Development of a lyophilized peptide drug product and assessment of alternative freeze drying methods

Stefan Schneid

Lyo Garmisch Conference
September 16 – 19, 2025

Bayer in Wuppertal

CNN travel DESTINA

Wuppertal, Germany

The Schwebebahn railway in Wuppertal is one of the world's coolest rail systems.

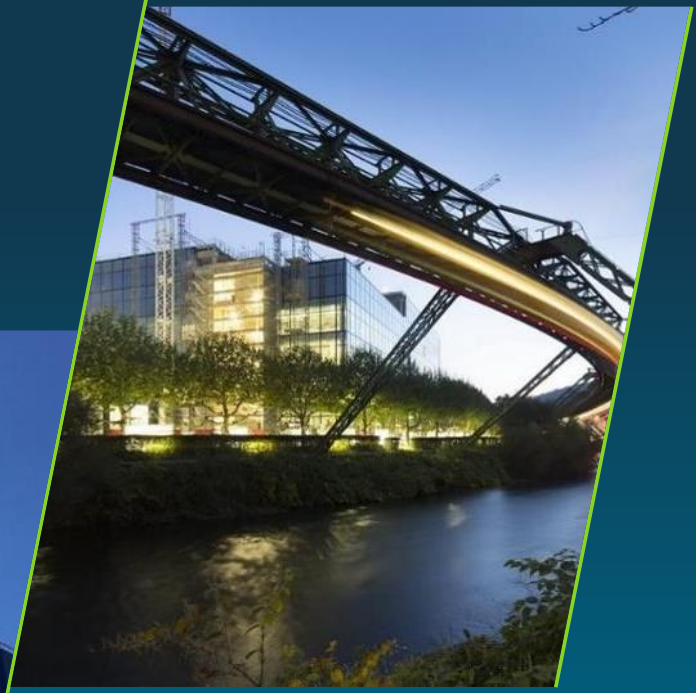
Ina Fassbender/AFP/Getty Images

An industrial city in western Germany may not sound like anyone's idea of a dream vacation, but Wuppertal has an extraordinary ace up its sleeve -- one of the world's coolest rail systems.

Newly repaired in 2019 after a six-month closure, the city's 120-year-old Schwebebahn suspension railway looks like something from the imagination of Jules Verne.

It's a steampunk vision of a mass transit system whose iron legs straddle the city's streets and waterways, whisking passengers high over traffic snarl-ups to stations just as sci-fi as the train that connects them.

It costs just a few dollars to ride the Schwebebahn, alongside the thousands of commuters that use it daily.



Agenda

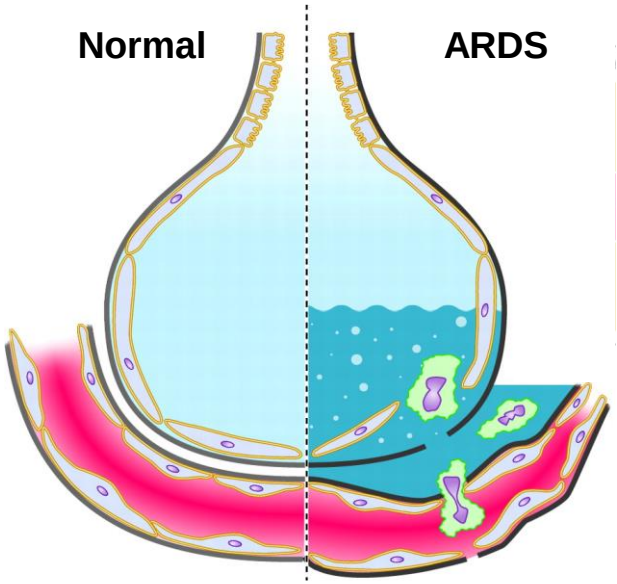
Introduction and API

Formulation Development

Process Transfer

Continuous Freeze Drying

Adrenomedullin pegol: Proposed Mechanism in ARDS



Vascular leakage

alveolar edema,
impaired lung function, reduced oxygenation

Invasive mechanical ventilation

High mortality (30-40 %)

Central MoA of BAY 1097761 might be beneficial as treatment option in ARDS

restore cell-cell junctions

reduce lung water

improve oxygenation

decrease mortality and need for mechanical ventilation

Negative efficacy data from Phase 2A study - project was terminated

pharmaphorum News Views & Analysis Deep Dive Webinars

Bayer pulls plug on respiratory drug PEG-ADM

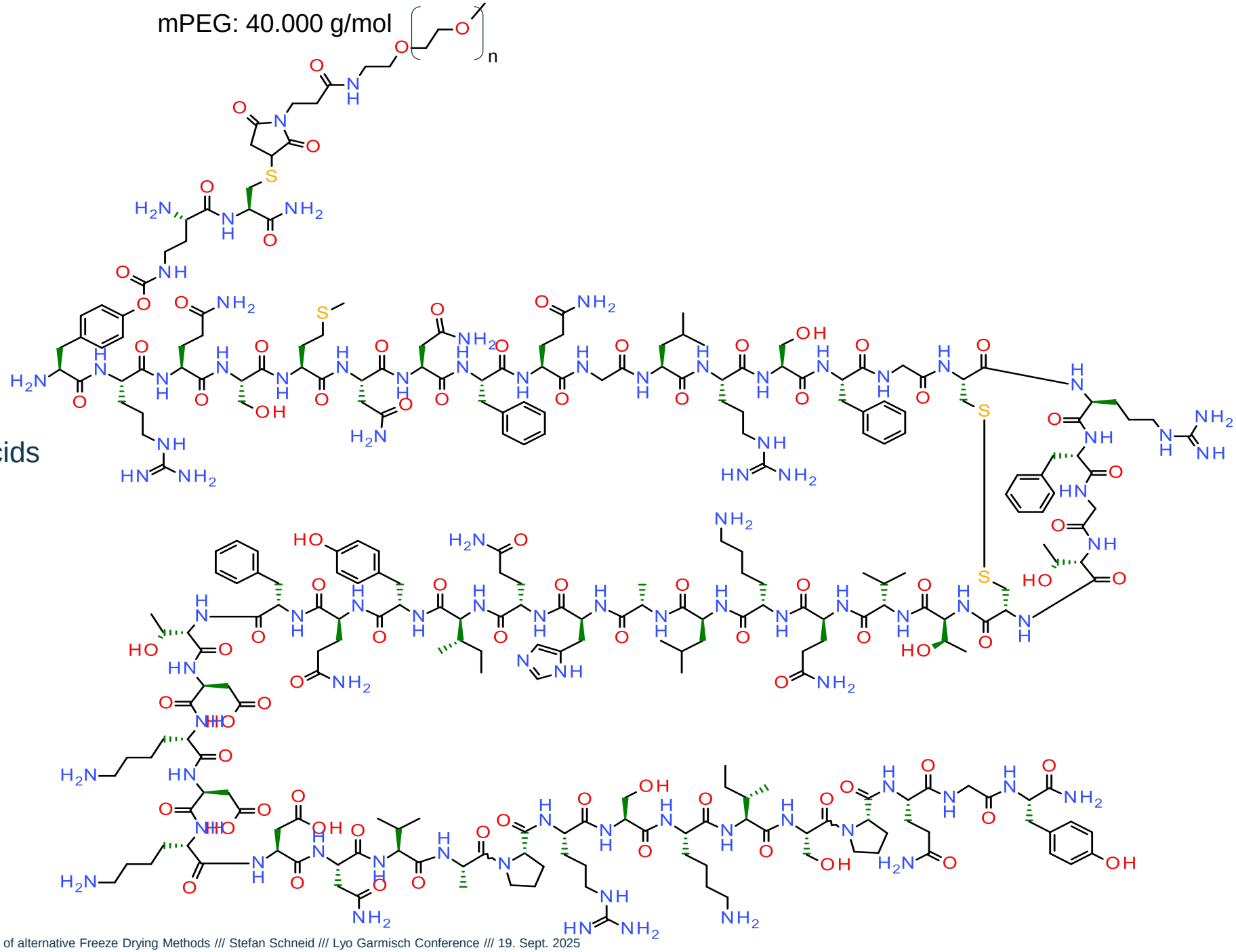
FIERCE Biotech Biotech Research Medtech CRO Special Reports Trending Fierce 50

BIOTECH

Bayer ends respiratory distress drug development in latest phase 2 cleanout

By James Waldron · Aug 8, 2023 9:40am

Adrenomedullin pegol



Some facts

Molar mass 46.3 kDa

85% of the mass is PEG

56 chiral centers, 54 amino acids

Intramolecular disulfide bond

10 g PEG-ADM (Prodrug)

= **1.3 g** ADM content

Adrenomedullin (ADM) is released upon pH increase

Drug Product and Device

Phase I

Drug Product: frozen solution in buffered saline, ADM conc. 0.48 mg/mL in application solution

Device: Aerogen Solo nebulizer with Ultra™ mouthpiece (self-breathing volunteers)

Phase II

Drug Product: lyophilisate formulation with matching placebo, ADM conc. 0.48 mg/mL in application solution

Device: Aerogen Solo nebulizer with T-piece (ventilation setup)

Regulatory requirements for synthetic peptide drugs

2009

- **Biologic Price Competition and Innovation Act (BPCIA)** amended the definition of a biologic product to include “proteins (except any chemically synthesized polypeptide)”
  - Chemically synthesized polypeptides = made entirely by chemical synthesis and greater than 40 but less than 100 amino acids in size

