Accelerating Pharmaceutical Process Development: From Microwell “Process-on-a-Deck” to Large Scale Manufacture

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Presentation Outline

• The need for microscale bioprocessing
• The basis – microwell engineering fundamentals
• The components – bioprocess unit operations
• The future – intelligent bioprocess experimentation
• Final remarks
The Pharmaceutical Sector is a Major Contributor to the UK Economy

• Leading industrial sector with a trade surplus of £3.7 billion

• Leading sector for R&D expenditure as a percentage of sales at 34.2%

• Employs 73,000 people, 37% of which are in R&D

• Global market predicted to increase in value to $1,300 billion by 2020 (PricewaterhouseCoopers, 2007)

[Source: www.abpi.org.uk/statistics]
However… The Sector is Facing Significant New Challenges

- Medicines are becoming more complex, failure rates remain high and markets are increasingly segmented (PWC, 2007)
- Rise in generic competition and loss of manufacturing to cheaper, more tax efficient, overseas locations
- Governments and insurance companies are working to contain healthcare costs
- Greater emphasis on **speed** to market and **improved** bioprocesses
How Microscale Biochemical Engineering Can Help…

• Able to address bioprocess issues early and at low cost

• Capacity to address whole bioprocess sequences as required by industry

• Automated microwell scale experimentation linked to bioprocess modelling can provide improved process understanding

• To be really useful the findings must be predictive of larger scale operations
The Microwell “Process-on-a-Deck” Concept

96 DSW  24 SRW  96 SRW

\[ V_{D} = 2000 \text{ ul} \quad V_{W} = 3859 \text{ ul} \quad V_{W} = 385 \text{ ul} \]
\[ \text{SA} = 0.64 \text{ cm}^2 \quad \text{SA} = 2.27 \text{ cm}^2 \quad \text{SA} = 0.385 \text{ cm}^2 \]

The UCL Advanced Centre for Biochemical Engineering (ACBE) Addresses the Three Main Classes of Future Medicines

- **Complex chemical pharmaceuticals**
  - e.g. multi-chiral centred drugs
- **Biopharmaceuticals**
  - e.g. proteins, genes, recombinant vaccines
- **Regenerative medicines**
  - e.g. human cells and engineered tissues

The microscale approach underpins each of these areas
Microwell Engineering Fundamentals

CFD Simulation of Fluid Shear and Energy Dissipation in Shaken Microwells (24SRW)

Visualisation and Quantification of Mixing Time, $t_m$, in Shaken Microwells (24SRW)

Critical shaking frequency, $n_{\text{crit}}$:

$$n_{\text{crit}} = \sqrt{\frac{\sigma d_w}{4\pi V_L \rho_L d_o}}$$

$n < n_{\text{crit}}$

$n > n_{\text{crit}}$

Measurement of Gas-Liquid Mass Transfer Rates and Prediction of $k_L a$

$$k_L a = 31.35 a_i Re^{0.68} Sc^{0.36} Fr^x Bo^y$$

Dissolved O$_2$ profile during mammalian cell culture...

Automation Characterisation: Jet Mixing in Static Microwells (96SRW, 96DRW)

$t_{95} = 40.0s$

$t_{95} = 0.23s$

Microwell “Process-on-a-Deck” Components: Microscale Unit Operations

**E. coli** Growth and Protein Expression at Microwell and 7.5L STR Scales ($k_La = 247 \text{ h}^{-1}$)

Production of a New *Neisseria lactamica* OMV Vaccine for Menningococcal Disease

Diplococci of *N. lactamica* Y92 NL1009 showing outer membrane vesicle (OMV) formation
Microwell Optimisation of Vaccine Production

<table>
<thead>
<tr>
<th>Major OMP</th>
<th>Properties</th>
<th>Weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PorB</td>
<td>Porin, limited diversity</td>
<td>37-42</td>
</tr>
<tr>
<td>RmpM</td>
<td>Reduction modifiable protein, conserved</td>
<td>---</td>
</tr>
<tr>
<td>NspA</td>
<td>Surface protein</td>
<td>18-22</td>
</tr>
</tbody>
</table>

Ciphergen 96-well protein chip-based, SELDI-TOF analysis of purified OMVs

![Graph showing protein concentrations](Image)

Recovery: Parallel Microscale Microfiltration

Custom Filter Plate Design

PLAN VIEW

FILTER INSERT
Quantification of Resistances

MEMBRANE RESISTANCE

\[ R_m = \frac{A \int \Delta P \, dt}{\mu V} \]

SPECIFIC CAKE RESISTANCE

\[ \alpha = \frac{dy}{dx} \cdot \frac{2A^2 \Delta P}{\mu c} \]
Quantification of Specific Cake Resistances

- Multiscreen filter plate (0.3 cm$^2$) under negative pressure
- Custom filter plate (0.8 cm$^2$) under negative pressure
- Membrane cell (3.8 cm$^2$) under positive pressure
- Membrane cell (3.8 cm$^2$) under negative pressure

![Chart showing specific cake resistances over time](chart.png)
Purification: Human Papillomavirus VLPs

L1 monomer (~55 kD)

Capsomere (~280 kD)

Virus Like Particle (~20,000 kD)

Non-infectious (no nucleic acid)

(Crystal structure coordinates courtesy of Prof. S. C. Harrison, Harvard University)
Micoscale Chromatography (PhyNexus Tips)

