Monitoring and control of reproducibility in quasi-continuous integrated production processes of Active Pharmaceutical Ingredients

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Monitoring and control of reproducibility in quasi-continuous integrated production processes of Active Pharmaceutical Ingredients
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Monitoring and control of reproducibility in quasi-continuous integrated production processes of Active Pharmaceutical Ingredients

Outline

The three levels and nine tasks of Process Analytical Technology

explained with a process development for potential Malaria vaccine production
DiCo – Diversity Covering Malaria proteins – *host construction*

Malaria vaccine candidates

**Apical Membrane Antigen 1**

**Merozoite Surface Protein 1**

- **AMA-1** (Dj)
- **MSP-1** (Mi)

**DiCo** – Diversity Covering *PfAMA1* and modified form of MSP1-19

**Pichia pastoris**

KM71H phenotype Mut⁸

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Enhanced process development – *The instrumentation level of PAT*

- **Process monitoring and automation**
- **Data acquisition, storage and processing**
- **Standard measurement and control equipment**
- **Off-line & on-line MVDA applications**
- **Additional on-line and in-line probes**
- **Advanced on-line data processing and monitoring**
- **Off-line cell mass, substrate and product analytics**
- **In-line spectroscopy**: NIR, Raman, 2D-fluorescence
- **At-line flow analysis for substrate and product detection**

*3 PAT levels: instrumentation*
Circular processing features with consistent production quality

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Cell specific reaction rates in reproducible experiments

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Industrial compatible *Integrated Scale-down Production Plant*
Unit operations of production of secreted pharmaceutical proteins
Enhanced process development – The process development level of PAT

3 PAT levels: instrumentation, process development

- process monitoring and automation
- data acquisition, storage and processing
- application of adaptive pO₂-control
- off-line cell mass, substrate and product analytics
- advanced on-line data processing infrastructure
- development of quasi-continuous integrated processes
- off-line & on-line MVDA applications
- development of Golden Batch process evaluation
- development of circular two stage expression strategies
- in-line spectroscopy NIR, Raman, 2D-fluorescence
- application of DoE-optimization methods
- standard measurement and control equipment
- additional on-line and in-line probes
- at-line analytics for substrate and product detection

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Up-scale into a two reactor strategy with sequential/parallel cultivation

K. Lögering
C. Müller

Lipase B from *Candida antarctica* (CALB)

Production

Precultivation

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Two reactor *sequential/parallel D1M1H production upstream strategy*

- **cell breeding**
- **protein expression**
- **refresh & harvest**
- **partial harvest & inoculation**
- **total harvest**

**Sequential processing** vs. **straightforward processing**

- Batch
- Induction

*Diagram details:*
- \( A_{P1M2at} \) [mAU]
- \( c_{XLj} \) [g/l]
- \( c_{S2Mj} \) [g/l]
- \( C_{XL1} \)
- \( C_{XL2} \)
- \( C_{P1M2at} \)
- \( C_{PtotM1} \)
- \( C_{PtotM2} \)

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Fully automated *integrated production* of Malaria vaccine D1M1H
Quasi-continuous process with *sequential/parallel production*

1\textsuperscript{st} day  2\textsuperscript{nd} day  3\textsuperscript{rd} day  4\textsuperscript{th} day  5\textsuperscript{th} day

- cell breeding
- protein production
- clarification
- microfiltration
- ultrafiltration
- purification
Process quality verification with test of conditions for reproducibility

1\textsuperscript{st} day 2\textsuperscript{nd} day 3\textsuperscript{rd} day 4\textsuperscript{th} day 5\textsuperscript{th} day

cell breeding
protein production
clarification
microfiltration
ultrafiltration
purification

process quality test?
are the single unit operations reproducible?

Multi-Variate Data Analysis-monitoring!
Time course of six circular reproduced cell breeding cultivations

Is the cell breeding process reproducible?
**Spectroscopic NIR-investigation** in production cycles reproducibility

Is the protein production reproducible, optimal and QbD-compliant?
Expansion of data processing with SIPAT®, MATLAB® and SIMCA®
MPCA – Multiway Principal Component Analysis: \textit{Observation Level M}

\[ D_{(o \times n \times m)} \rightarrow \text{reorganize} \]

\[ X_m \rightarrow \]

\[ \text{PCA} = T_M + P_m^T + E_M \]

\[ \text{OLM} \rightarrow \text{Observation Level Model} \]

\[ \text{batch} \quad \text{induction} \]

\[ p_{c_t} \]

\[ 0 \quad 12 \quad 24 \]

\[ t \text{ [h]} \]

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1st task: **Autoscaling of Matrix D into X** for principal component analysis

- transform *process data matrix X* into *score matrix T*
- and *loading matrix P*
- summarize *m (22) informations* in a few *variables r (2 or 3) only*
2\textsuperscript{nd} task: \textit{Principal Component Analysis} of autoscaled Data Matrix X