2020

- **Further Consolidated Appropriations Act (FCAA)** further amended the definition of a biologic product by removing the phrase “(except any chemically synthesized polypeptide)”
  - Final Definition of a protein effective 2020-March-23:

Protein: any alpha amino acid polymer with a specific defined sequence that is greater than 40 amino acids in size, including any **chemically synthesized polypeptide**

Adrenomedullin pegol is considered a BIOLOGIC in the US, but a SMOL in all other countries

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Formulation Development

Previous product versions:

- Mannitol-based lyo formulation for infusion
- Buffered saline frozen solution for inhalation

Planned new product version:

- Stable lyophilizate for inhalation
- Suitable for nebulization
- Additional item: evaluation of lyophilized DS

US 20230149553A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2023/0149553 A1**

UNGER et al. (43) **Pub. Date: May 18, 2023**

(54) **LIQUID PHARMACEUTICAL FORMULATIONS POLYETHYLENE GLYCOL-BASED PRODRUGS OF ADRENOMEDULLIN AND USE**

(71) Applicant: **Bayer Aktiengesellschaft**, Leverkusen (DE)

(72) Inventors: **Florian UNGER**, Haan (DE); **Stefan Christian SCHNEID**, Düsseldorf (DE); **Hans-Walter MOTZKUS**, Schildow (DE); **Carina HAASBACH**, Wuppertal (DE)

(73) Assignee: **Bayer Aktiengesellschaft**, Leverkusen (DE)

(21) Appl. No.: **17/916,778**

(22) PCT Filed: **Mar. 31, 2021**

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A61K 47/60 (2006.01)
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(52) **U.S. Cl.**
CPC ***A61K 47/60*** (2017.08); ***A61K 9/08*** (2013.01); ***A61K 47/542*** (2017.08); ***A61K 9/0073*** (2013.01)

(57) **ABSTRACT**

The present invention relates to novel liquid pharmaceutical formulations, preferably for inhalation, comprising polyethylene glycol (PEG)-based prodrugs of Adrenomedullin (PEG-ADM) and the use thereof for the treatment and/or prevention of acute lung injury/acute respiratory distress syndrome (ALI/ARDS).

Specification includes a Sequence Listing.

US 20230364245A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2023/0364245 A1**

UNGER et al. (43) **Pub. Date: Nov. 16, 2023**

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(21) Appl. No.: **17/905,747**

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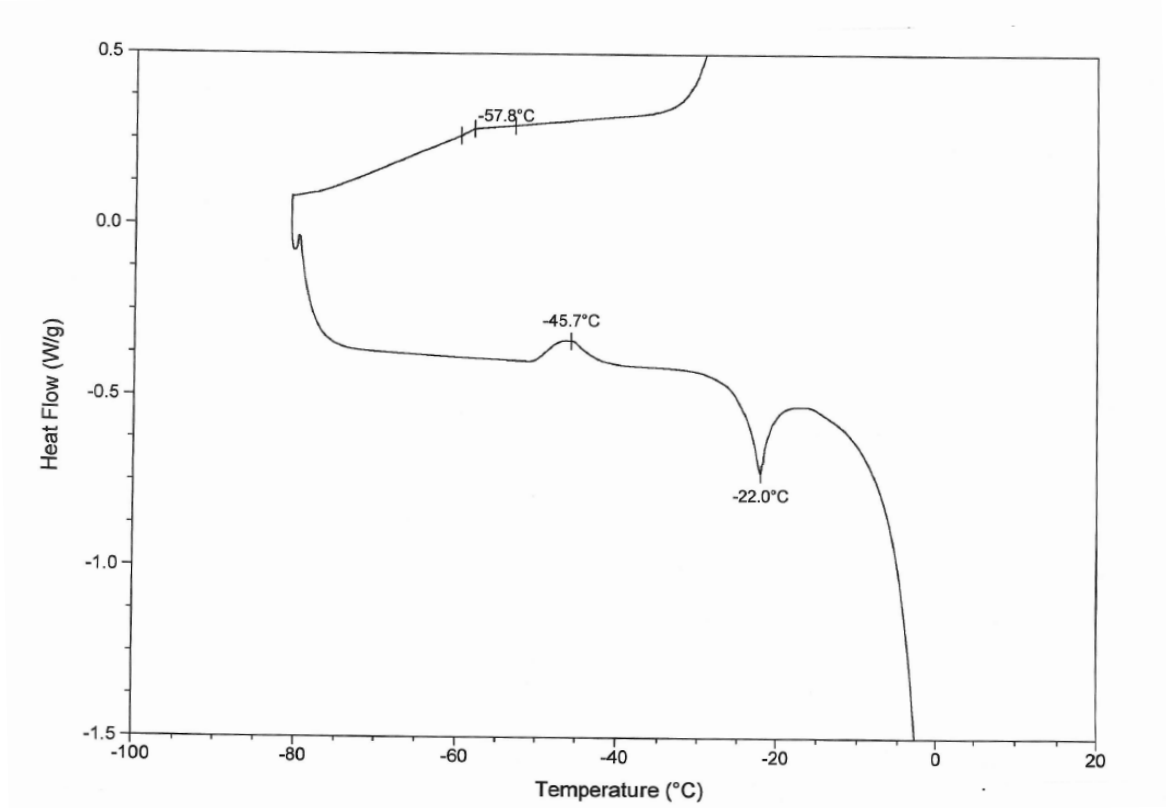
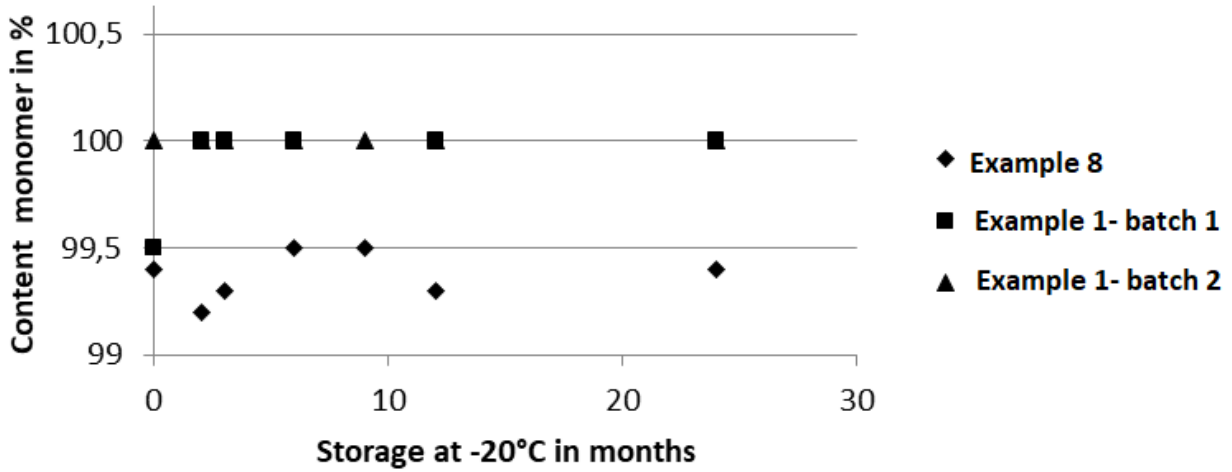
Specification includes a Sequence Listing.

Frozen Solution

- Citrate buffered solution at pH 4 containing NaCl
- Very low T_g' value of -58°C, and eutectic melting at -22°C
- However, the product was perfectly stable when stored at < -15°C (effectively -20 +/- 5°C)
- Stability was also confirmed after freeze-thaw cycling with 5 consecutive freezing and thawing steps
- Incomplete freezing in this case not detrimental for product quality and stability





