compact sizes of both the 4500 MiD® MS and the MiDas™ allowed the system to be used on the bench, at the point-of-need, next to a bioreactor autosampler, allowing close to real time monitoring.

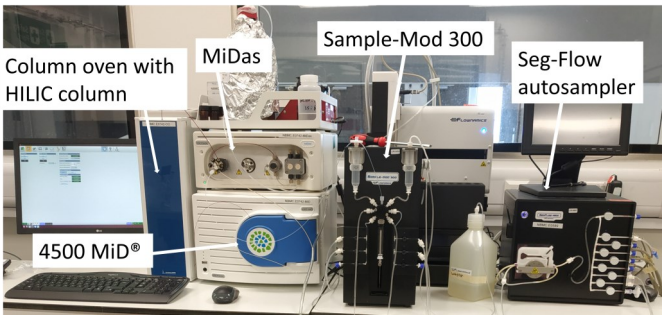

FIGURE 1: Experimental configuration of the Microsaic Metabolite Monitoring PAT system – The autosampler, consisting of a Seg-Flow autosampler coupled to a Sample-Mod 300 (right), sampled from the 10 L bioreactor at a pre-defined time interval and delivered these filtered cell culture samples to the 1 µL injection loop on the MiDas™ equipped with a ceramic pump head (left). Samples were separated using HILIC chromatography prior to analysis by the point-of-need 4500 MiD®

Masscape®, Microsaic's control software, was used to control the Microsaic Metabolite Monitoring PAT system. The workflow was programmed to communicate with the Open Platform Communications (OPC) server with various system states and to carry out tasks, including system start-up, column equilibration and loop cleaning, automatically upon receiving a trigger from the OPC server. The Flownamics Seg-Flow 4800 autosampler, supplemented with a Flownamics Sample-Mod 300, sampled the cell media from the bioreactor at regular intervals, clarified the samples by filtration, then delivered the cell culture samples to the modified Microsaic MiDas™ (Figure 1). Filtered samples were delivered directly to the 1 µl sample loop on the modified MiDas™ and subsequently injected onto a HILIC column, to separate out the metabolites of interest, prior to detection by the Point-of-Need 4500 MiD® MS for analysis. The processes were controlled and communicated via the OPC server using Remote Operations Protocol (ROP) commands.

Prior to analysing the cell broth samples from the bioreactor, a 10-point calibration was performed using matrix-matched standard samples. For this work only glucose, glutamine, glutamate and lactate were monitored and quantified regularly in the Microsaic Metabolite Monitoring PAT system. The concentrations were calculated automatically using Masscape®, where purpose designed algorithms for automatically analysing metabolite concentrations, were used to export data directly to the PharmaMV software.

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Anti-Her2 IgG1 expressing CHO cells were used and cultivated in the 10 L Biostat B-DCU bioreactor (Sartorius Stedim Biotech, Germany) at the Centre for Process Innovation (CPI). The pH, dissolved oxygen (DO), temperature and agitation were controlled at constant values, whereas the glucose level in the bioreactor was monitored by the Microsaic Metabolite Monitoring PAT system and the results were used by the PharmaMV software to control glucose concentrations to a pre-defined target of 4000 mg/L. Further metabolites, for example amino acids, were separated on the column and could be observed using the 4500 MiD® MS but were not quantified.

RESULTS AND DISCUSSION

Using the Microsaic Metabolite Monitoring PAT system allowed monitoring of the concentrations of the four metabolites concurrently from a single injection, reducing the required sample volume and analytical time for each analysis . The measurement of metabolites with orders of magnitude difference in concentrations, for example glucose and glutamine, was achievable simultaneously because of the wide dynamic range (3-4 orders of magnitude) of the Point-of-Need 4500 MiD® mass spectrometer. This allows filtered cell broth samples to be injected into the system directly without additional dilution steps (Figure 2).