- transformed \( m \) process data \( x_j \) into \( r \) (3) time dependent scores \( t_i \) (principle components \( pc_i \)) with \( m \cdot r \) loadings \( p_{ij} \)

\[
\begin{align*}
\mathbf{T} & = \mathbf{X} \cdot \mathbf{P} \cdot \left( \mathbf{P}^T \cdot \mathbf{P} \right)^{-1} \\
\end{align*}
\]
Golden Batch tunnels with three sigma limits for process evaluation

Golden Batch tunnel:
Take principle component $pc_{1p}$ and add $\pm 3 \sigma_{1p}$-standard deviation

$pc_{1p}$ [-]

breeding pre-induction production

$p = 1$

$+3 \sigma_{11}$ $pc_{11}$ $-3 \sigma_{11}$

$p = 2$

$+3 \sigma_{12}$ $pc_{12}$ $-3 \sigma_{12}$

score data loadings

$T = \mathbf{X} \cdot \mathbf{P} \cdot \left[ \begin{array}{cc} m & r \\ m & m \end{array} \right]^{-1}$

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MPCA – Multiway Principal Component Analysis: *Batch Level B*

\[
\begin{align*}
\mathbf{D} &= (o \times n \times m) \\
\text{time interval} &= i \\
\text{batch} &= k \\
\text{variable} &= j
\end{align*}
\]

1. **Reorganize**
2. **PCA**
   - \( T_B (o \times r) \)
3. **BLM**
   - \( E_B (o \times nm) \)
   - \( P^T_B (r \times nm) \)

\[
\begin{align*}
X_B (o \times nm) &= t^2_k \left[ - t^1_k \right] + X
\end{align*}
\]
Batch level score scatter plot ($pc_2$ vs $pc_1$) for different campaigns $XX_{ic1y}$

Are these models QbD-compliant?
Enhanced process development – *The QbD-compliance level of PAT*

- **process monitoring and automation**
  - development of automated cyclical expression strategies
  - pass a risk analysis
    - define TPP
    - identify CQA

- **standard measurement and control equipment**
  - application of adaptive pO₂-control

- **data acquisition storage and processing**
  - establishment of DoE-optimization plants & methods

- **additional on-line and in-line probes**
  - link QA – Quality Attributes with PP – Process Parameters
  - identify Critical Process Parameters and Design Spaces

- **at-line analytics for substrate and product detection**
  - operation of optimal quasi-continuous integrated processes
  - implement adjustable Control Spaces

- **off-line cell mass, substrate and product analytics**
  - development of Golden Batch monitoring & control
  - on-line monitoring, prediction & control of process quality

- **QbD-compliant off-line & on-line MVDA models**

- **in-line spectroscopy**
  - NIR, Raman, 2D-fluorescence

- **3 PAT levels: instrumentation, process development, QbD-compliance**
Evaluation of optimization potential in secretion productivity PRD of D1

\[ y_k = PRD_k = \left[ c_{P1Mkn} \cdot V_{Lkn} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkn}) - c_{P1Mk0} \cdot V_{Lk0} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLk0}) \right] \]

+ \sum_{j=1}^{n} c_{P1Mkj} \cdot \Delta V_{Skj} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkj}) \cdot (\rho_Z \cdot (t_{kn} - t_{k0}) \cdot V_{Lkn})^{-1}

Volumetric secretion productivity PRD – process evaluation performance criterion

\[ pH_{w1} = 4, \quad g_{lw1} = 18 \, ^{\circ}C, \quad c_{S2Mw1} = 4.5 \, g^{-1} \]

0.93 mg(lh)^{-1}
Define Critical (Process) Quality Attribute: *Product secretion productivity*

\[ y_k = \text{PRD}_k = \left[ c_{P1Mkn} \cdot V_{Lkn} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkn}) - c_{P1Mk0} \cdot V_{Lk0} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLk0}) \right] + \sum_{j=1}^{n} c_{P1Mkj} \cdot \Delta V_{Skj} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkj}) \cdot (\rho_Z \cdot (t_{kn} - t_{k0}) \cdot V_{Lkn})^{-1} \]
**Multi-bioreactor DoE-plant:** BIOSTAT® Bplus with a BIOSTAT® Qplus 6

H.-P. Bertelsen  
U. Scheffler  
J. Fricke
Optimal Critical Process Parameters in Malaria vaccine productions

D1: \( \text{PRD}_{\text{max}} = 0.019 \text{ AUsh}^{-1} \)

\[
\begin{align*}
\vartheta_{\text{Lopt}} &= 25.8 ^\circ \text{C} \\
\text{pH}_{\text{opt}} &= 5.55 \\
C_{\text{S2Mopt}} &= 1.0 \text{ gl}^{-1}
\end{align*}
\]

D1M1H: \( \text{PRD}_{\text{max}} = 9.0 \text{ mg(lh)}^{-1} \)

\[
\begin{align*}
\vartheta_{\text{Lopt}} &= 22.0 ^\circ \text{C} \\
\text{pH}_{\text{opt}} &= 5.55 \\
C_{\text{S2Mopt}} &= 1.0 \text{ gl}^{-1}
\end{align*}
\]

