Microsaic Systems

Using Mass Spectrometry for Point-of-Need Bioprocessing

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INTRODUCTION

For any monoclonal antibody to be efficacious and safe, it is important that the molecule remains stable from the time it's produced until it is used. Although freezing has been the preferred method of storing proteins in bioprocessing industries (1,2), it has also been identified as the cause of ice-water surface denaturation, cryoconcentration and cold denaturation of proteins over time (3). The drug substances sometimes need to be stored for extended periods before conversion into drug product. At around 200 K, proteins undergo Glass transition below which most of the protein's functions are altered (4). Glass transition is a phenomenon that occurs at lower temperatures as the result of change in dynamic behaviour of individual protein and viscosity during glass formation.

Applications of mass spectrometry (MS) in the pharmaceutical industry are well known. The new demands of modern bioprocessing and the implementation of QbD and PAT methods require a deeper understanding of these processes, MS is well placed to provide the information rich data required. However, traditional mass spectrometers are large, expensive, and require operation by specialists in centralised laboratories. Integrated real-time MS, decentralised and at the point-of-need, could provide important process information quickly and at reduced cost. This project will discuss the use of an innovative point-of-need microscale electrospray ionisation mass spectrometer (ESI-MS), to analyse the effect of storage time and conditions, feeding strategies and medium type on the IgG quality derived from Perfusion (Frozen) and Fed-Batch (Fresh) CHO cell cultures.

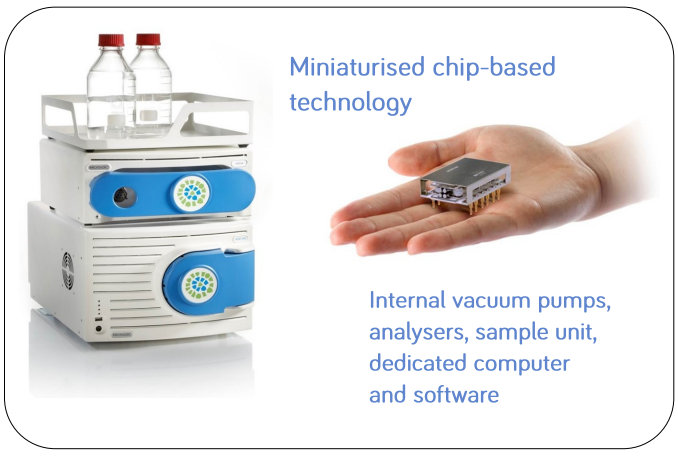

FIGURE 1. MiD® *ProteinID mass spectrometer and MiDas™ sampling interface unit.*

FOCUS

Analysis of the effect of different storage conditions on IgG samples and evaluation of the MiD® ProteinID for inclusion in bioprocessing operations.

EXPERIMENTAL

Conditions	Frozen Perfusion IgG sample	Fresh Fed Batch IgG sample
Culture Duration	18 days	12 days
Sampling Time	Everyday	On 12th day
Medium Type	OptiCHO™ (Thermo Fisher)	Dynamis™ (Thermo Fisher)
Feed Supplements	None	Feed A and Feed C
Storage Time and Temp	2 years at –20°C	A week at –20°C

TABLE 1. *Experimental samples.*

All samples were clarified using 0.2 micron filter and purified using Pure Proteome Protein A magnetic beads (Sigma-Millipore). The elution was done with 1% (v/v) formic acid. Samples were injected with a 2 µL loop into the MS. All the injections were done in triplicates. Cell media were also spiked with standard IgG to produce spiked recovery controls. The mobile phase used was Water:MeCN at 60:40 ratio along with 0.5% (v/v) formic acid.

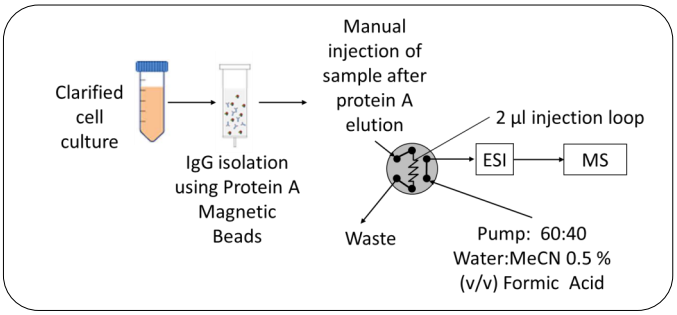

FIGURE 2. *Overview of sample preparation and workflow.*

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RESULTS AND DISCUSSION

The spiked recovery control sample of standard IgG showed a consistent mass of 148.2 kDa across the injection (Figure 3). The Fresh Fed Batch IgG sample also exhibited a consistent mass 147.2 kDa. However, the injection of Frozen Perfusion IgG sample showed considerable variation of masses across the injection, with IgG of mass~146.5 kDa eluting early in the injection and IgG of mass~148 kDa eluting towards the end of the injection.

