Monitoring CHO cell Cultures

CHO cells are the most common mammalian cell line used for mass production of therapeutic proteins. Aber Radio Frequency Impedance (RFI) probes are commonly used to both monitor and control these processes.

A study was carried out by Millipore (US) to assess the performance of both the multi-use annular probe and the disc style probe with flush platinum electrodes in a 3 L rigid plastic bioreactor (Carvell et al., 2011). The disposable probe was connected to a light weight "Mini-Remote Futura" pre-amplifier and the bio capacitance was converted into cells/ml by using a simple linear correlation factor. The calculated cell concentrations were compared with off-line measurements from the Vi-cell (Beckman Coulter, USA).

The capacitance measurements from both probes show a similar growth trend which correlate with the off-line measurements up to the peak cell concentration on day 6. In the death phase there was a small divergence between the on-line and off-line data. This divergence has been studied in more detail by a number of groups (Braasch et al., 2013, Lee et al., 2015) and it is indicated that the differences during the death phase can relate to changes in bio-volume or it can be attributed to the bio capacitance method picking up changes at the cellular level due to apoptosis at a much earlier phase than the trypan blue method. When investigating the online capacitance and offline measurements closely, there could be a strong argument that the RFI measurement provides a signal that is more closely related to the metabolic activity of the cells (Braasch et al., 2013).

The Mini-Remote Futura electronics have since been branded under the Sartorius Stedim Biotech (SSB) BioPAT ViaMass trade name. Sartorius now produces the SU biomass sensor disc under license (Figure 1), the company integrates this sensor into its SU Flexsafe bioreactors and markets these products in a dual-branding arrangement with Aber Instruments.

Figure 1 – BioPAT® ViaMass electronics supplied by Sartorius Stedim Biotech

Figure 2 shows the results of a CHO cultivation in a Sartorius Flexsafe® RM 50 L bag fitted with a single use RFI disc sensor. The capacitance signal is compared with the offline viable cell density measurements using a Cedex HiRes (Roche, Switzerland) as a reference. In addition, the Cedex measurement principle reveals the average viable cell diameter (µm). This was multiplied with the viable cell density for each sample. The result is the viable cell volume (cm^3/ml) or the viable cell volume as percentage of the total viable volume (%).

Additionally, the wet cell weight is plotted with the data relating to cell viability (from the Cedex). Observing the results shown in figure 2, the first 8 days which represent the exponential phase of growth, excellent correlation was observed between the capacitance signal of the online biomass measurement and the viable cell density, the viable cell volume and wet cell weight. After this phase, the cell diameter begins to increase rapidly and the viability drops. The deviation between capacitance and cell density increases with increasing cell diameter however, the calculated viable cell volume and capacitance correlate well up to the end of the cultivation. It is evident that the online measurement of the biomass (as percentage of the volume) shows every small effect of biomass change during the cultivation process, eg: each dilution due to additional feed medium and growth rate changes in the stationary and dying phase of the process.

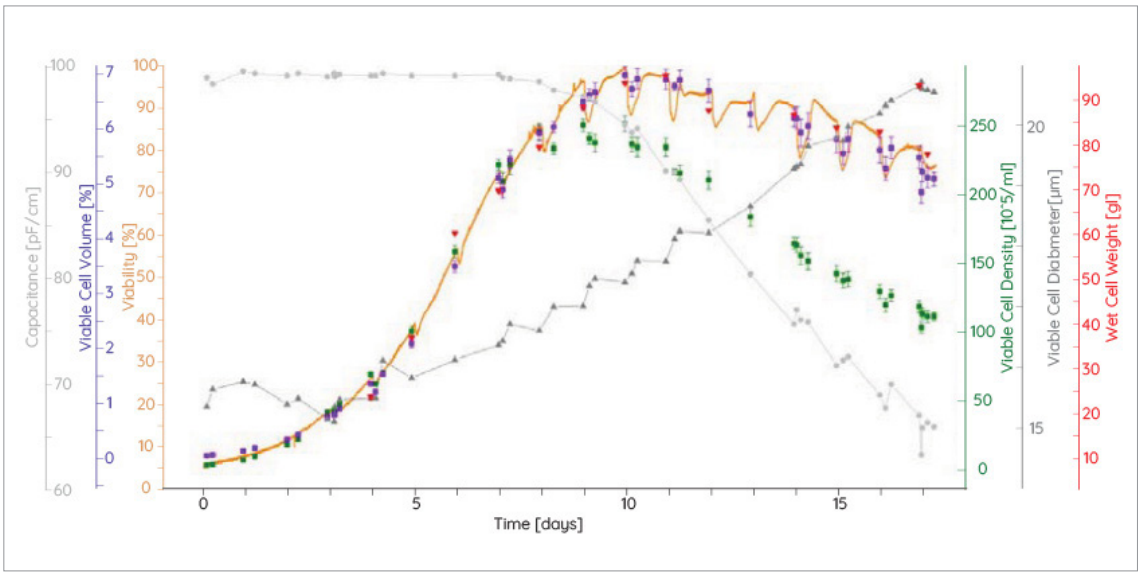

Figure 2 – Online Biomass measurement and offline reference for CHO fed-batch cultivation with single use BioPAT® ViaMass at the 50 L scale (Courtesy S. Ruhl, & J. Scholz. Sartorius Stedim Biotech, Germany)

