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Formulation Challenges of Protein Drugs

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Development of Biotech Drug Products

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Introduction

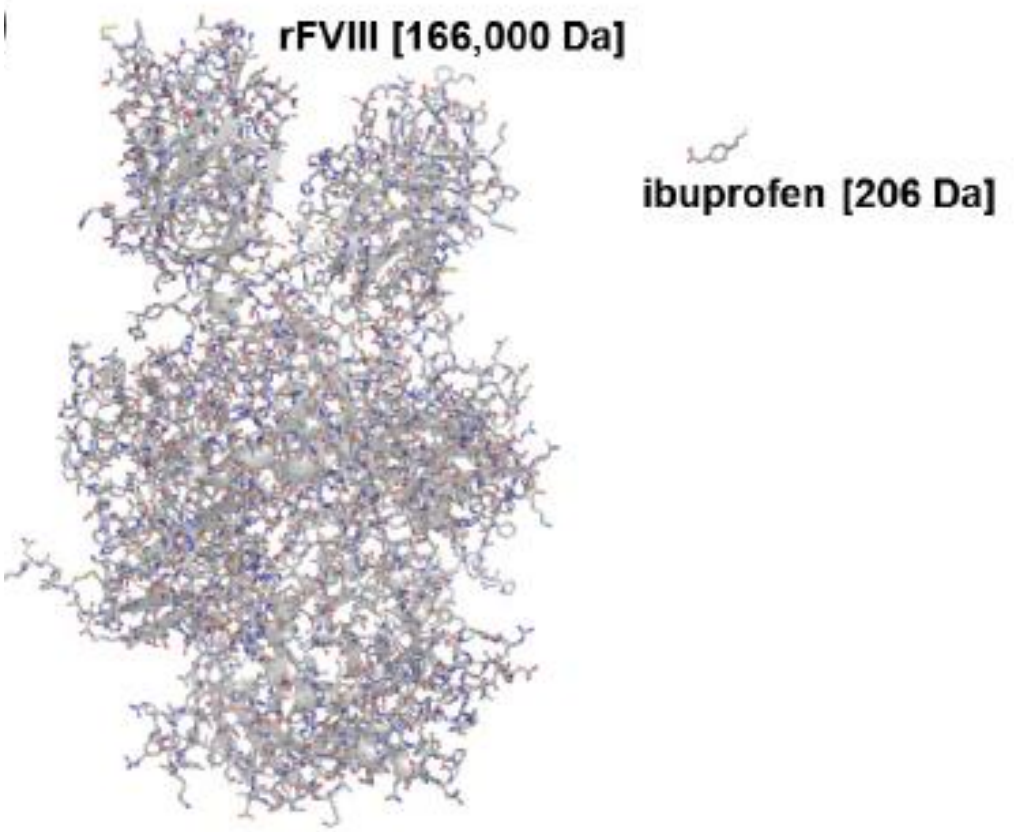
- Protein-based therapeutics represents the fastest growing sector of the innovator pharmaceutical industry
- Therapeutic targets historically difficult to address with small-molecule drugs
- The global market for protein drugs reached $174.7 billion in 2015 and it is predicted to reach $248.7 billion by 2020 at a compound annual growth rate (CAGR) of 7.3% through 2020

Introduction

- 239 FDA-approved peptides and proteins *(THPdb: Database of FDA-approved peptide and protein therapeutics, 2017)*
- 62 FDA approvals from Jan. 2011 through Aug. 2016
  - ~ 50% Monoclonal Antibodies
  - 26% oncology
  - 29% hemethology
- 1300 recombinant pharmaceuticals under development *(May 2015)*

Introduction

- Formulation and stability: very complex problems for proteins



Protein Instability

- Proteins are prone to a variety of physical and chemical degradation pathways
- Liquid, frozen, and lyophilized states
- The glycosylation state can significantly affect degradation
- In many cases, multiple degradation pathways can occur at the same time
- The degradation mechanism may change depending on the stress conditions

