Maximizing the Benefits of PEGylation by Optimizing the Conjugation Process

The Crossroad of Biotechnology

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Optimizing PEGylation

- Avantium Technologies
- PEGylation
- PEGylation downside
- PEGylation optimization
- Case study
Benefits of high-throughput experimentation

- Increase in R&D productivity
- Increased speed, significant time savings
- Better data consistency
- Better coverage of experimental space
- Higher knowledge return from R&D investments
PEGylation

- Chemically coupling PEG-agent to protein drug

- Marketed products:
  - Neulasta/Amgen
  - Somavert/Pfizer
  - Pegasys/Roche
  - PEG-Intron/Schering-Plough
PEGylation

• With the aim to:
  – Prolong circulation half-life (lower dosage frequency)
  – Decrease immune reactions and other side-effects
  – Reduce the amount of protein needed for the same therapeutic effect
PEGylation downside

- Losses during the PEGylation process of 25% in late stage/commercial production to 50-75% in early clinical production are not uncommon

- Proteins as well as PEG agents are expensive
PEGylation downside

• Example of Marketed Product:
  Annual sales of $250 million and COGS of 20% =>
  Each 10% yield improvement represents a value of $5 million per year.

• Example Early Clinical Product:
  Price per batch of $2.5 million =>
  Each 10% yield improvement represents a $250K cost reduction per batch.
PEGylation downside

- Maximizing the benefits requires Yield improvement!
  *but usually not performed due to time constraints*
PEGylation Process Optimization

Screening
50-500 exp
@1-5 mL

Optimization
25-75 exp
@20-50 mL

3-5 exp
@0.5-2 L

Verification

Start to Finish in 2-3 months
Case study

Yield / Cycle time optimization

Accelerating R&D
The Process

• Pegylation of a polypeptide (NH$_2$ specific conjugation)

\[
\text{mPEG-O} \quad \text{linker} \quad \text{H} \quad \text{N} \quad \text{Polypeptide} + \quad \text{H}_2\text{N} \quad \text{Polypeptide} \quad \xrightarrow{\text{reducing agent}} \quad \text{mPEG-O} \quad \text{linker} \quad \text{H} \quad \text{N} \quad \text{Polypeptide}
\]

- pH 7.5
- 25°C
- >100 h
The Problems

- Process step has long cycle times and low yields
  - Reaction time >100 h
  - Yield 76%
  - Conversion 80%

- R&D at the customer’s site:
  - Cycle time reduced to 40 h
  - Yield reduced to 73%
  - Conversion increased to 85%
  - Undesired impurity profile
  - pH important for impurity profile
The Objectives

- Reduce cycle time (<40 hrs)
- Minimize impurities (not exceed current process)
- Maximize yield (>85%)
Project Scope

- Phase 1: Rational screening of co-solvents and reducing agents
- Phase 2: Optimize process parameters
Rational screening

• How to select the co-solvents (=categorical variable)?

• How to ensure diversity?
The rational HT screening approach

- **Topological**
  - Use of the connection table representation of the chemical structure.
  - Employ methods drawn from mathematical graph theory.

- **Geometrical**
  - Calculated from 3D molecular models.

- **Electronic**
  - Calculated from semi-empirical or ab-initio calculations.

- **Hybrid Representations**
  - Encode the molecule's ability to interact with other compounds.
  - Encode the molecule's ability to form other species (e.g. complexes).

- **Measured**
  - Physicochemical (e.g. based on Spectroscopy or Thermodynamic)
The rational HT screening approach

- Co-solvents represented as points in ‘property space’
- Selection based on existing knowledge
Rational Screening

• Experiments:
  – All combinations of 15 solvents and 10 reducing agents
  – Other conditions fixed based on current process conditions
  – 150 pegylation reactions within 2 weeks (including analysis)
Rational screening

• Screening results (Yield)
Conclusions Phase 1

• Highlights:

  – Yield improved to 78% with encouraging impurity profile (conversion of 84%)

  – Co-solvent and reducing agent selected for optimization phase

  – Time frame screening phase: 2 weeks
Scope

- Phase 1: Rational screening of co-solvents / reducing agent
- Phase 2: Optimize process parameters
Response surface modeling

- Evaluate process parameters (main effects study)
  - Equivalents of reducing agent
  - Equivalents of PEG reagent
  - Reducing agent addition rate
  - Reaction concentration
  - Stirring speed
  - Stirring method (mechanical vs. orbital shaking)
  - Reaction time
  - Temperature
  - pH
  - Ionic strength
Response surface modeling

- Optimize significant process parameters and their interactions using statistical design of experiments (DoE)
  - Reaction concentration
  - Reaction time
  - Equivalents of PEG reagent
  - pH

- I-optimal design (48 reactions including replicates)

- Results in a response surface model (RSM)
Response surface modeling

![Response surface modeling diagram](image-url)
Response surface modeling
Conclusions Case study

- Optimal co-solvent/reagent combination and 4 main process conditions identified and explored

- Response Surface Model indicating achievable yield of 92% within process time of 50 hrs (instead of target 40 hrs)

- Potential yield improvement of >15% representing a COGS reduction of > $5 mio per annum

- Acceptable impurity profile

- Time frame: <2 months
Summary

- PEGylation benefit of lower amount needed for same therapeutic effect can be optimized by yield optimization

- PEGylation yield improvement represents significant value

- Rational design, high-throughput experimentation is a very effective and efficient way to optimize PEGylation process