PAT in Bioprocessing

Innovative pharmaceutical development, manufacturing and quality assurance is a main concern of FDA for the future. Understanding and controlling a bioprocess by application of latest advances in science and technology is strongly recommended. This accelerates technological progress on a broad basis. For development and implementation of the required tools, biological knowledge has to thoroughly meet technical and engineering competence.

TEXT **HELMUT TRAUTMANN**

We are midst of explosive growth in the number of biotechnology medicines, offering great promise for a broad spectrum of diseases. Currently, over 130 biopharmaceuticals are on the market including thirteen blockbuster drugs, representing an overall annual market value of $ 48 bn (1).

Up to date, these substances are favourably produced by their natural producers, the mammalian cells, which exhibit high demands on process management. Ensuring safe, reproducible and predictable processes is an aspired goal. However, up to 30% of processes cannot be used or have to be reprocessed for quality reasons, leading to a significant financial loss.

Additionally, due to a strong increasing demand in biopharmaceuticals, recent projections predict an increasing shortfall in production capacity during the next three years. Industry analysts assume that inability to fulfil an actual production request causes potentially a later-on 10fold loss of profit over time (2).

In conjunction with the vital necessity to lower expenses in health care, bioprocessing industry is under pressure to reduce their production costs, and speed up time-to-market. Hence, increasing overall quality and thus efficiency of bioprocesses is an urgent demand. With PAT (3), FDA stimulates with a scientific and risk-based framework pharmaceutical manufacturers to innovate towards this demand.

PAT – a non-typical FDA guidance

Process Analytical Technology (PAT) is a non-typical FDA guidance encouraging industry for voluntary development and implementation of innovative pharmaceutical development, manufacturing, and quality assurance using the latest scientific advances in technology. The FDA's main goals are:

- Ensuring a predefined product quality at a low variability at the end of the production process, leading to real-time release of the product
- Reducing production cycle time and costs by using on-, in- or at-line measurements and controls
- Preventing rejects, scrap and reprocessing

According to W. Edwards Deming's rules of quality management (4), "quality cannot be proved into products; it should be built-in or should be by design". His postulation to „manage the cause not the result" focuses on FDA's intention „to design and develop well understood processes that will consistently ensure a predefined quality at the end of the manufacturing process".

It is a clear demand to increase process knowledge largely and to transfer it consistently, beginning from R&D up to production. Further, the steady increase in knowledge during the entire lifetime of a product should contribute to permanent progress in process management.

This demonstrates a shift in FDA's paradigm from an originally static, end-product orientated claim to an overall dynamic object-orientated quality concept.

Bioprocess Technology – Biology meets Engineering

By asking for efficient, robust and easy-to-use process analyzers and control tools operating on highly sophisticated latest technologies FDA contributes generally to an acceleration in technological development from state of the art to state of routine.

In particular, PAT is encouraging for

- Multivariate tools for design, data acquisition and analysis
- Process analysers
- Process control tools
- Continuous improvement and knowledge management tools

For development and implementation of such tools, biological knowledge has to meet technical and engineering competence. A substantial progress can only be achieved by close and interactive collaboration of these disciplines.

INFO **THE AUTHOR**

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Dr. Helmut Trautmann (1958) graduated from Albert-Ludwigs-Universität Freiburg, Germany in pharmacy in 1985 where he was awarded his Ph.D. in Pharmaceutical Technology in 1990. He worked as a postdoc and leader of the mammalian cell culture group at ETH Zürich's Institute of Biotechnology and joined Biospectra in 1993. Since January 2005 he works as an independent consultant in bioprocess technology and process analytics.

