AMINO ACIDS AND ANTIBODY N-GLYCOSYLATION BASED ON RAMAN SPECTROSCOPY IN HIGH CELL DENSITY PERFUSION CULTURE

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Raman spectroscopy based on a probe is easy to mount in a bioreactor and has been proven to be an interesting process analytical technology (PAT) in bioprocesses. The information about important parameters of the culture is extracted from Raman spectra often based on partial least square regression models (PLS). While numerous applications of PAT by Raman have been reported for fed-batch cultures, to our knowledge, no similar study has focused on steady-state perfusion process. This latter type of process has typically a stable metabolite profile, which is not conducive to modelling due to the absence of variations of the culture parameters. Here we present an approach where a dynamic culture environment favorable for the Raman model calibration was obtained during the process development.

Antibody producing CHO-K1 cells were cultured in 200 mL bioreactors with ATF2 and mounted with Raman probes (Kaiser+Endress). Different steady-states were studied at cell specific perfusion rates (CSPR) between 10 and 40 pL/(cell*day) by varying the cell density and the perfusion rate. The process performances were not influenced by the cell density studied between 2 at 100 x 1E6 cells/mL but the CSPR had an impact on the metabolism, antibody productivity and N-glycosylation.

Predictive least square regression models were developed for the concentrations of cells, lactate, ammonia and amino acids, and validated with new runs performed at multiple or single steady-states, showing high prediction accuracy in cultures between 2 and 100 x 1E6 cells/mL [1]. A high correlation of the amino acid profiles and the antibody N-glycosylation pattern was observed, and a new PLS model was created exploiting this correlation. This latter model provided an evaluation of the glycosylation profile in real-time in cross-validation [1]. The present approach joining a dynamic process development and PLS model identification based on Raman spectra can provide a highly valuable PAT for monitoring and control of steady-state perfusion processes.