RamanFest November 09-10 2023 Paris | FRANCE

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Raman spectroscopy as a part of the PAT approach for in-line bioprocess monitoring

Context and Objective: Successful industrial bioprocess, in particular that concerning production of therapeutic antibodies (TAB) by animal cells in culture, requires the possibility of controlling a multitude of parameters, those of the process (CPP as critical process parameters) and those of the product (CQA as critical quality attributes). CPPs correspond to the concentrations of nutrients, catabolites and the density of the living cells. The CQAs correspond to the characteristics of the TAB produced (post-transcriptional modifications, aggregation state). Monitoring CPP and CQA in real time allows corrective actions to be carried out without leaving the quality margins. Even more interesting is to measure them without sampling (in situ), thus reducing the risks and costs linked to analyzes and helping to better understand the mechanisms involved in the successful process (Quality by Design approach).
Such a strategy becomes possible by process analytical technology (PAT) approaches, namely using molecular optical spectroscopy (MOS) where Raman spectroscopy may have a particular role. As part of a CLIMBIN collaborative project aiming to develop a PAT-MOS solution for bioprocessing, the present work evaluatied the Raman spectroscopy performance in quantitative analysis of bioprocess ingredients.
Methods: CHO cells (ExpiCHO) suspensions were cultivated in a commercially purchased nutritive medium (ExpiCHO™), at controlled conditions (pH, temperature, gas equilibrium), for up to one week, either in flasks or in a Bioreactor (Primo, Pierre Guérin). The culture medium was analyzed either as in-line kinetics, or off-line, as the model solutions prepared to correspond to different moments of the cell culture. Both in-line and off-line Raman measurements were performed via immersion probes coupled by optical fibers with a Viserion Raman spectrometer (INDATECH, France) equipped with a 785 nm laser source and CCD detector. The Raman data have been then pre-processed and analyzed using MATLAB® (Mathworks, USA). The same samples were also analyzed by conventional off-line methods, to establish viable cells density (NucleoCounter® SCC-100™, Chemometec) and molecular composition of the medium (biochemical analyser Gallery, Thermo; HPLC, Agilent).
Results and Conclusions: Operating conditions were optimized for recording Raman spectra from both the off-line model solutions and the in-line bioreactor monitoring. The multivariate statistics modes were established to predict from the Raman spectra the concentrations of several molecules (nutrients, metabolites or TAB) and the cell density. The performance of the Raman-based vs conventional methods-based analysis has been compared.

Acknowledgment

The authors acknowledge the financial support from BPI France (CLIMBIN project), from Académie Orléans-Tours and DGESIP, France (DIMAMAB project), the co-funding of the thesis of A. Rayyad from LabEx MabImprove, Tours and Fondation Philippe Maupas, Tours. We also thank Laëtitia Grenat from IMT group, Tours, France, for technical assistance.