Lyophilisate Development

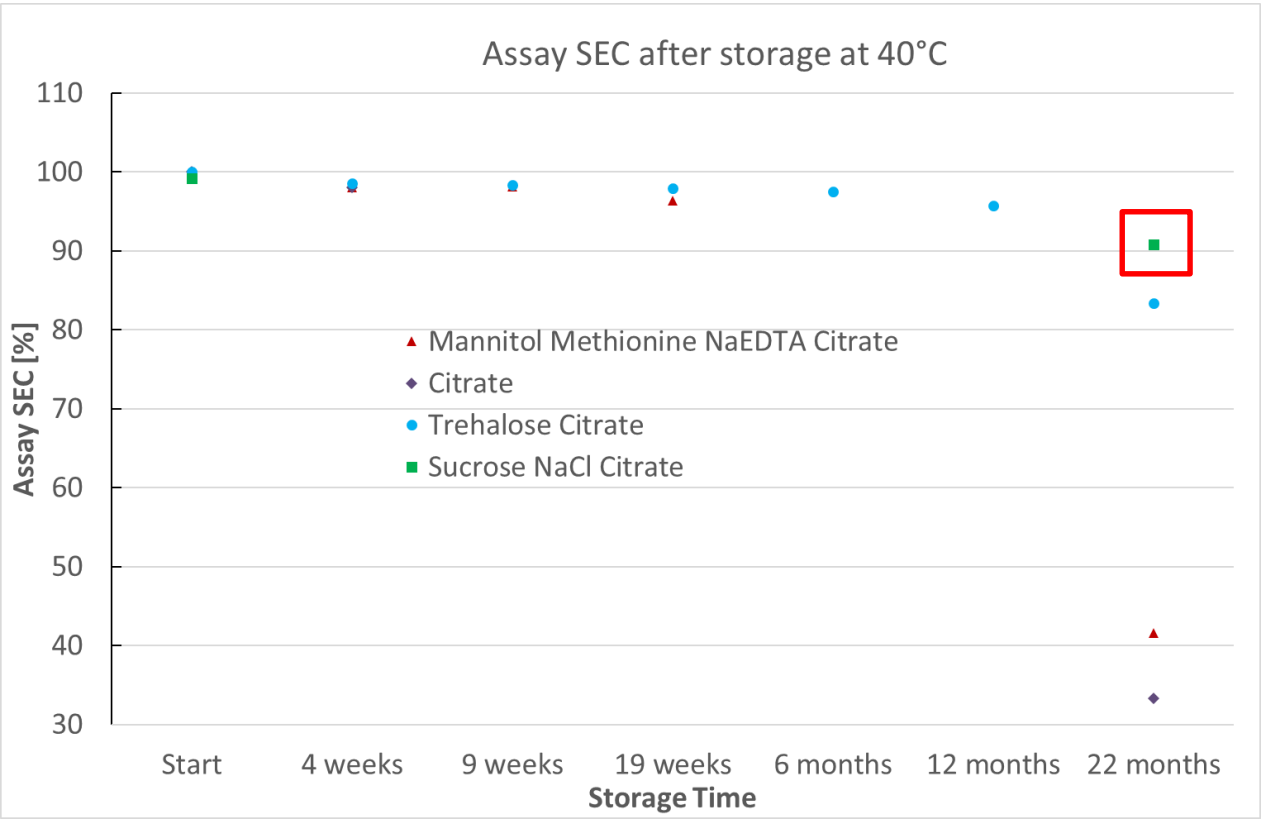
Objective for new product version:

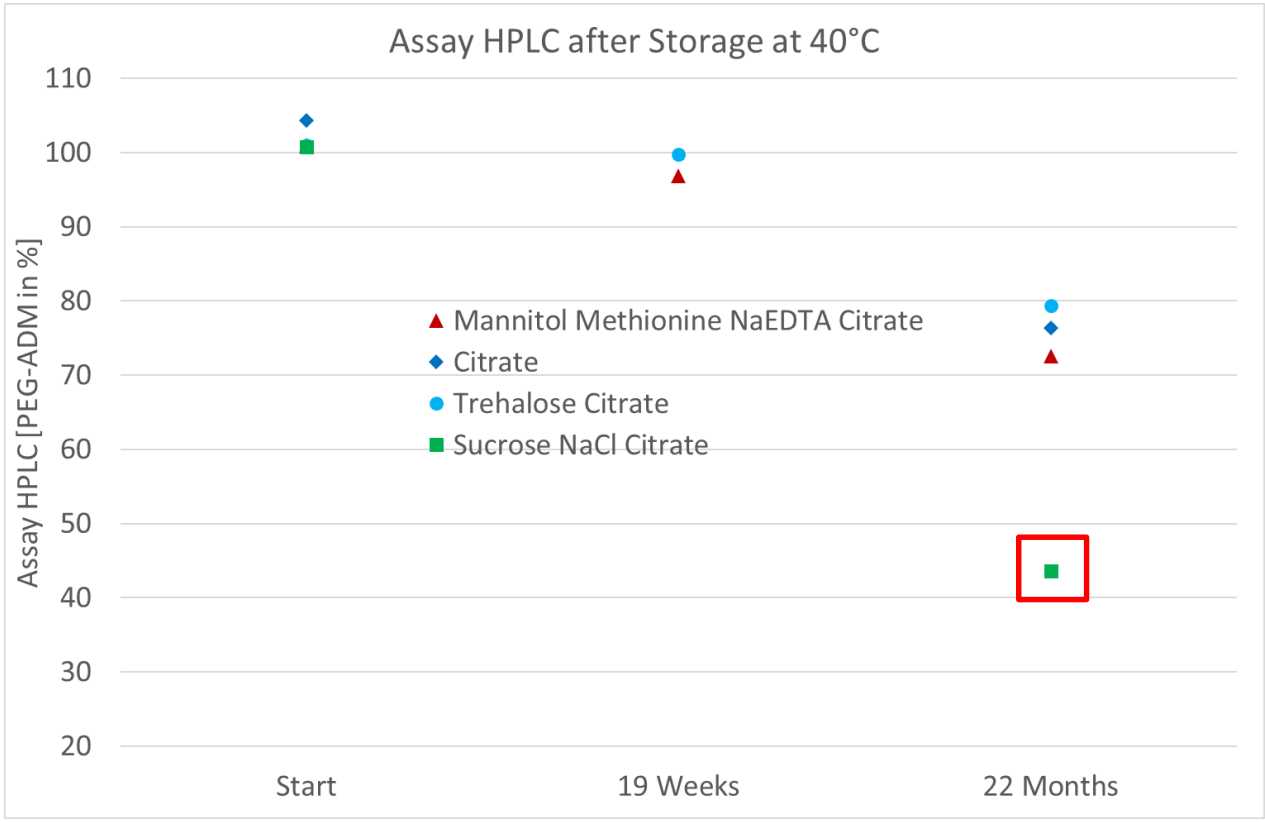
- Stable lyophilisate suitable for inhalation application
- Low pH of 4 required due to prodrug properties, limited buffer options
- Some NaCl needed in the final application solution to avoid cough reflex
- Compatible with nebulization using commercial nebulizer, several nebulizations per device (3 treatments per day)
- Avoid development of separate reconstitution solution

Formulation Screening:

- Citrate selected as buffer system
- Screening of cryo- and lyostabilizers, surfactants, antioxidants, chelating agents
- Stress testing in liquid and lyo state
- Addition of surfactant was found to be not required based on mechanical stress data
- Low T_g' of formulations (due to NaCl) needed to be increased to manageable level for freeze drying
- But: high concentrations of cryo-/lyostabilizer and increased viscosity impaired nebulization properties (reduced throughput, increase of droplet size)