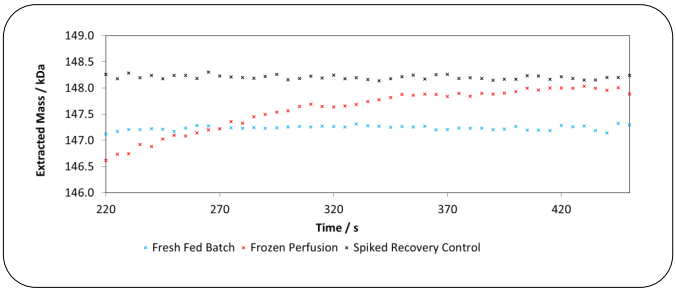

FIGURE 3. *Spiked recovery control IgG, Fresh Fed Batch IgG and Frozen Perfusion IgG mass distributions across the injection.*

The IgG produced in Fresh Fed Batch and Frozen Perfusion samples were different in terms of mass distribution. Figure 4 shows the injection of the Fresh Fed Batch and Frozen Perfusion samples. Different injection profiles can clearly be seen for the Total Ion Chromatogram (TIC). Observation of the mass-to-charge spectra at different time-points showed differences between the samples. At the peak maximum (300 sec) clear IgG spectra can be seen for both samples. In the peak tail (650 sec) little IgG can be observed in the Frozen perfusion samples, which is dominated by the presence of other multiply charged species.

Looking at the Frozen perfusion sample in more detail (Figure 5) a heterogenous distribution of the masses can be seen across the TIC. Different IgG masses (146.6 kDa and 147.4 kDa) were seen at the peak front and middle of the injection. An increase in signal between 750 and 1750 m/z in the peak tail, and the presence of extra multiply charged species was also observed. The uniform distribution of the IgG mass across the Fresh Fed Batch injection and the uneven masses of the IgG sample from the Perfusion sample suggests that the Fresh IgG from the Fed Batch was better in quality. The long storage of Perfusion samples is suspected as the major cause of IgG deterioration. The presence of this heterogenous population of IgGs can be further studied by glycan profiling these samples.

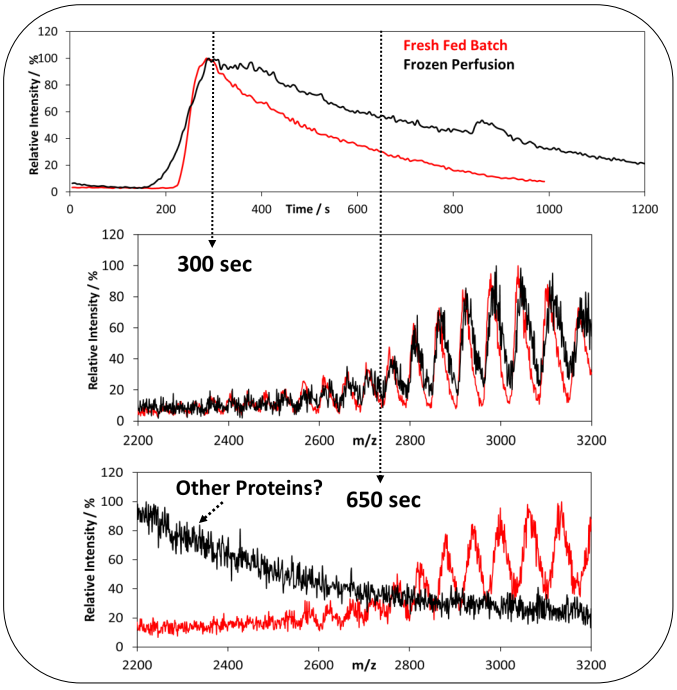

FIGURE 4. *Total Ion Chromatogram (TIC) of Fresh Fed Batch and Frozen Perfusion Samples (top). Corresponding mass-to-charge spectra (bottom) for the TIC at 300 and 650 seconds.*

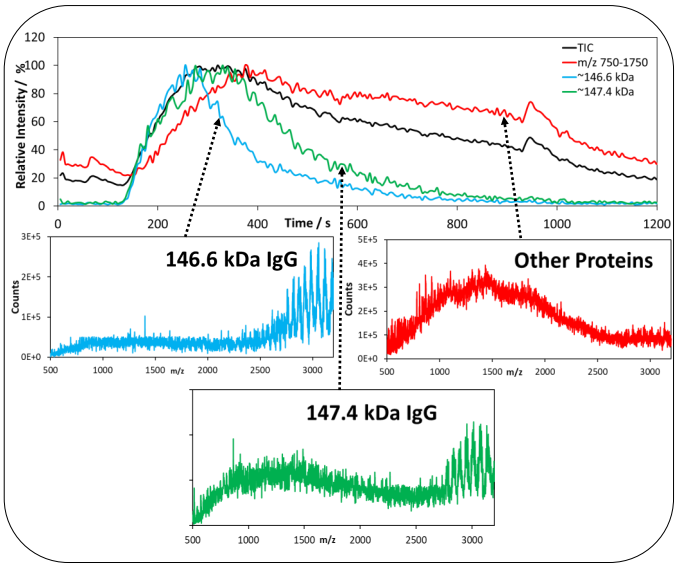

FIGURE 5. *IgG mass distribution in the Frozen Perfusion sample. Top shows the Extracted Ion Chromatogram (EIC) during the injection of Frozen sample. Blue represents the intensity of IgG species with mass~146.6 kDa over the injection and green represents the intensity of IgG species with mass of 147.4 kDa over the injection. Red represents the intensity of signal between 750—1,750 m/z for other observed multiply charged species.*

CONCLUSIONS

Proof-of-concept that the novel minaturised point-of-need mass spectrometer is able to monitor IgG and protein fragments, and offers the opportunity to determine product quality. Significant differences in the mass distributions of Fresh Fed Batch IgG and Frozen Perfusion samples, which were stored for one week and two years respectively, were observed. The vast difference in the masses suggests that the proteins may have deteriorated over time. The ice-water surface denaturation and the cryoconcentration (which are outcomes of storage conditions across time) may have contributed to the observed heterogenous mass distribution. However, further study and experimentation to identify which medium or feeding strategy works better for optimisation of IgG Production is required.

Acknowledgements

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