D2M2D3H: \( \text{PRD}_{\text{max}} = 1.3 \text{ mg(lh)}^{-1} \)

\[
\begin{align*}
\vartheta_{\text{Lopt}} &= 24.7 ^\circ \text{C} \\
\text{pH}_{\text{opt}} &= 6.2 \\
C_{\text{S2Mopt}} &= 1.0 \text{ gl}^{-1}
\end{align*}
\]
Implementation of Control Space – *Mathematically fixed adjustment*

\[
\left( \frac{\theta_L - \theta_{Lcen}}{\Delta \theta_L} \right)^2 + \left( \frac{\text{pH} - \text{pH}_{cen}}{\Delta \text{pH}} \right)^2 \leq 1
\]

Characterisation of an ellipsoid shape

\[
\theta_{Losp} = 26.6 \, ^\circ\text{C} \quad \Delta \theta_{Losp} = 0.7 \, ^\circ\text{C}
\]

\[
\text{pH}_{osp} = 5.47 \quad \Delta \text{pH}_{osp} = 0.1
\]

product: D1

\[
\theta_{Losp} = 22.0 \, ^\circ\text{C}
\]

\[
\text{pH}_{osp} = 5.55
\]

product: D1M1H
QbD-evaluation – Golden Batch models of Design Space production

observation level

protein production – product: D1M1H

batch level

pH2 [-] xMV1 [°C] xMV2 [°C]

DModX [-]

Hotellings T² [-]

T²crit (99%) = 44.8

T²crit (95%) = 23.5

OSP
Resulting multivariate limits for future on-line process evaluation

protein production – product: D1M1H

observation level

batch level

DModX [-]

Hotellings $T^2$ [-]

pH$_2$ [-]

$\varrho_{L2}$ [°C]

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Final evaluation of a new production with „optimal“ settings for $X_{MV}$
On-line evaluation of MVDA models with SIPAT® and SIMCA® Q
On-line monitoring of Golden Batch variables in both sub-processes

Is it possible to look into the future of process behaviour?
Process prediction and model predictive control with SIMCA®online
What is to do, if we are leaving the Golden Batch tunnel?
Quality control: **BOBYQA – Bound Optimization BY Quadratic Approximation**

**cell breeding – product: D1M1H**

**BOBYQA – Minimize the quadratic deviation from an optimal process behaviour $J$**

$$
\min J \Rightarrow J = \theta_{X_{MV}} \cdot (X_{MVgb} - X_{MV})^2 + \theta_Y \cdot (Y_{SP} - Y_{pred})^2 + \theta_{DModX} \cdot (DModX)^2 + \theta_{T2} \cdot (T^2)^2
$$
Conducting an experiment with „out of design space“ settings for $X_{MV}$
MPMC-Model Predictive Multivariate Control with the monitoring model

protein production – product: D1M1H

observation level

batch level

MPMC on

pH₂ [-]$

X_{MV1}$

$\theta_{L2}$ [°C]$

X_{MV2}$

0 10 15 20 25 30 35

4.5 5.0 5.5 6.0 6.5 7.0

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60

MPMC on

MMCA

gb

osp

MPCM on

XX2514_9

XX2514_8

XX2514_7

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Control Model cm – Golden Batch with „playing around“ set points
MPMC – Model Predictive Multivariate Control with the control model

![Graphs showing protein production and control model results](image)

- Observation level: Protein production with product D1M1H.
- Batch level: Control model visualization with parameters pH, temperature, and concentration.

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Conclusions: *Development of QbD-compliant quality-controlled ICB*

- **process monitoring and automation**
- **standard measurement and control equipment**
- **additional on-line and in-line probes**
- **at-line analytics for substrate and product detection**
- **in-line spectroscopy**
  - NIR, Raman, 2D-fluorescence
- **off-line cell mass, substrate and product analytics**
- **QbD-compliant off-line & on-line MVDA models**
- **data acquisition storage and processing**
- **advanced on-line data processing infrastructure**
- **operation of optimal quasi-continuous integrated processes**
- **implementation of Golden Batch monitoring & control**
- **development of automated cyclical expression strategies**
- **pass a risk analysis define TPP identify CQA**
- **link QA – Quality Attributes with PP – Process Parameters**
- **identified Critical Process Parameters and Design Spaces**
- **implementation of adjustable Control Spaces**

3 PAT levels: *instrumentation* **process development** *QbD-compliance*
Sequential/parallel production of potential Malaria vaccines – A direct way from single batch to quasi-continuous integrated production

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ABSTRACT

An intensification of pharmaceutical protein production processes can be achieved by the integration of unit operations and application of recurring sequences of all biochemical process steps. Within optimization procedures each individual step as well as the overall process has to be in the focus of scientific interest. This paper includes a description of the development of a fully automated production plant, starting with a two step upstream followed by a four step downstream line, including cell clarification, broth cleaning with microfiltration, product concentration with ultrafiltration and purification with column chromatography. Recursive production strategies are developed where a cell breeding, the protein production and the whole downstream is operated in series but also in parallel, each main operation shifted by one day. The quality and reproducibility of the recursive protein expression is monitored on-line by Golden Batch and this is controlled by Model Predictive Multivariate Control (MPMC). As a demonstration process the production of potential Malaria vaccines with Pichia pastoris is under investigation.

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