M^3C Workshop ESBES-8 Bologna

Sunday 5 September 2010, 14.00-15.45

Process Analytical Technology for Biopharmaceuticals

Chair: Carl-Fredrik Mandenius

The workshop is organized by the ESBES Working Group for Measurement, Monitoring, Modelling and Control (M^3C) and highlights recent progress in Process Analytical Technology for production of biopharmaceuticals. The first five presentations bring up monitoring and control aspects on a variety of product examples. Focus is predominantly on mammalian bioprocesses.
The last last presentation intends to bridge the topics of this workshop with an adjacent area: biochemical engineering for waste treatment where M^3C methods also have significant impact.

1. Process control and Systems Biology: application to mammalian cell cultures

Rui Oliveira

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The progress in the "-omic" sciences has allowed a deeper knowledge on many biological systems with industrial interest. This knowledge is still rarely used for advanced bioprocess monitoring and control at the bioreactor level. In this work, a bioprocess control method is presented, which is designed on the basis of the metabolic network of the organism under consideration (Teixeira et al., 2007). The metabolic network is decomposed into elementary modes (EMs), which are the simplest paths able to operate coherently in steady-state. Metabolic reduction strategies are employed to identify dominant pathways and respective environmental control variables. A dynamical model integrating material balance equations, EMs stoichiometry and kinetics is formulated. EMs kinetics have a mechanistic term and an empirical term identified from data, in a process control perspective. This approach allows the quantification of fluxes associated to dominant pathways as function of environmental conditions. The methodology was employed to control a recombinant Baby Hamster Kidney (BHK-21A) culture expressing a fusion glycoprotein (IgG1-IL2). The identified EMs kinetics demonstrated typical glucose and glutamine metabolic responses during cell growth and IgG1-IL2 synthesis. Finally, an online optimisation study was conducted in which the optimal feeding strategies of glucose and glutamine were calculated after re-estimation of model parameters at each sampling time. An improvement in the final product titer was obtained as a result of this online optimisation control scheme.

Ana P Teixeira, Carlos Alves, Paula M Alves, Manuel JT Carrondo and Rui Oliveira (2007) Hybrid elementary flux analysis/nonparametric modeling: application for bioprocess control, BMC Bioinformatics 2007, 8:30

2. Multivariate data analysis for industrial CHO bioprocess development

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Achievements in bioprocess development can be attributed to the use of high-producer cell clones with enhanced cell productivity and to the optimization of key process characteristics such as advanced process control and well-designed nutrient compositions that are integrated into a platform technology for biopharmaceutical manufacturing processes.
In order to identify potential process parameters which have an impact on critical quality attributes (CQAs), the process-developer is forced to identify and understand a set of critical process parameters (CPPs). One possible approach is to integrate multivariate techniques directly into instruments or equipment, allowing the user to gain high value information from the techniques without expert knowledge. Here, we present some examples for the application of multivariate data analysis in high titer CHO fed batch processes using commercial software from Umetrics.

3. PAT in cell culture monitoring

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Quality by design and PAT methodologies have been at the forefront of bioprocess modelling and monitoring research literature for a number of years now and a number of research publications dealing with advanced monitoring of cell culture processes increases rapidly.
This contribution will present results of a number of case studies involving industrial bioprocess development and using relevant data analysis techniques for given application. In particular the benefits and issues related to the application of near infra-red spectroscopy in process decision making during cell culture manufacturing processes will be discussed. The ability of multivariate data analysis techniques, such as Principal Component Analysis (MPCA) and Partial Least Squares (PLS), to reveal important features relating to processing will be highlighted. Issues around multiplexing probes and limited data availability will be discussed in light of the ability to produce models fit for purpose.
Reducing lead time from R&D to full manufacturing process is a critical objective of biopharmaceutical industry. Extracting maximum knowledge from the early development experiments is a key element of gaining process knowledge required to achieve building quality by design into the product and thus enable a more systematic and robust process development. It will also enable the company to gain FDA approval on PAT basis with significant implications for manufacture.

4. Recombinant protein production in *E. coli* – process understanding the base for implementation of PAT and QbD in upstream processing

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Understanding and control of the manufacturing process are coinciding key criteria of process development and the FDA's PAT and QbD initiative. These mutual interests are impaired by the complexity of bioprocesses and the challenge of in-, on- and/or at-line measurement of physiologically relevant variables. An opportunity to overcome this problem is the application of a chemometric approach to integrate and correlate physical and chemical variables with a broad spectrum of off-line data sets. The implementation of novel on-line sensor systems like multi-wavelength on-line fluorescence spectroscopy or proton transfer reaction mass spectrometry and the expansion of off-line analysis to high-through-out off-line techniques (omics-technologies) strongly support this concept. Physiology relevant off-line data enable assignment of simple to acquire on-line signals to cellular reactions thereby providing increased real time process understanding. Altogether, the application of PAT and chemometrics forms the base for identification, monitoring and/or prediction of critical process parameters (CPP) that have significant influence on critical quality attributes (CQA) the fundamental parts for implementation of QbD concepts.

5. Associating physical parameters to the biology of cells using the propagation of acoustic waves in thick shear mode acoustic devices.

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Acoustic wave sensors have been shown to be highly effective functional devices to monitor the process of cell adhesion to surfaces in real time, to study several aspects of cell biology, and to evaluate the effect of different chemicals at the cellular level. The thickness shear mode (TSM) acoustic wave sensor is the most popular acoustic wave device, generally known as Quartz Crystal Microbalance (QCM). The uses QCM devices to study mammalian cell adhesion onto chemically modified surfaces have been increasing in the recent years. Upon real time monitoring the variation of the sensor resonance frequency and motional resistance, or dissipation factor, the morphological alterations occurring during cell adhesion have been signalled and identified for different cell types.

In this work we develop methodologies to use QCM devices to study different aspects of cell biology. Multilayer sensor devices that include a quartz substrate, a cell–substrate interfacial layer and a cell layer are constructed to associate physical parameters, namely resonance frequency, resistance and impedance, to biological events in order to directly evaluate morphological and physiological alteration of the adsorbed cells. As such, the dynamic process of cell adhesion as a function of cell seeding densities, type and density of adhesion molecules at the sensor surface is monitored using the acoustic wave sensor. Impedance spectroscopy analysis of the acoustic sensor is used to assess the variation of the relevant parameters, namely the motional resistance, induction, and resonance frequency. Specific sets of parameter variations are correlated with the different cellular status, morphology and phenotype. The physical model predictions of the acoustic wave sensor are further used to identify and elucidate the cellular mechanisms that are responsible for the frequency and resistance variations.

In this communication we show the data reflecting cell adhesion as well as how to use the physical parameters to evaluate the viscoelastic properties and their modifications during the life cycle of adsorbed cells. As the viscoelastic properties of the cells are expected to change considerably upon stem cell differentiation we expect that this technology is suitable to directly monitor and study stem cell differentiation.

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6. Mechanistic modeling in the frame of PAT applications

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Implementation of PAT (Process Analytical Technology) on a pharmaceutical production process requires a good understanding of the manufacturing process as well as the on-line use of this knowledge, via a suitable control strategy, to ensure the desired product quality. The recent scientific literature on PAT covers many examples of application of on-line equipment for process monitoring and analysis, where chemometric methods such as PCA and PLS play a key role. However, only a few studies deal with the use of mechanistic models in a PAT context. The objective of this presentation is therefore to highlight the potential application of mechanistic models in PAT context. Examples will be taken from recent publications of the PROCESS group, and will have focus on fermentation modelling, uncertainty and sensitivity analysis and design of PAT systems.