Orienting Stability with Preliminary Lyo Formulations

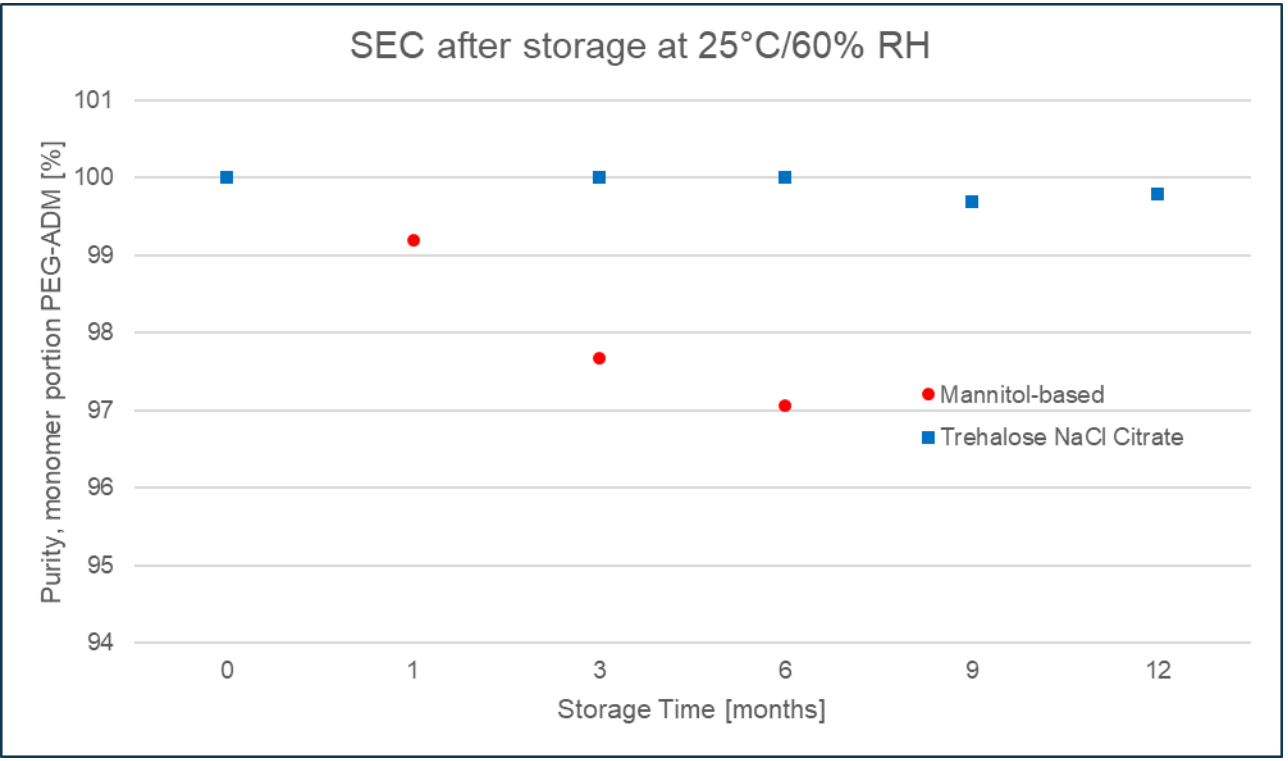


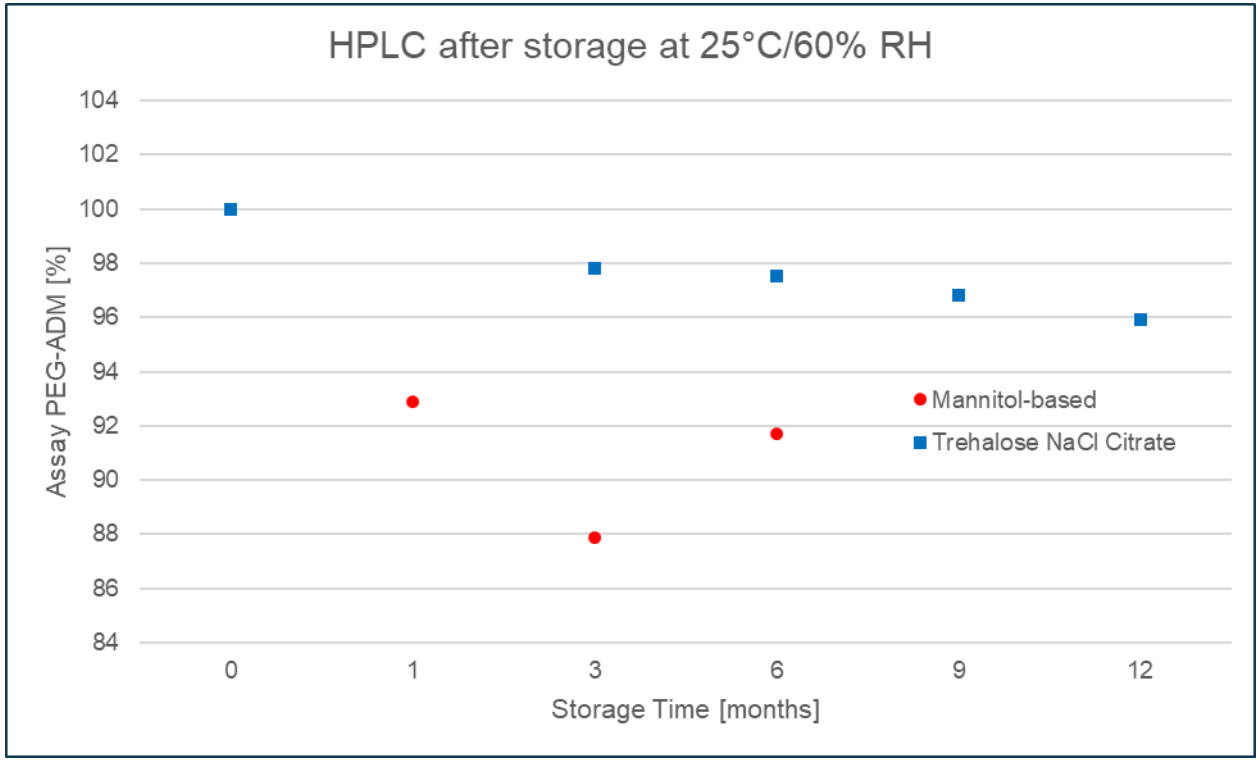


Partially contradicting results for different analytical methods were caused by formation of sucrose degradation products after extended storage at pH 4 which impacted the UV detection

Overall, a trehalose-based formulation showed the best stability and was selected

IND-enabling Stability Studies for Lyo Formulations (further iteration)





Comparison of the IMPD stability studies for the old Mannitol-based i.v. formulation and the Trehalose-based inhalation formulation confirm superior stability of the new product version

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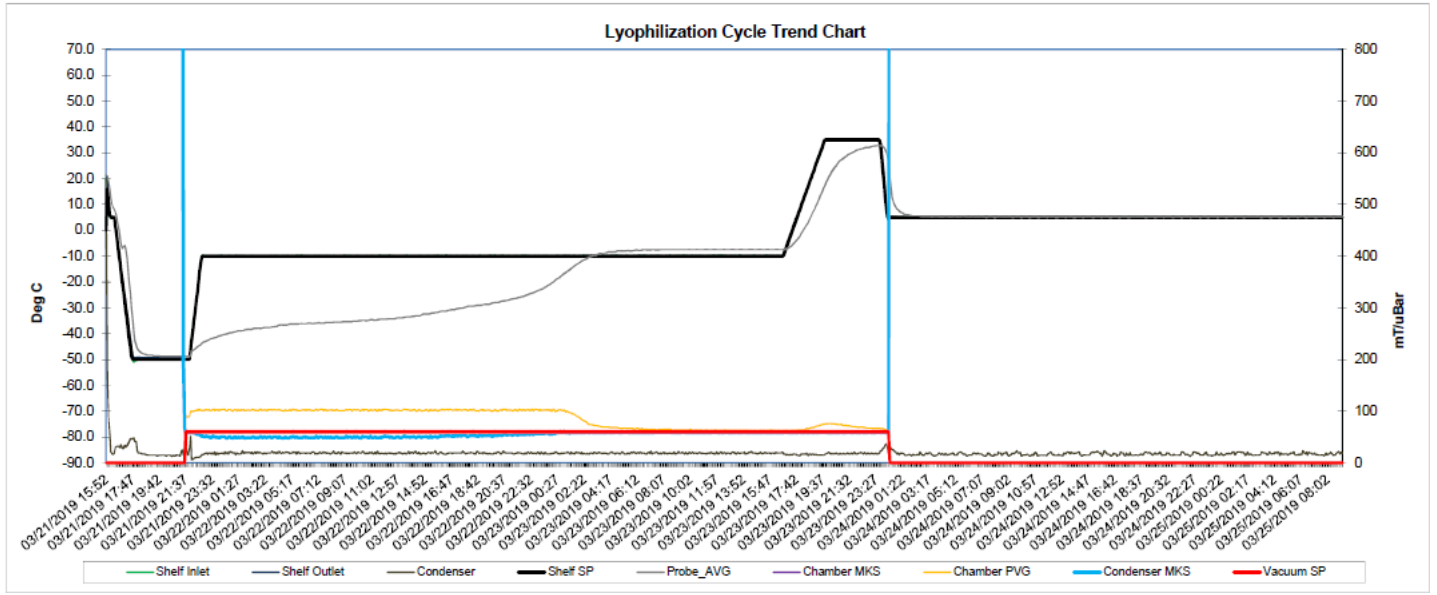
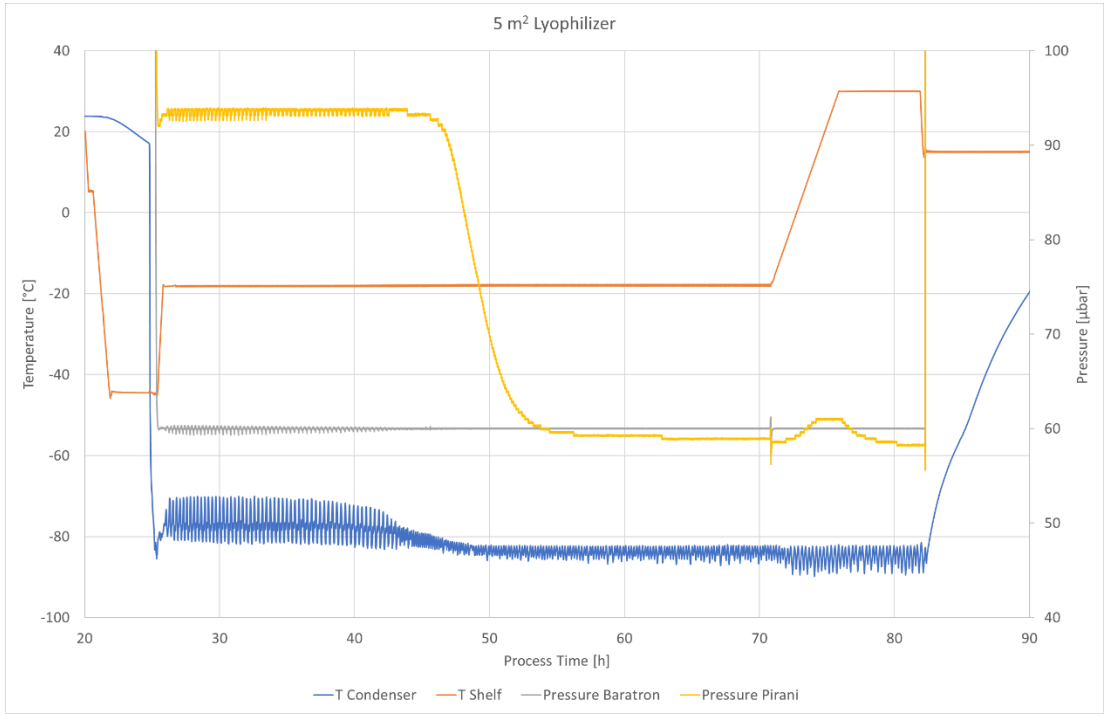
Lyo Process Transfer

- Process Development performed in lab freeze dryer
- Characterization of collapse temperature and T_g' for active and placebo (~ -35°C and -37°C)

→ Lower shelf temperature used for placebo than for active process to ensure comparable appearance

