Primary Clarification Step Evaluation
Implementation of 3M Technologies During Transition to Late Stage Process Development

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Agenda

• Biologics Development at Bayer
• Overview of Early Stage Primary Clarification Process
• Assessment of Phase 1 Depth Filter Performance
• Assessment of 3M Zeta Plus™ Depth Filter Performance
  • Additional Benefits of 3M Zeta Plus™
  • Emphaze™ Hybrid Purifier
• Looking Forward
Bayer
Our Business Areas

**Pharmaceuticals**
- Prescription drugs

**Consumer Health**
- Over-the-counter medicines, dietary supplements, dermatology products, foot care and sunscreen

**Crop Science**
- Innovative crop protection and seeds
The Bay Area: Bayer’s Biotechnology Hub

We are one of the largest biotech employers in the Bay Area

Berkeley Development & Manufacturing

Mission Bay Research
The BD group at Bayer has the overall responsibility for the CMC development of new biological entities. Process development covers all areas from the expression construct to the final drug product including process development for commercial manufacturing and technical support for commercial product supply.

Furthermore, BD supplies Preclinical/Clinical Development with drug products for their studies. The development and validation of analytical methodologies for in-process release, product comparability testing, and drug product release testing as well as the generation of drug product related regulatory and GMP documentation are additional responsibilities of BD.
Challenges for Early and Late Stage Process Development

**Early development**
- Fast
- Cheap
- Predictive of commercial success

**Late development**
- Pressure to launch
- Robust
- Well characterized
- Low CoG

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**Development Success Rates**

- NME Success Rates By Phase And Overall 2007-2011 Industry Portrait, Pure

- Percent Calculated To Achieve 1 Approval
  - Preclinical: 3%
  - Phase 1: 5%
  - Phase 2: 12%
  - Phase 3: 54%
  - Registration: 83%

- Success Rate For Each Phase
  - Preclinical: 64%
  - Phase 1: 44%
  - Phase 2: 22%
  - Phase 3: 1.9
  - Registration: 1.2
A Phase 1 clinical process was developed utilizing existing materials and methods for primary clarification. As the project moved towards late stage development, process improvement evaluation was performed on the Phase 1 clinical clarification process with the objectives of:

- Improving step yield
- Improving upon cell culture impurity removal
- Increasing process flow rates
- Decreasing burden on capture chromatography
Overview of Primary Clarification Step
Phase 1 Process Before Evaluation

- Clarification of HCCF with uncharged depth filters (1.5 - 20 µm) at a conservative process flow rate (Flux: ~30 LMH) based on observed pressure at bench scale.
- Filter surface area also sized conservatively to accommodate potential scale-up load variability.
- Buffer chase step executed to minimize product loss from hold-up in the depth filter housings.
- Resulting impurity levels (HCD and HCP) in CCCF are high.
- CCCF loaded directly onto capture column.
- Results are acceptable but there is substantial area for improvement.
Phase 1 Clarification Performance with Single Stage, Uncharged Depth Filters

Four Phase 1 clinical lots of the product (IgG₂) produced using the single stage, uncharged depth filters resulting in a) approximately 80% step yield due to high filter area requirements, and b) limited host cell impurity removal and resultant high burden on capture chromatography.

- **Step Yield (%)**
  - 1: 80%
  - 2: 100%
  - 3: 100%
  - 4: 100%

- **Host Cell DNA**
  - 1: 175%
  - 2: 75%
  - 3: 75%
  - 4: 75%

- **Host Cell Protein**
  - 1: 100%
  - 2: 50%
  - 3: 50%
  - 4: 50%
Various sizes of 3M Zeta Plus depth filters were tested with a two stage system ultimately implemented.

- Stage 1 – 05SP01A - sized (10 – 1.5 µm) to remove cells and large cellular debris
- Stage 2 – 60ZB05A – smaller nominal pore size (3 – 0.2 µm) along with a positively charged membrane for improved DNA clearance
- Development evaluation showed an increase in both the filter loading capacity and flux rate
Modified Primary Clarification Process
2 Stage, 3M Zeta Plus™ Depth Filters

- Process moved to 2 stage depth filtration: Stage 1 05SP01A - to remove major cell mass, Stage 2 60ZB01A - charged properties for DNA removal.
- Filter sizing evaluation: Required filter surface area ↓ by 10%.
- Process flow rate set point ↑ by 25% based on pressure/flow observed.
Primary Clarification Performance with Two-Stage, Charged Depth Filters

One clinical lot of the product was produced using the two stage, charged 3M filters resulting in a) an increase of 10% step yield due to decreased filter area requirements, and b) a significant reduction in HCD due to the charged properties of the Stage 2 filters.

However, no observed improvement in HCP level of HCCF pool.
The change to 3M depth filters also provided benefits for Scale-Up considerations:

- An approximate 25% increase in process flow rate capabilities resulting in shorter run times.
- Lower capsule requirements with a decreased footprint for process equipment.
- Maintained comparable cost per batch consumable requirements.
Next Step: Integration of Emphaze hybrid purifier technology. Potential process advantages:

- **Significant reduction (~30%) of HCP**
- Virtually clear HCD (4-5 LRV) prior to capture step
- Not pressure/flow limited allowing variable surface area scaling (Multiple Stage 1 Filters/1 x Emphaze)
- Potential to remove a polishing step and go to a streamlined, 2 chrom. step purification process.
Primary clarification step for 3 projects was evaluated with and without Emphaze and demonstrated significant reduction of both HCD and HCP. HCD levels in the CCCF are often below **100 ppb**. HCP levels in the CCCF across projects were between **25-40%** lower after Emphaze.

<table>
<thead>
<tr>
<th>% HCD in CCCF after Emphaze</th>
<th>% HCP in CCCF after Emphaze</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - No Emphaze</td>
<td>1 - w/Emphaze</td>
</tr>
<tr>
<td>2 - No Emphaze</td>
<td>2 - w/Emphaze</td>
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<tr>
<td>20.0%</td>
<td>40.0%</td>
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<td>100.0%</td>
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<tr>
<td>100.0%</td>
<td>&lt;0.02%</td>
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Where is the tipping point for value added by depth filtration assemblies versus complexity and cost? Does an elaborate clarification train make sense?

Goal: Combine 2 stages of filter media without separation via manifolds in order to maximize the nominal pore size and surface chemistry combinations used.

Can we make a 05SP + 60ZB or 10 SP + 90ZB within a single capsule? Another step on the way to a shorter, simpler purification process.
Summary

The implementation of 3M Zeta Plus depth filters improved the primary clarification process for a late stage project by:

- Increased HCD clearance and Process Flow Rate
- Decreased Filter Area Requirements
- But, no observed improvement in HCP clearance

Further improvement with the Emphaze Hybrid Purifier:

- Substantial increase in both HCD and HCP clearance
- No observed pressure/flow limitations

As we move forward, the goal to maximize combinations of pore sizes and surface chemistries while minimizing operational complexity is key with an eye on removing polishing steps from processes wherever possible
Questions?
Forward-Looking Statements

This presentation may contain forward-looking statements based on current assumptions and forecasts made by Bayer management.

Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer’s public reports which are available on the Bayer website at http://www.bayer.com/.

The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.
Thank you