Novel liquid-liquid scalable extraction/chromatography technology

December 3rd, 2008

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Delft, Utrecht, Rotterdam, BPP2005

- Introduction to industrial scale centrifugal liquid-liquid extraction and its potential for the purification and manufacture of proteins and biologics
- Reviewed Hydrostatic & Hydrodynamic Countercurrent Chromatography
- Prospect for true moving bed chromatography
Outline of Today’s Talk

• Some advantages of working with liquid stationary phases
• Some additional not well known versatilities
• Quick review of high-resolution, hydrodynamic counter-current extraction
• Quick introduction to aqueous two-phase systems (ATPS)
• Focus on high-resolution, hydrostatic counter-current extraction using ATPS in isocratic mode
• Introduction to intermittent true moving bed extraction
• Other high resolution continuous extractions schemes in the offing
Key Advantages of a Liquid Stationary Phase

- **Total (100%) sample recovery** – liquid-liquid nature of the process means it is gentle and versatile and there is no irreversible adsorption to a solid support
- **Improved sample solubility** – both upper and lower phase available
- **Quick due to higher throughput** – High flow, low pressure system
- **Inexpensive & a Green Technology** compared to HPLC (significantly less solvent usage and no expensive solid supports)
- **Crude extracts**, including particulates can be handled reducing the number of separation steps in a process and reduced sample preparation requirements
- **High loading capacity** due to liquid nature of stationary phase
- **Easy & predictable scale-up** so that scale-down to scale-up philosophy can be applied
- **Low pressure system** – so columns can be lengthened to increase resolution and widened to increase capacity and throughput
- **Normal and reversed-phase operation** and the switching between them gives expanded operational capability – elution extrusion, dual mode, true moving bed
Other Little Known Advantages

• **Isocratic operation**
  – Either phase can be mobile (normal or reverse phase)
  – The process can be switched halfway through a run
  – The process can be stopped and the retained components pumped out (elution/extrusion – Berthod)

• **Co-current Chromatography/Extraction**
  – Both phases can be pumped at the same time in the same direction

• **Counter-current Chromatography/Extraction**
  – Both phases are simultaneously flowed in opposite direction
  – Continuous processing of closely related binary compounds
Hydrodynamic Counter-current Extraction
New Dynamic Extraction/Chromatography Technology

- Developed now as a fast, robust, continuous process with separations in minutes as opposed to hours with previous technology
- Small molecules – Natural Products
- Potential for application to proteins, peptides, plasmids and other biologics
Coil Planet Centrifugation producing mixing and settling zones along a continuous tube
Twist free connection to rotor
DE Centrifuges - Predictable Scale-up

<table>
<thead>
<tr>
<th>CCC-Device</th>
<th>Bore</th>
<th>Area</th>
<th>B</th>
<th>Mean Flow</th>
<th>Flow at SP Retention (Sf) given below</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>92.5</td>
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<tr>
<td>Mini-DE</td>
<td>0.8</td>
<td>0.50</td>
<td>-37.01</td>
<td>0.50</td>
<td>0.04</td>
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<tr>
<td>Midi-DE1</td>
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<td>2.00</td>
<td>0.45</td>
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<td>Midi-DE2</td>
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<td>10.64</td>
<td>-3.13</td>
<td>10.00</td>
<td>5.74</td>
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<tr>
<td>Maxi-DE</td>
<td>10</td>
<td>78.54</td>
<td>-0.82</td>
<td>80.00</td>
<td>83.66</td>
</tr>
</tbody>
</table>

Milli-CCC – R=50mm, Vc=5-17ml  Brunel-CCC – R=110mm, Vc=0.1-1 litre  Maxi-CCC – R=300mm, Vc=4.6 litre
Hydrodynamic Counter-current Extraction

- Continuous tube with wave mixing
- Stop the pump & liquid phases move to opposite ends of the tubing
- Ideal with low viscosity aqueous organic phases
- Hydrodynamic equilibrium between the mobile phase and the retained liquid stationary phase
  - Kg scale in days have been demonstrated with aqueous/organic phase systems
  - Feasibility of continuous countercurrent extraction has now been demonstrated at a pilot scale
Aqueous Two-Phase Polymer Systems (ATPS)
Aqueous Two-Phase Systems (ATPS)

- Numerous polymer-polymer, polymer/salt combinations
- Gentle host medium for biologics (ref Albertsson, Kula, Hubbuch)
- Low interfacial tension
- ATPS more viscous than aqueous/organic phase systems
Cascade v. Wave Mixing

Fig. 1. Synchronous coil planet centrifuge—cascade mixing.