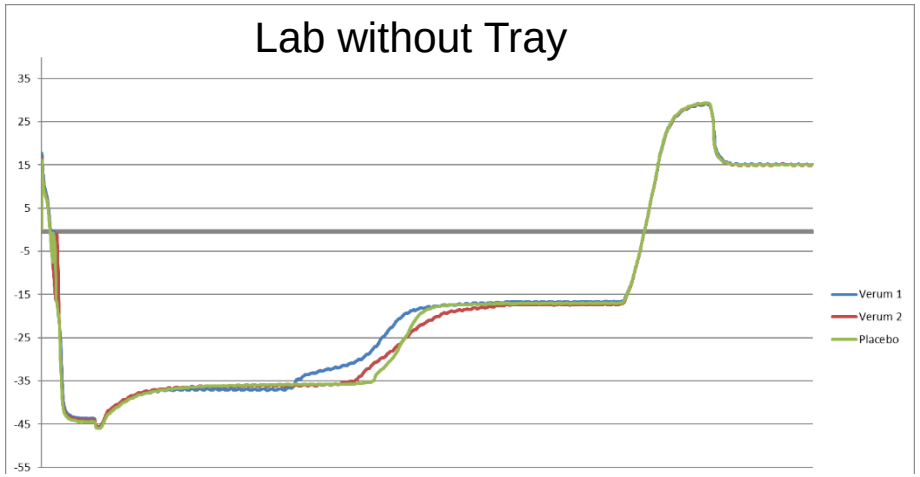
- Successful transfer to Bayer-internal clinical supply line with two freeze dryers (~ 5 m^2 each)
- Subsequently, temporary transfer to clinical CDMO required due to internal shutdown, with different lyo and configuration (vials on trays)

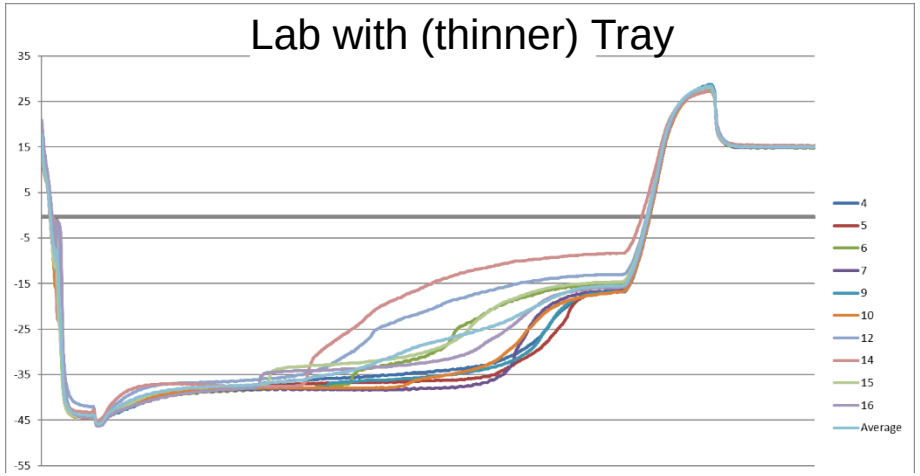


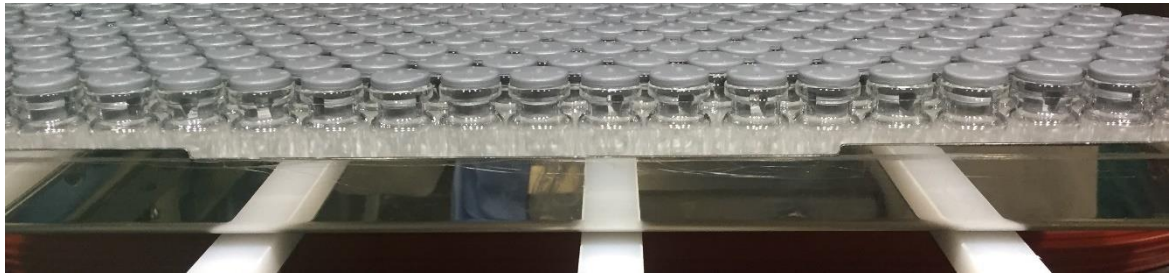


Process Adaptations to compensate for Trays

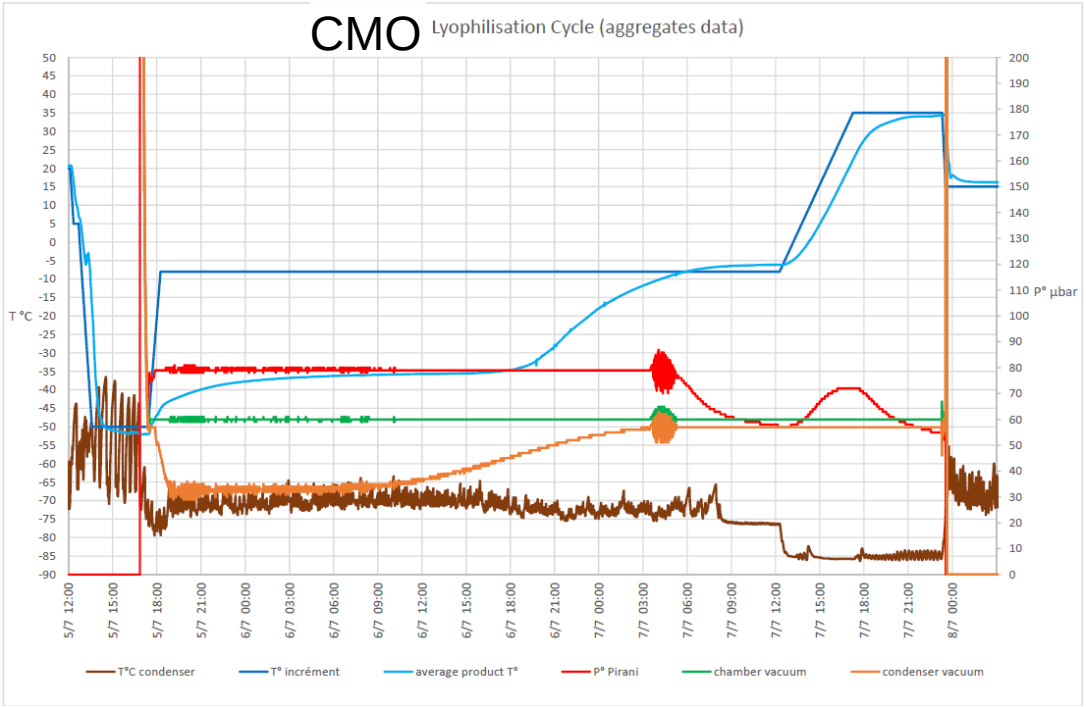
Tests performed at Bayer => impact on Tp,
Drying duration and RM
+ use of OQ data from CMO on lyo performance







Freeze drying recipe adapted



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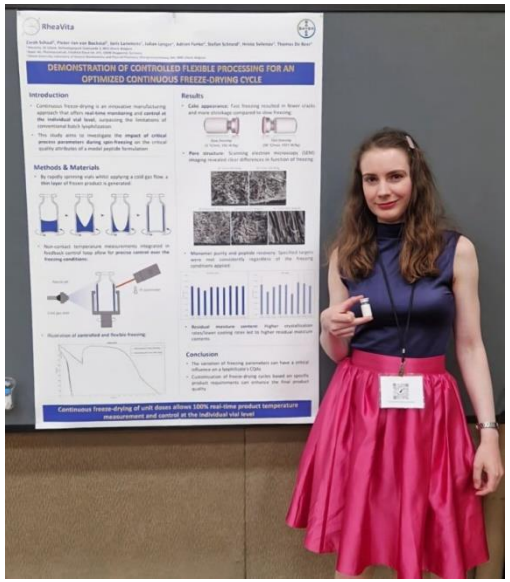
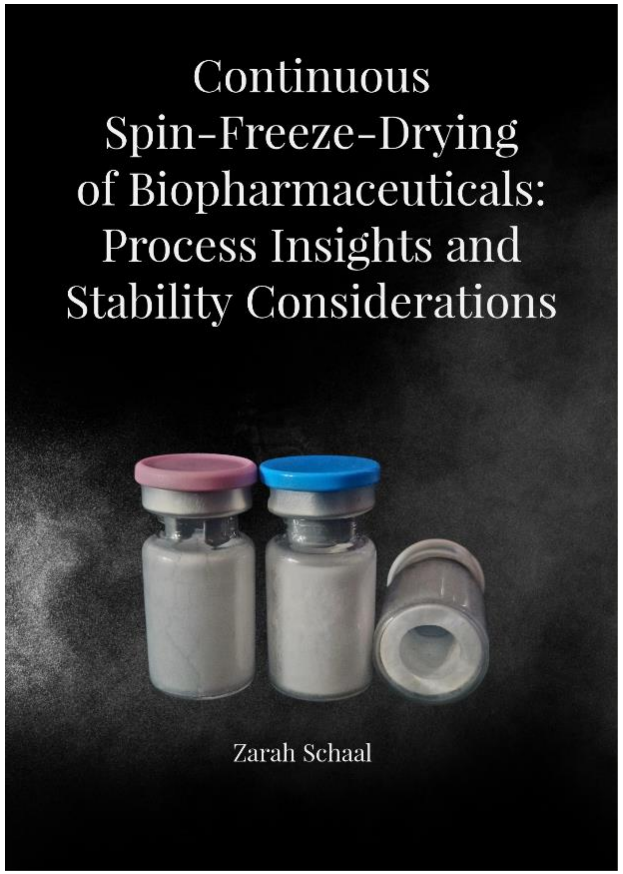
Ph.D. Collaboration with Rheavita, Ghent