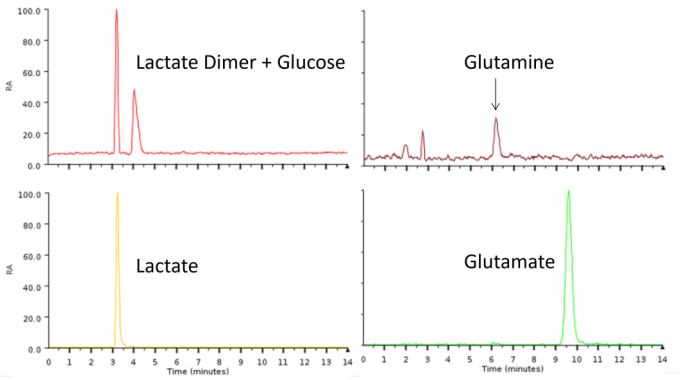

FIGURE 2: Typical chromatogram showing the simultaneous metabolite profiling of the filtered cell broth sample delivered from the bioreactor via the autosampler.

During the cell culture, the Microsaic Metabolite Monitoring PAT system demonstrated a 100% start-up success rate upon receiving 68 triggers from the OPC server. The error handling ability of the system has also been tested and demonstrated during the experiment, a critical feature towards a truly automated PAT system with minimum human input.

Continuous monitoring of the metabolites within the bioreactor is critical for optimising the condition of the cells. The measurements were taken at fixed time interval during the experiment and the metabolite concentration profiles are shown for glucose, lactate, glutamine and glutamate in Figure 3. With this increase in sampling frequency, afforded by automation, the changes in metabolite concentrations can be monitored in more detail. For glucose feeding, the higher sampling frequency also allows more frequent feeding, preventing the glucose from deviating too far from the target value of 4000 mg/L. This allows better control of glucose concentrations and improves the consistency of biologic Critical Quality Attributes (CQAs).

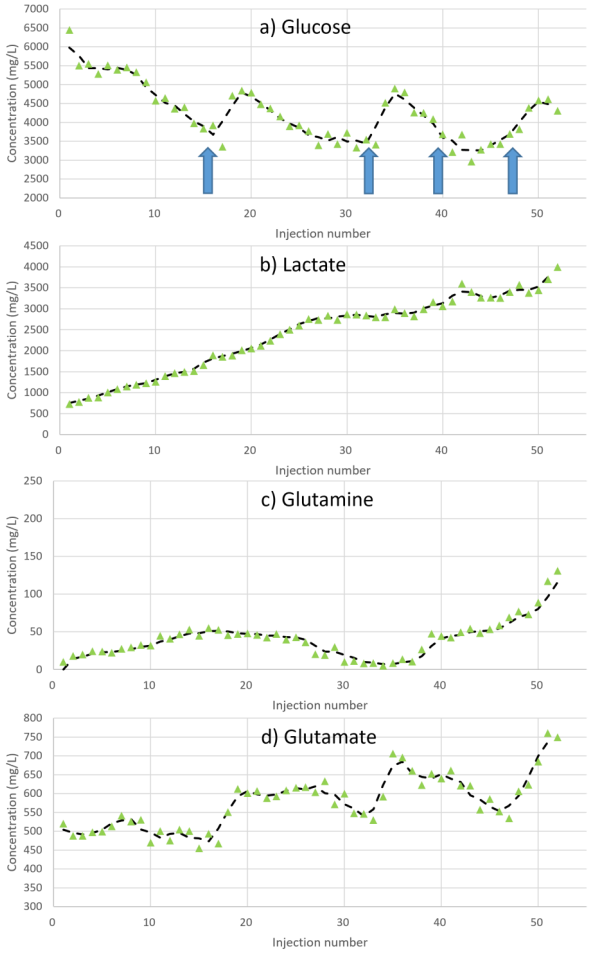

FIGURE 3 Metabolite concentration profiles during the 10 L cell culture when testing the Microsaic Metabolite Monitoring PAT system. Glucose feeding (large bolus) interventions are shown as arrows

CONCLUSION

We have described the integration of a low cost, small footprint mass spectrometer, the 4500 MiD®, in combination with a HILIC workflow for the automated monitoring of metabolites in upstream bioprocessing. The small footprint of the Microsaic Metabolite Monitoring PAT system enables measurements to be undertaken at the point-of-need, away from centralised laboratories, allowing close to real time monitoring. The successful demonstration of this PAT tool also offers the potential of lower running costs than currently used photometric assays. The methodology described here also has the potential to be combined with workflows for monitoring the biologic product CQAs alongside cell culture metabolites.

REFERENCES

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