Biological Demands

Where are the specific problems in bioprocessing compared to chemical processes? Compared to a chemical process with usually a limited number of educts, intermediates, products and by-products standing in relation to an almost explicit arranged set of stoichiometric and kinetic equations, a bioprocess requires the consideration of the behaviour and reactivity of a multiplicity of living cells:

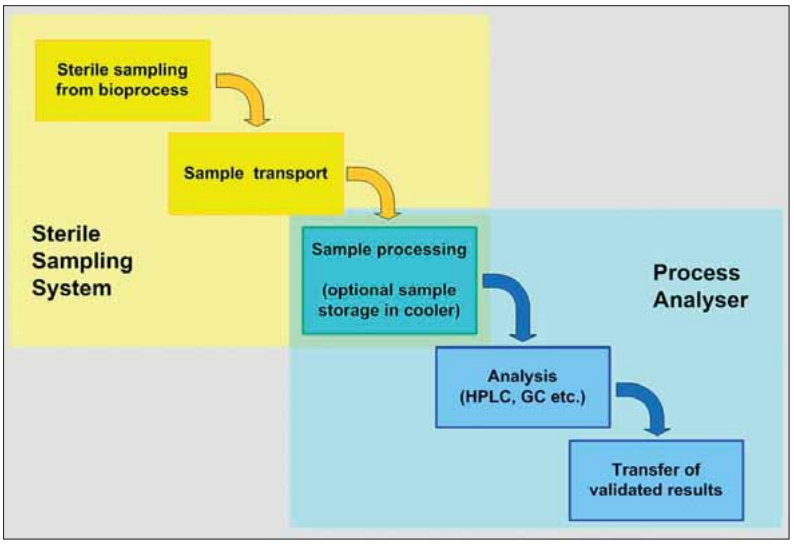

Fig. 1: Complete workflow for automated bioprocess monitoring with routine-lab-equipment. Reliable sterile sampling, sample transport and subsequent sample processing is a prerequisite for any further analysis. Finally, validated results are visualized and can be transferred to superior expert systems, where they serve as input values for soft-sensors and potential control loops.

With the sum of its biochemical main-reactions, being represented by a highly complex and also dynamic network of pathways, genetic regulations, cell cycle-dependent performance, potential cell-cell-interactions etc., biological systems are up to date not fully understood. In order to achieve a practical and manageable approach for process design and control, diverse models – imaging the cellular behaviour of interest in a simplified but correct way – are applied.

In conventional stirred and aerated bioreactors, biochemical and biological function of single heterotrophic cells in suspension is mainly affected by the physical and chemical conditions of their micro-environment. Therefore, all relevant micro-environmental conditions have to be identified in a qualitative and quantitative way in order to achieve a precise basis for subsequent process strategies.

Critical Control Issues in Cell Culture Micro-Environments

Mammalian cells can react very sensitive to even small changes in their culture micro-environment. Compared to microorganisms, they are much more complex and further-on not designed by evolution to survive as single cells in an adverse environment. They tolerate only a narrow range in physical and chemical conditions without turning to undesired – and even partly irreversible – physiological states.

In order to guarantee for an intended physiological behaviour, main nutrients should be available in optimal concentrations. For longer cultivation periods, controlled feeding strategies are preferable guaranteeing for minimal accumulation of toxic metabolic by-products.

Why is this so important? Metabolic by-products concern the vital functions of basic cellular growth and metabolisms as well as – in a not so obvious but even more sensitive way – the glycoform variability of secreted glycoproteins. Achieving and maintaining an at most human-like glycosylation pattern – in particular with the desired high amount of terminal sialic acid residues, avoiding undesired fast removal of the applied drug by the patient's liver – is a challenge (5).

Glycosylation is not template-driven like protein-biosynthesis but mainly a result of complex enzymatic reactions during the protein-transport through the different compartments of the Golgi apparatus, prior to secretion. Maintaining optimal enzyme activities is a major target for process control: Sialyltransferases show a tremendous decrease in activity due to ion-trap-mediated intracellular pH shifts in the Golgi, which has a slightly lower pH than the cytosol enabling consequently accumulation of basic substances. Therefore, extracellular ammonia, occurring as a by-product from glutamine metabolism, affects sialylation even in concentrations far below the level that affects cell growth! This single example demonstrates the urgent need for subtle glutamine feeding strategies in order to minimize accumulation of ammonia whilst maximizing cellular growth and performance.

Aspired Technical and Engineering Solutions

In order to fulfil the demands of the biological systems, engineers are challenged to provide and implement the corresponding technical solutions. Which technical solutions are ready-to-use in routine applications?