Zarah Schaal
Pharmacist,
Ph.D. Candidate Ghent



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journal homepage: www.elsevier.com/locate/ejps

Optimization of continuous spin-freeze-drying: The role of spin-freezing on quality attributes and drying efficiency of a model peptide formulation

Zarah Schaal [a,b], Pieter-Jan Van Bockstal [a], Joris Lammens [a], Julian H. Lenger [c], Adrian P. Funke [d], Stefan C. Schneid [c], Hristo L. Svilenov [c,1], Thomas De Beer [a,b,*]

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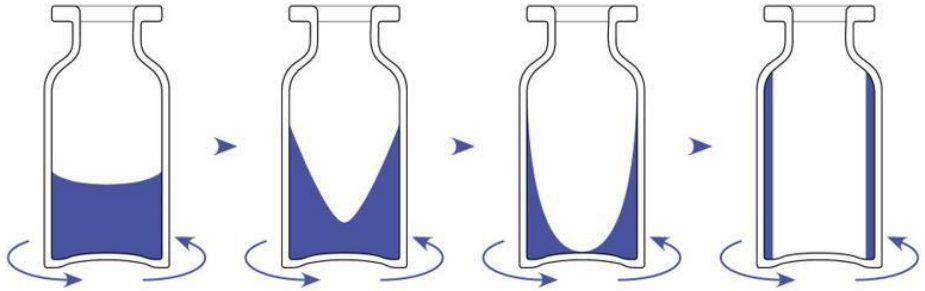
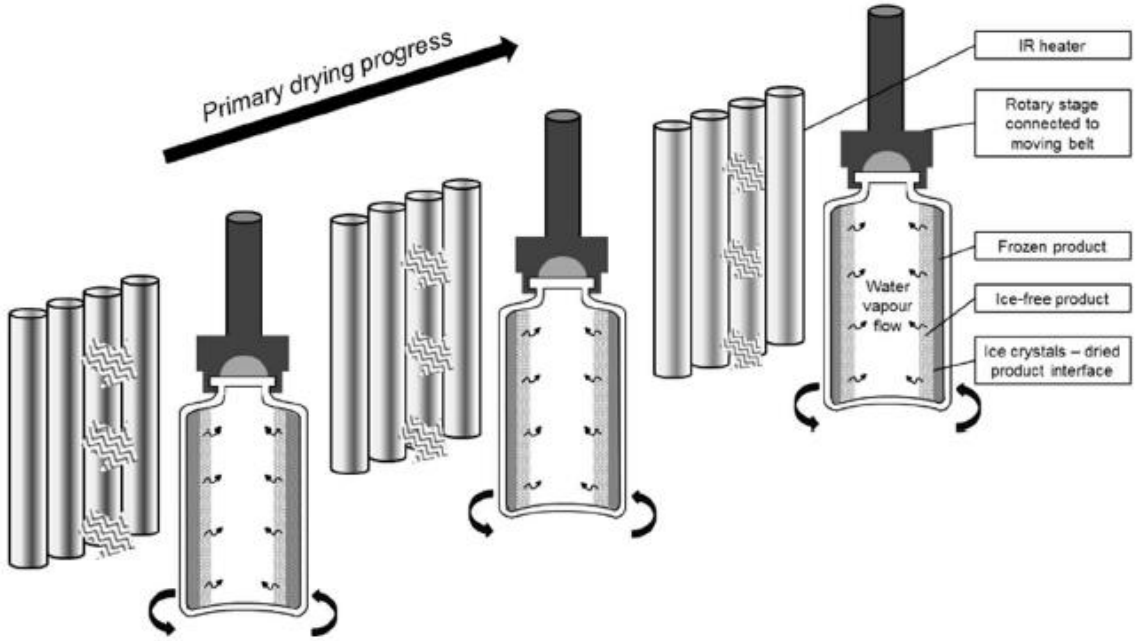
Impact of spin-freezing parameters and excipient composition on product stability of a PEGylated peptide formulation

Zarah Schaal [a,b], Pieter-Jan Van Bockstal [a], Joris Lammens [a], Julian H. Lenger [c,1], Adrian P. Funke [d], Stefan C. Schneid [c], Thomas De Beer [a,b,*]

Z. Schaal, P.J. van Bockstal, J. Lammens, J. Lenger, A. Funke, S. Schneid, H. Svilenov, T. De Beer. Continuous spin-freeze-drying of a PEGylated peptide formulation: evaluating the role and impact of annealing and radiative cooling. Submitted to AAPS Open

Continuous Freeze Drying – Rheavita Technology





Schaal Z, van Bockstal PJ, Lammens J, Lenger J, Funke A, Schneid S, Svilenov H, De Beer T. Freeze Drying of Pharmaceuticals & Biologics Conference 2023, Breckenridge CO

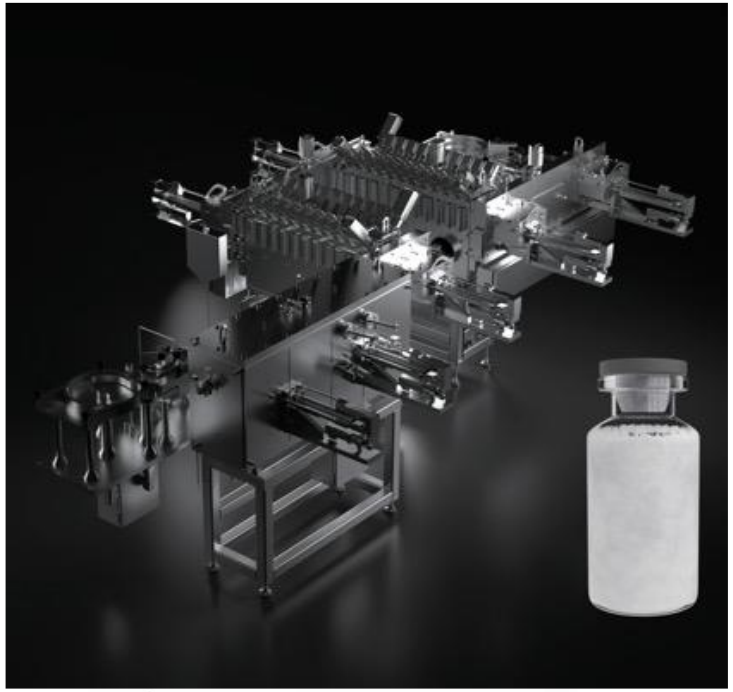
Scale-Up and Transfer

SVU

R&D equipment including software & digital twin for fast product & process development with very low product consumption

MVU

Available for evaluation studies by pharmaceutical companies for stabilitiy analysis and formulation optimization



GMP-FLEX

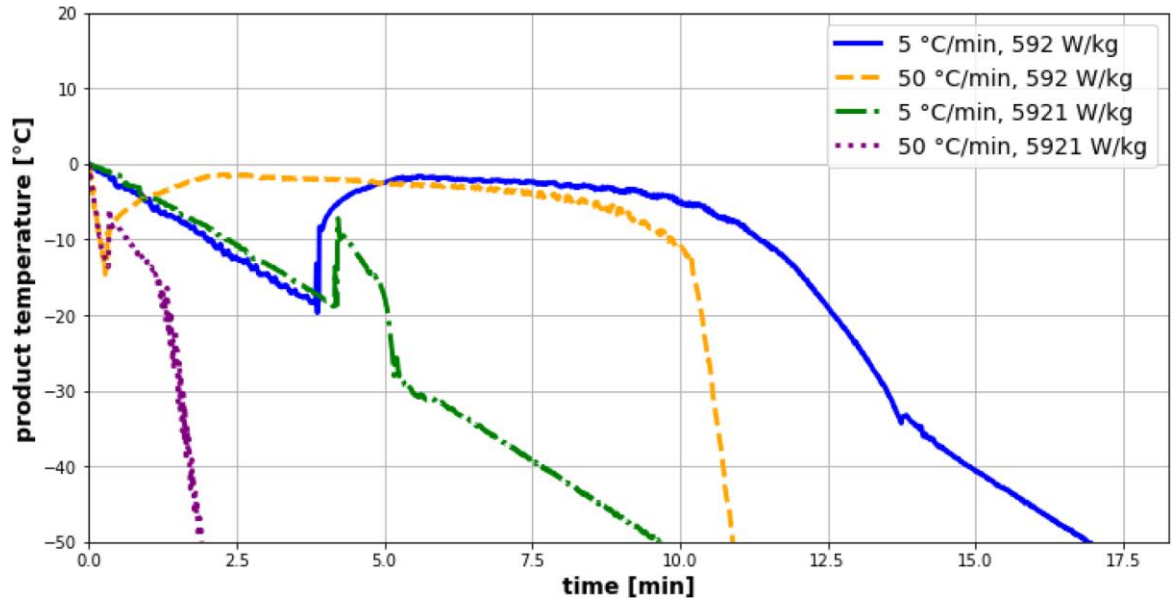
GMP production scale continuous freeze-dryer - custom made assembly