Fig. 2. Epicyclic coil planet centrifuge—wave mixing.
Cascade v. Wave Mixing

Hydrodynamic systems retain ATPS but wave mixing is too gentle for large proteins.
Cascade v. Wave Mixing

Large scale toroidal hydrostatic systems being constructed but not yet tested

Hydrodynamic systems retain ATPS but wave mixing is too gentle for large proteins
Hydrostatic Counter-current Extraction
Hydrostatic Counter-current Extraction

- Often discrete chambers with narrow bore interconnecting tubes
- Cascade mixing (like waterfalls)
- Stop the pump and the stationary phase stays where it is – trapped in the chambers
- The focus today - Centrifugal Partition Chromatography (CPC)
CPC: ... Without solid support ...
Sanki HPCPC, analytical scale

1 channel $\approx 90 \mu L$

Armen Elite, industrial scale

Twin cells $\approx 12$ mL

For the 12.5 liter instrument
Earth gravity modulates the Coriolis acceleration and promotes spray formation.

Earth gravity: possible sedimentation of the lower phase in deep channels.

Stroboscopic flash and fast response camera.

Visual CPC II


Flow rate (mL/min)

BuOH / Water

Ascending Mode

From 0 to 500 mL / min in 1 min then back to 0 mL / min in 1 min

506 rpm

1290 rpm
Advanced Bioprocessing Centre (ABC)

Analytical Lab

Applications Lab

Hazard’s Lab

Control Room
1 litre Hydrostatic CPC Set-up at BIB
Run Conditions

- **Centrifuge**: Armen Elite 1 litre CPC
- **Capacity**: One rotor only - volume 500ml, cell volume 429ml
- **Phase System**: ATPS: 12.5% PEG1000-12.5% K$_2$HPO$_4$
- **Sample**: 90mg lysozyme, 90mg myoglobin in 40ml (~10% CV) 50:50 ATPS mix
- **Speed**: 2000 rpm (224g)
- **Flow**: 10 ml/min
- **Mobile Phase**: lower salt phase
- **Stationary phase retention (Sf) at breakthrough**: 52%
- **Stationary phase retention (Sf) at end**: 19%
Analytical Runs

- **Lysosome**
- **Myoglobin & Apomyoglobin**

- $k=0.59$, $S_f=40\%$, 80 CCD steps
- $k=1.91$, $S_f=19.5\%$, 80 CCD steps

$Rs=1.28$
Analytical Runs

Note: theoretical prediction from test tube experiments
Transfer to Pilot Scale Unit in France
Armen 12.5 litre CPC at Archimex
Run Conditions

- **Centrifuge**: Armen Continuum 12.5 litre CPC
- **Capacity**: One rotor only - volume 6.25 litre
- **Phase System**: ATPS: 12.5% PEG1000-12.5% K$_2$HPO$_4$
- **Sample**: 1.1g lysozyme, 1.1g myoglobin in 500ml (~10% CV)
  50:50 ATPS mix
- **Speed**: 1293 rpm (224g)
- **Flow**: 125 ml/min
- **Mobile Phase**: lower salt phase
- **Stationary phase retention (Sf) at breakthrough**: 63%
- **Stationary phase retention (Sf) at end**: 22%
Some of the team at Archimex
Fraction collection & on-line monitoring
Armen 12.5 litre CPC at Archimex