Multivariate tools for design, data acquisition and analysis represent the basic equipment and are at the same time a fundament for implementation of a series of advanced process tools. Therefore, concepts and equipment on this level may not be limited to often restricted present needs but must be open for higher demands and progressive evolution.

Process analyzers – for a deeper insight into cellular performance

Besides the routinely accessible phys-

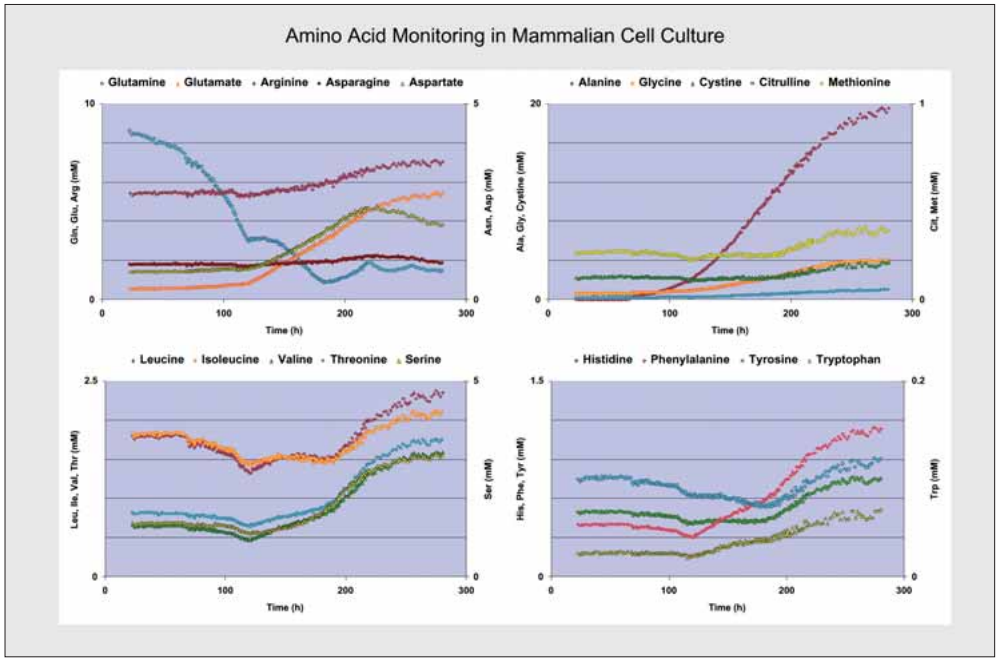

Fig. 2: Amino acid monitoring from mammalian cell cultures serves as a basis for rational feeding strategies (6).

ical process state variables precise and broad information from biochemical and biological state variables is a prerequisite for any further rational consideration.

In the field of «hard-sensors», different analytical in-line, on-line or at-line tools can be applied. In-line and on-line sensors, for example spectroscopic devices based on NIR- or Raman-technology, are easy to apply but frequently have to be recalibrated, offering more a fingerprint result than being fully comparable to the routine off-line lab results. Biosensors offer interesting potentials but often exhibit problems in terms of stability and matrix-independence. The exhaust-gas stream from a process can be used without the risk of affecting sterility for diverse gas analyzers or a mass spectrometer (MS). Oxygen, carbon dioxide and the whole spectrum of volatile metabolic products become accessible.

For robust and reliable analysis from liquid biosamples, at-line analytics combining validated routine-lab-equipment (HPLC, GC, optional coupled to powerful MS-detection) with automated sterile sampling and sample-preparation units is applied. Replacement of manual interaction offers an increased degree in accuracy, security, and reproducibility during 24 hours a day. In extent to the analysis of small molecules, new reverse phase HPLC technologies allow fast and precise characterization of large biomolecules (monoclonal antibodies, virus particles etc.). Even for DNA-analysis a broad range of microarray technologies becomes available. Biological state variables are accessible with microscopy-CCD-units combined with subsequent image analysis.

As each process has its specific demands, modular devices (units for basic fluid handling, extraction, mixing, heating, filtration etc.) have to be combined tailored to the actual needs. A complete system has to have a well-elaborated logical structure operating on programmable logic controllers with the corresponding security algorithms being fully implementable into regular process control systems.