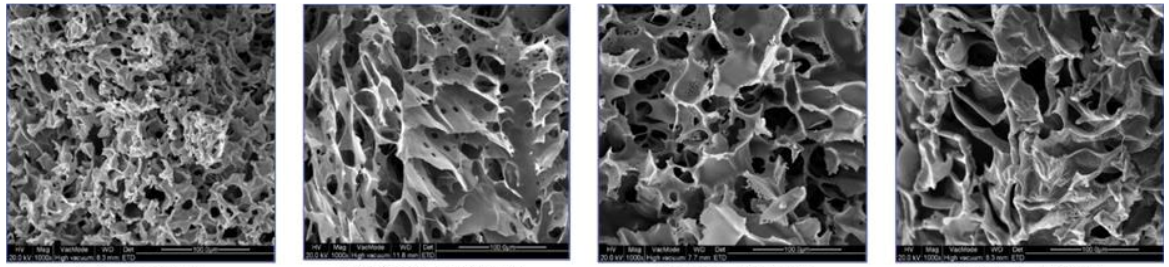
RheaVita - 2022 Confidential – all rights reserved

Spin-Freezing Conditions influence Cake Appearance

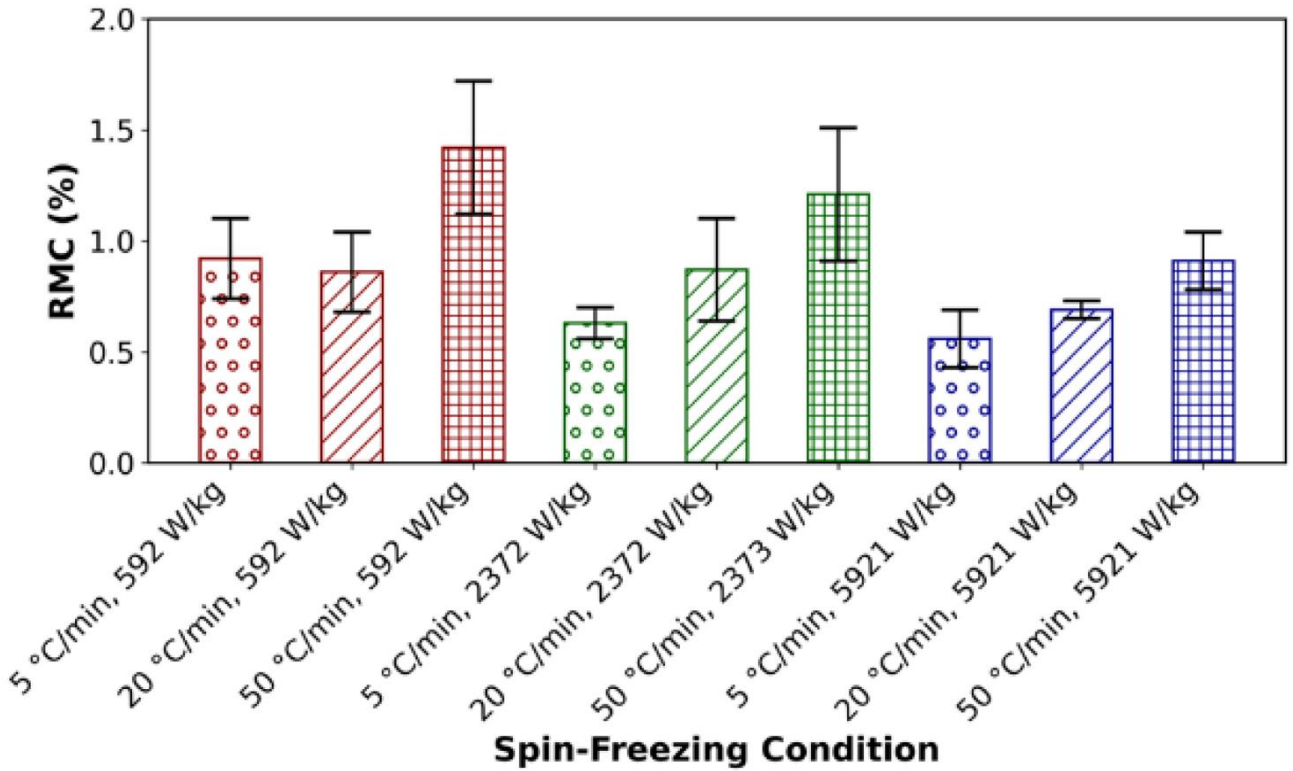
Variation of Cooling Rate and Crystallization Rate resulted in changes in product appearance and cake morphology; fast crystallization rate beneficial for avoidance of cracking

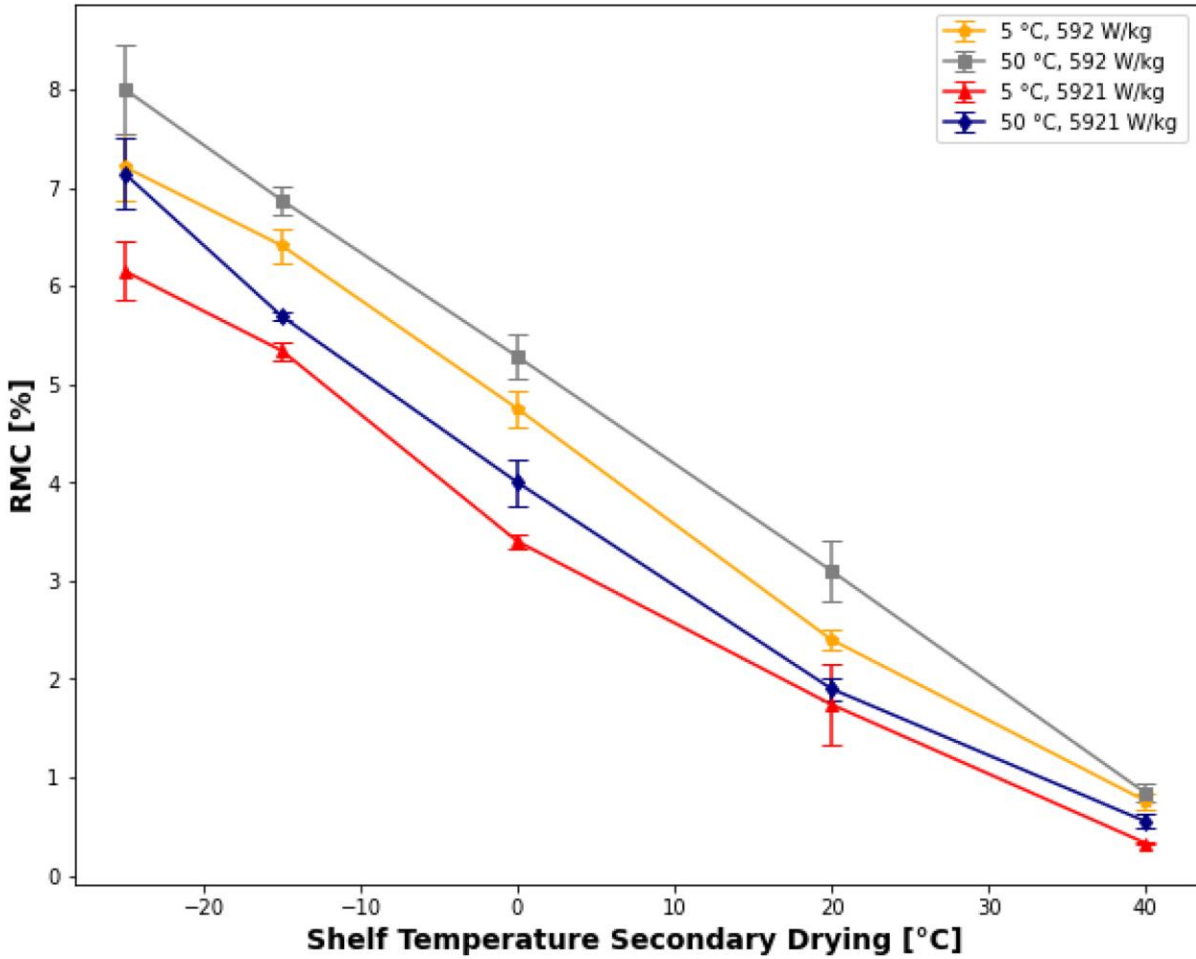
Most product cQAs (assay, purity, reconstitution time) and drying time were not impacted. RM → next slide





Spin-Freezing Conditions impacted Residual Moisture Content

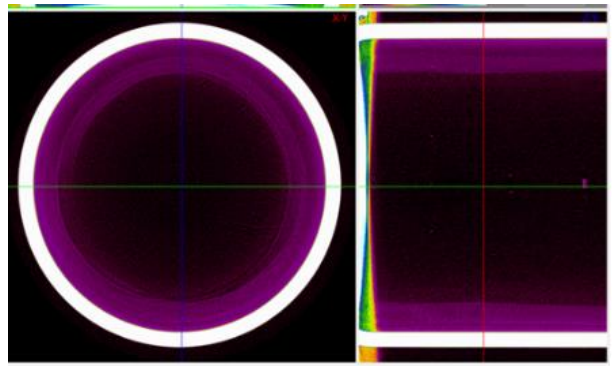




Annealing resulted in unexpected results

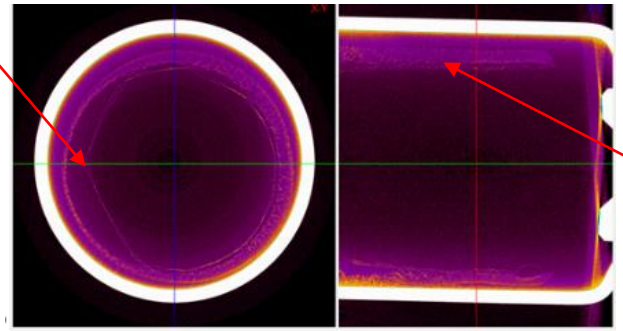
- **Annealing**: skin formation, collapsed/heterogeneous cakes, and increased product resistance
  → unsuitable for this formulation and integration appears impractical for this technology
- **Peptide Stability:** unaffected by annealing after the drying process (no stability data)

Left to right: unannealed formulation, unannealed placebo, annealed formulation, and annealed placebo



µ-CT – no annealing

skin formation



more irregular pores

µ-CT - annealing

Reformulation Stability Study

Research Objective:

Do spin-freezing conditions affect the long-term stability of the PEGylated peptide formulation?