Mechanical Cell Disruption

Debris Removal
(Centrifugation or Microfiltration)

CEX Chromatography (80 µL)

Polishing Chromatography (40 µL)

1000-fold scale-down

Minimal plate manipulations
(= reduced automation complexity)

Dynamic flow
(= Improved mass transfer)

Engineering Considerations and Purification Comparison

Flow Characteristics

- Up, down
- Screens attached to the plastic tip body
- Separation media encased between the two screens

5 - 20 µL/sec = linear velocity of 270 – 1080 cm/hr (avg, 80 µL)
The “Bioprocess-on-a-Deck” Concept

Plate Prep. → Fermentation → Enzyme Recovery → Bioconversion → Analysis

Pre-culture Medium

96DSW → THERMOMIXER → SONICATION (off line) → CENTRIFUGATION → Clarified lysate

Whole cell

Cell debris

HPLC

Automated Microwell TK Process Sequence

Plate Prep. → Fermentation → Enzyme Recovery → Bioconversion → Analysis

Pre-culture medium

Whole cell

Clarified lysate

Cell debris

SONICATION (off line)

CENTRIFUGATION

[Worklist.gwl (containing pipetting commands for next sample)]

[Thermomixer]

[PLATE READER]

[ResultsFile.xls]

Automated Microwell TK Process Sequence

Plate Prep. → Fermentation → Enzyme Recovery → Bioconversion → Analysis

Pre-culture Medium

96DSW → THERMOMIXER → CENTRIFUGATION → WORKLIST.GWL

(containing pipetting commands for next sample)

SONICATION (off line)

Clarified lysate → Cell debris

HPLC

Microplate Bradford Assay (automated)

SDS Page (off-line)

Storage -20C

New Directions: Intelligent Bioprocess Experimentation

(Image courtesy of Tony Newcombe, Protherics)
Application of DoE For Rapid Protein Expression Optimisation

Choose factors (Hi + Lo only)
- Media (GM9Y, LB, TB)
- Plate (48-f, 24-r, 24-p)
- Temperature
- Agitation
- Volume
- Growth period
- Induction period
- Induction temperature
- Induction agitation

Determination of relevant factors

Adjust factors (and levels)
- Agitation
- Volume
- Growth period
- Induction period
- Induction temperature
- Induction agitation

Determination of optimal settings

Screening

Single response [Protein]

Optimisation

Multiple responses [Protein] [Cell]

270 rpm
2 ml
4 h
18 h
30 °C
330 rpm
Combining DoE and Scale-up Fundamentals

Microwell Optimisation

Predictive Scale-up to 75L Bioreactor ($k_L a$ 247 h$^{-1}$)

Model Driven Experimental Design: Rapid Bioconversion Kinetic Model Generation

Full kinetic model (ping-pong bi-bi mechanism):

\[
\frac{d[Q]}{dt} = \frac{k_{\text{cat}}E_i[A][B]}{K_i[A](1 + \frac{[A]}{K_{ia}}) + K_a[B](1 + \frac{[B]}{K_{ib}}) + [A][B] + \frac{K_a}{K_{iq}}[B][Q] + \frac{K_aK_{ib}}{K_{iq}}[Q]}
\]

- High substrate concentration kinetic data
- Preliminary \( k_{\text{cat}}, K_a, K_b \)
- Simplified model
- Preliminary \( K_{ai}, K_{bi} \)
- Full kinetic model
- Ignore \( K_{ai}, K_{bi} \)
- Substrate inhibition negligible data
- Final \( k_{\text{cat}}, K_a, K_b, K_{ai}, K_{bi} \)

## Model Driven Experimental Design: Experimentation and Resource Benefits

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Conditions</th>
<th>Number of experiments</th>
<th>Required HPA (mg)</th>
<th>Number of experiments</th>
<th>Required HPA (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For $k_{cat}$, $K_a$, $K_b$</td>
<td>$[GA] = 30, 50, 100$ mM, $[HPA] = 10, 20, 30, 40, 50$ mM</td>
<td>15</td>
<td>495</td>
<td>25 (Figure 4)</td>
<td>12.8</td>
</tr>
<tr>
<td>For $K_a$</td>
<td>$[GA] = 10, 20, 30, 40, 50$ mM, $[HPA] = 200, 300, 400$ mM</td>
<td>15</td>
<td>4950</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For $K_b$</td>
<td>$[GA] = 200, 300, 400, 500$ mM, $[HPA] = 10, 20, 30, 40, 50$ mM</td>
<td>20</td>
<td>660</td>
<td>9 (Table 2)</td>
<td>170.7</td>
</tr>
<tr>
<td>For $K_{aq}$</td>
<td>$[ERY] = 0, 20, 40, 60, 80$ mM, $[GA] = 20, 30, 50, 50$ mM, $[HPA] = 10, 20, 30, 40$ mM</td>
<td>80</td>
<td>2200</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>130</strong></td>
<td><strong>8305</strong></td>
<td><strong>34</strong></td>
<td><strong>183.5</strong></td>
</tr>
</tbody>
</table>

Benefits of linking modelling and automated microwell experimentation:

- 4x decrease in experiments
- 45x decrease in materials
- >20x increase in throughput

Final Remarks

• Microwell results can be quantitative and predictive of larger scale performance

• Enabling HT bioprocess development represents a new application area for laboratory automation

• Microwell and automation technologies established for discovery applications provide a good basis but further refinement (and ingenuity!) is required
Acknowledgements

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