As a result, the actual process state conditions (nutrient-, product-, by-product-concentrations, biological variables) can be transferred from such devices as validated results to superior expert systems. Here, they serve also as input values for «soft-sensors» calculating online a complete set of related process state variables, e.g. yields, specific uptake- and production-rates.

Closed loop control of bioprocesses – a new milestone

The entire online information can be used as input values for desired control loops, thus enabling the important step from open loop to closed loop control of bioprocesses. Previously, discontinuous and slightly time-shifted analyzer-data were favourably critically evaluated and then transformed into a continuous and prospective signal curve representing the basis for subsequent control algorithms.

As a gain for production processes variables become now parameters guaranteeing for almost controlled cellular behaviour – and thus product quality. In R&D, a complete new strategy for designing and performing automatically a multiplicity of bioprocess experiments will be accessible (see below).

Continuous improvement and knowledge management tools

Evaluation of a multiplicity of historical data sets originating from R&D experiments or production cycles by using powerful data-mining tools allow the extraction of even hidden information. On a non-mechanistic basis, a variety of statistical operations elucidate critical process events with respect to their consequence on the end-product. By this means, increasing experience is used to a maximal extent.

Faster from Clone to Product – High Throughput Bioprocessing

Today, molecular biologists generate a huge amount of promising clones which they test up to the multi-well plate level for the targeted metabolic performance. However, this is not an adequate basis for the proper decision which clone will finally have the best performance in large production bioreactors.

In order to explore cellular behaviour with respect to genetic stability, energy

metabolism, production performance, etc. over a wide range of controlled micro-environmental conditions, process development in fully instrumented bioreactor systems is inevitable. But, here we observe generally a bottleneck in capacity for carrying out the urgent required number of experiments. Consequently, only a few clones can be tested further-on, others are wasted or - with nearly the same effect – are stored in liquid nitrogen for undetermined time.

The aspired solution is to overcome the classical time and labour-intensive 3-step procedure:

1. Design of experiment and preparation of equipment (1-2 days)
2. Experimental phase (several days to 2 weeks)
3. Postprocessing of equipment, evaluation of results, reporting (1-2 days), representing the basis for a new experimental design (subsequent step 1)

Modern fully automated and computerised multi-bioreactor systems (6) can overcome this limitation by combining above-mentioned technologies in an ingenious way, offering overall a ten- to hundred-fold performance. The applied strategy is twofold:

First, the whole workflow described above is completely automated for an increased number of fully equipped precision bioreactor units, including in-, on- and at-line measurements corresponding to the requirements of the process.

Second, all units are controlled by an expert system allowing new experimental strategies for precise evaluation of physiological behaviour of tested organisms: Pulse-shift-experiments under steady evaluation and controls enable for target-oriented testing of a broad spectrum of micro-environmental conditions. Information is generated, even under non-equilibrium conditions, within shortest time during each experimental run and finally presented by automatically generated reports.

Besides clone selection, these systems are generally open for any kind of process development, e.g. multi-factorial approaches for media development, or even for scale-down analysis in order to mimic later conditions in large production bioreactors.

With these advantages, such systems aim to fulfil in an innovative manner the requests from PAT for quality- and efficiency-orientated bioprocessing. □

References

1. Biopharmaceuticals - Current Market Dynamics & Future Outlook, Novis, Industry & Science News, AS insights, May 2005
2. Andersen, R. and Mynahan, R., The protein production challenge, *In Vivo*, May 2001, 1-5
3. PAT – A framework for innovative pharmaceutical development, manufacturing, and quality assurance, FDA / Center for Drug Evaluation and Research, Office of Pharmaceutical Science, Rockville, MD, USA, September 2004
4. W. Edwards Deming, Out of the Crisis (The MIT Press) Cambridge, Massachusetts 1982.
5. Zopf, D. and Vergis, G., Glycosylation: A critical issue in protein development and manufacturing, Pharmaceutical Visions, Spring 2002, 10-14
6. Biospectra, various technical papers and application notes; www.biospectra.ch

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