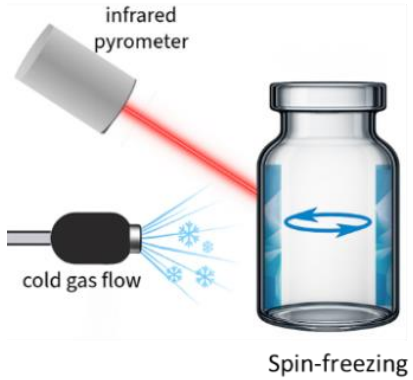
How does replacing trehalose with alternative excipients influence stability?

→ Four different spin-freezing & two different storage conditions (50 °C & 2 – 8 °C) examined

Trehalose-based: 3.7 mg/mL API, 7.5 mg/mL citrate, 1.7 mg/mL NaCl, 50 mg/mL Trehalose

Mannitol-based: 3.7 mg/mL API, 7.5 mg/mL citrate, 1.7 mg/mL NaCl, 50 mg/mL Mannitol

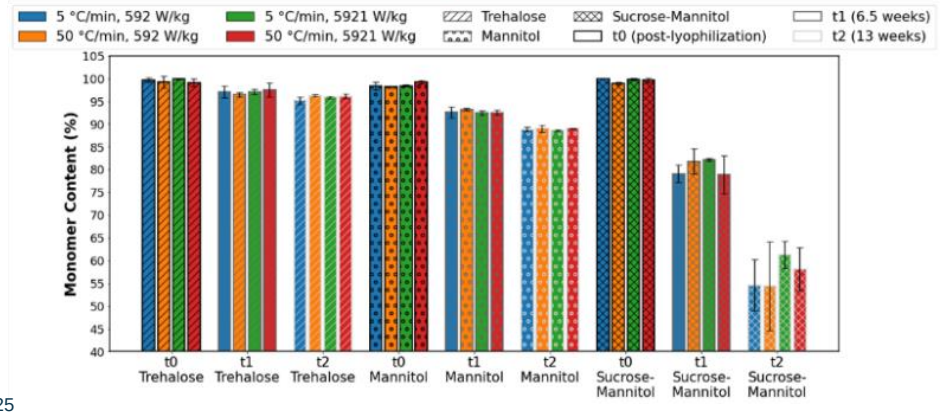
Sucrose/Mannitol-based: 3.7 mg/mL API, 7,5 mg/mL citrate, 1.7 mg/mL NaCl, 37.5 mg/mL Sucrose, 12.5 mg/mL Mannitol



conservative drying in a batch-freeze-dryer

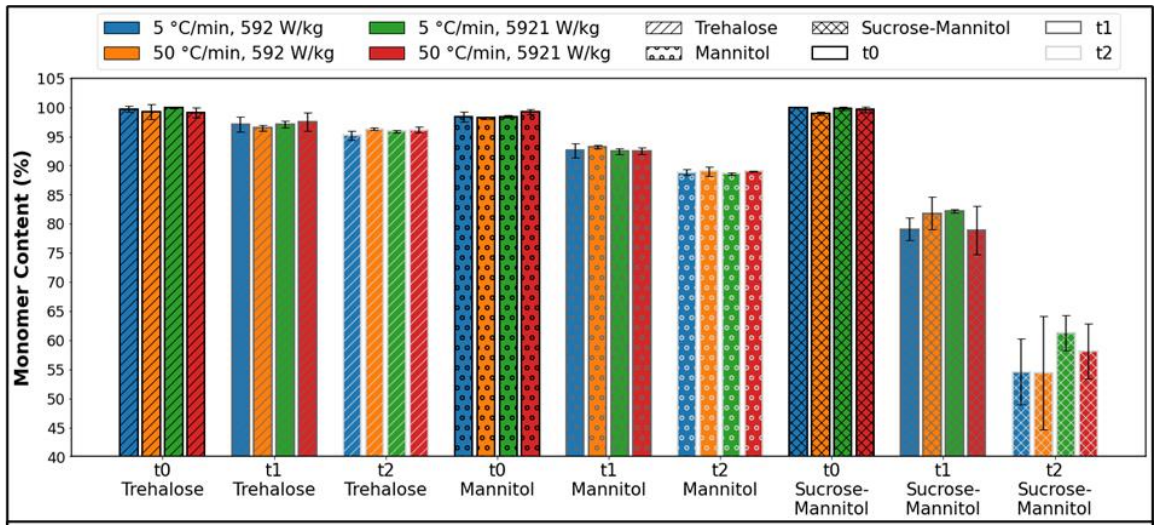
After 13-week storage at 50 °C

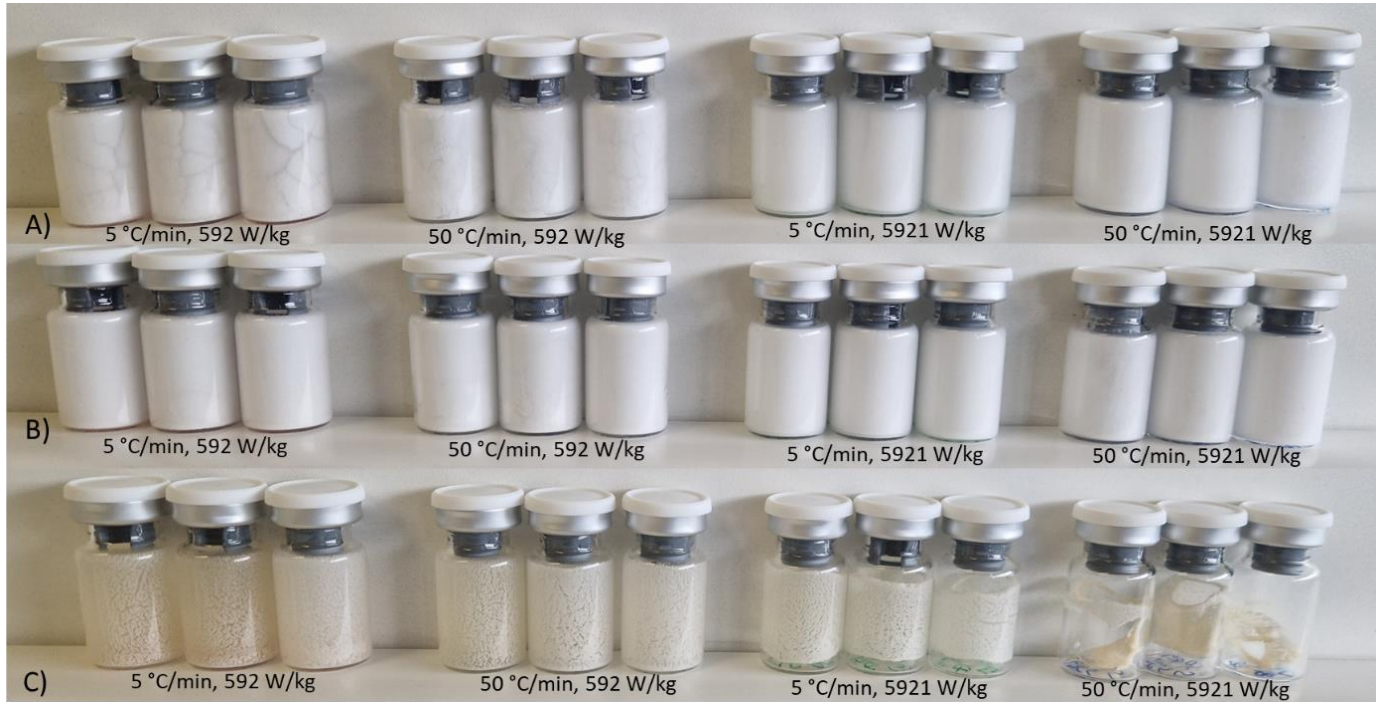
Chromatographic Analysis



Reformulation Stability Study

- **Trehalose:** again most stable formulation, high peptide concentration and low aggregation even at 50 °C
- **Mannitol:** moderate degradation at 50 °C, no assay interference
- **Sucrose–mannitol (75:25):** collapse → sugar hydrolysis/Maillard → UV-interference, high aggregation
- **Spin-freezing parameters:** stability mainly affected by excipients & storage, not freezing parameters





A) trehalose, B) mannitol, and C) sucrose-mannitol samples after 13 weeks at 50 °C

Thank you! Questions?

Acknowledgements

Zarah Schaal

Thomas de Beer

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