HPLC Peak Area

- Lysozyme
- Myoglobin + Apomyoglobin

Rs = 1.88, sample 10%CV

Rs = 1.28
Theoretical Predictions – Process Scale

- **Lysozyme**
- **Myoglobin + Apomyoglobin**

Theory:
- $k=0.59$, $S_f=52.5\%$, 80 CCD steps
- $k=1.91$, $S_f=24.2\%$, 80 CCD steps
ATPS Stripping Characteristic – Scale-up

ATPS Stripping Characteristic During Scale-up

- 6.25 litre rotor
- 0.5 litre rotor

Fraction of Column Volume

Time (mins)
Conclusions – Batch Isocratic

- Linear scale-up feasible
- Robust technology – easily transferred from the test tube to a production run
- Note that with Hubbuch’s robotic high throughput screening with ATPS systems, combined with this rapid scale-up approach – small footprint, rapid new approaches to manufacture are becoming feasible
Intermittent – True Moving Bed (i-TMB)
True Moving Bed

Sutherland, Scientific Update, San Francisco
March 21-22, 2005
Novel Liquid-liquid Extraction Technology

Injection

Ascending mode period

Descending mode period

Injection of A + B
Run Conditions

- **Centrifuge**: Armen Elite 1 litre CPC
- **Capacity**: Two rotors - volume 1000ml, cell volume 858ml
- **Phase System**: ATPS: 12.5% PEG1000-12.5% K$_2$HPO$_4$
- **Sample**: Continuous flow of 2.2mg/ml lysozyme, 2.2mg/ml myoglobin at 3ml/min in UP (ascending) and LP (descending) – 0.79g/hr – total 1.2g in 1.5 hours
- **Speed**: 2000 rpm (224g)
- **Flow**: 10 ml/min (Ascending) 5 minutes; 10ml/min (Descending) 5 minutes and so on…..
- **Mobile Phase**: alternating between between UP & LP
Armen CPC i-TMB System at Archimex
Optimising Biomanufacturing
December 2-3, 2008

Novel Liquid-liquid Extraction Technology

Descending Elution

HPLC Peak Area (arb. units)

Fraction Number

- Lysozyme
- Myoglobin & Apomyoglobin
Ascending Elution

- **Lysozyme**
- **Myoglobin + ApoMyoglobin**
Novel Liquid-liquid Extraction Technology

**Descending Elution**

- **Lysosome**
- **Myoglobin & Apomyoglobin**

**Ascending Elution**

- **Lysosome**
- **Myoglobin + Apomyoglobin**
Elution control monitoring during run
Descending Elution - Myoglobin
## Summary – Batch v. i-TMB

<table>
<thead>
<tr>
<th>Centrifuge*</th>
<th>g/hour</th>
<th>g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 litre CPC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>0.14</td>
<td>3.24</td>
</tr>
<tr>
<td>i-TMB</td>
<td>0.79</td>
<td>19.01</td>
</tr>
<tr>
<td>12.5 litre CPC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>1.69</td>
<td>40.50</td>
</tr>
<tr>
<td>i-TMB</td>
<td>9.90</td>
<td>237.60</td>
</tr>
</tbody>
</table>

* Non-Optimised
Conclusions

• Rapid scale up from the test tube (Hubbuch) to pilot scale (kg/day) is feasible for model systems in batch and continuous process mode

• Now needs evaluating with real systems
  – Paula Rosa, Lisbon, Portugal (BPP2007)
  – Emma Bourton, Brunel, UK (PhD – Syngenta)
  – Other volunteers welcome
Challenges

- **New liquid-liquid phase systems**
  - Ionic liquids (QUILLS)
  - ATPS with higher solubility (Hans-Olef Johansson, Sao Paulo, Brazil)

- **Increased sample concentrations in ATPS**
  - Non-equilibrium sample loading & extraction (NESLE)
  - New continuous tube hydrostatic approach

- **Development of small footprint, versatile, multi-product facilities for “niche markets”** highlighted by Dr Dana Andersen of Genentech
  - Initially for high value added products

- **UK Government – future emphasis on basic and “user-driven” research**