The final example in this section shows how two different designs of a RFI probe perform when used in to measure a batch cell culture. Figure 6 shows a comparison study between the 12 mm Annular probe and the 7.5 mm Pico for measuring a CHO cell culture process. Both probes were placed in a Dasgip (Eppendorf, Germany) benchtop bioreactor. The performance of both probes was highly comparable and the cell concentration trends followed each other throughout the growth curve. In addition, both probes correlated well with offline viable cell density measurement. The performance of the small pico probe is particularly important if small bioreactors are being used in scale up studies and it is important to have the same pF/cell biovolume for an individual scale for the small design compared with the probe designs used in production cGMP vessels. Not only can the capacitance probes be used to measure cell concentration in different scale up platforms, but the capacitance trends obtained can be used to determine the success of a scale up process or strategy.

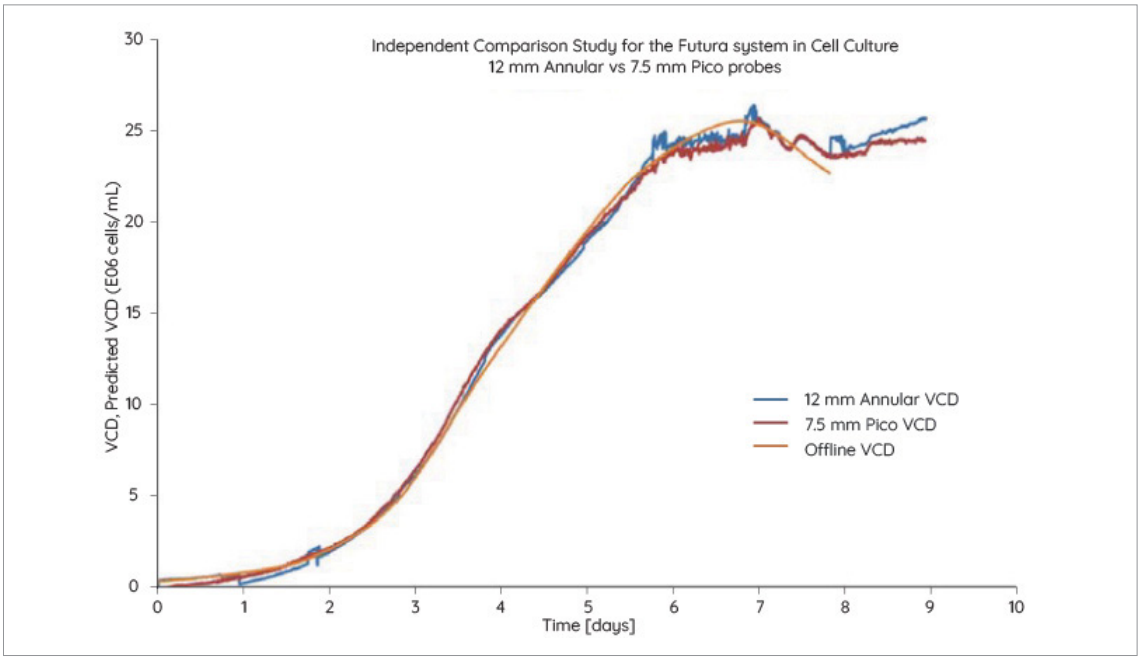

Figure 3. Comparison study between the 12 mm diameter Annular probe and the 7.5 mm Pico for measuring a CHO cell culture process.

Summary of the benefits:

- Monitor CHO cells in situ and in real time in a variety of bioreactor platforms (reusable and single use)
- Eliminates/reduces need for sampling
- Perform cell density measurements non-disruptively
- Perform measurements across different scales
- Obtain fingerprint of the process in real time
- Can be used to measure a variety of processes - batch, fed batch, perfusion and continuous processes

References:

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Carvell, J.P., Williams, J., Lee, M. and Logan, D., 2012. On-line monitoring of the live cell concentration in disposable bioreactors. In Proceedings of the 21st Annual Meeting of the European Society for Animal Cell Technology (ESACT), Dublin, Ireland, June 7-10, 2009 (pp. 315-318). Springer, Dordrecht.

Lee, H.W., Carvell, J., Brorson, K. and Yoon, S., 2015. Dielectric spectroscopy-based estimation of VCD in CHO cell culture. Journal of Chemical Technology & Biotechnology, 90(2), pp.273-282.

For further technical information please contact:

support@aberinstruments.com

or alternatively contact sales@aberinstruments.com