Protein Instability: An Overview

Protein

Chemical Instability

- Deamidation (pH!!!)
- Racemization
- Hydrolysis (pH!!!)
- Oxidation
- Disulfide exchange
- Dimerization and polymerization

Physical Instability

- Denaturation
- Aggregation
- Precipitation
- Surface adsorption

Microbiological Instability

Analytical Development

- No individual analytical method can be viewed as "stability indicating" for all proteins
- Each method is to be evaluated for its stability indicating nature
- The stability of proteins must be assessed through a combination of analytical methods

Chromatographic Methods for Protein Stability Evaluation

Method	Variations that can be highlighted
SEC	Fragmentation, aggregation, oligomerization
IEC	Deamidation
RP	Oxidation (cysteine or methionine), disulfide exchange, racemization

Denaturating Conditions

- Heat
- pH variations
- Organic solvents
- High salt concentration
- **Detergents**
- Mechanic stress
- **Lyophilization**

- These are factors that can interfere with the interactions that stabilize the native structure
  - Hydrogen bonds
  - Hydrophobic interactions
  - Salt bridges

Aggregation

Lyophilization can induce aggregation and aggregation is a common occurrence during storage

Formulation	% Aggregates (SEC)		
	Solution to be freeze-dried	Freeze-dried Time zero	Freeze-dried After 3 months at 40°C
5 mM Succinate, pH 5	0.2%	1.4%	~ 11%
5 mM Succinate, pH 5 + 60 mM Trehalose	0%	0%	< 2%
5 mM Histidine, pH 6	0.4%	1.1%	~ 15%
5 mM Histidine, pH 6 + 60 mM Sucrose	0%	0%	< 2%

- rhMAb 25 mg/mL 5; Tween 20 (similar residual moisture for all freeze-dried products)

J. Pharm. Sci., 90, 310 (2001)

Formulation of Proteins

- Complex and exciting challenge
  - Structural complexity
  - Multiplicity of degradation pathways
  - Conformational instability
  - Degradation/Denaturazion not a simple single-step reaction
  - Arrhenius approach questionable
  - Need to use several analytical techniques

⇒ **Specific approaches** (different from those commonly used for "small and rigid" organic molecules)

Formulation of Proteins

- Several products are supplied as freeze-dried forms
  - “Without lyophilization, nearly 60% of biopharmaceuticals including plasma, vaccines and antibodies could not be commercially available. With a greater trend to outsource manufacturing and more biologicals requiring freeze-drying, this market is set to maintain its year-on-year double digit growth”.

    John Shah, 3rd Annual Lyophilization, Boston, 2010

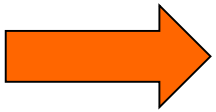
Liquid Versus Lyophilized Formulations

- **Liquid Formulations**
  - **Pros**
    - Easy to manufacture
    - Less expensive
    - Convenient administration
  - **Cons**
    - Less stable
    - Concentration limitation

- **Lyo Formulations**
  - **Pros**
    - Can be used as a concentration step
    - Slower degradation rates
    - Longer shelf life
    - Short-term temperature excursions and mechanical stresses (e.g., during shipping) usually not problematic
  - **Cons**
    - More expensive
    - Less convenient administration
    - Several “stresses”, possibly leading to denaturation

Protein Stability Improvement

- Proteins are highly unstable in aqueous media



REMOVAL OF WATER

BUT …

- Removal of water **DOES NOT** mean absolute stabilization!!!
- Processes to remove water themselves introduce factors of instability

Destabilizing Factors: An Overview

- **Freezing Step**
  - Low temperatures
  - Increased protein and solute concentration
  - pH shifts
  - Ice/water interface

- **Drying Step**
  - Water removal (dehydration)
  - Excessive mobility (i.e., *reactivity*)

- **Storage**
  - Water content
  - Excessive mobility (i.e., *reactivity*)

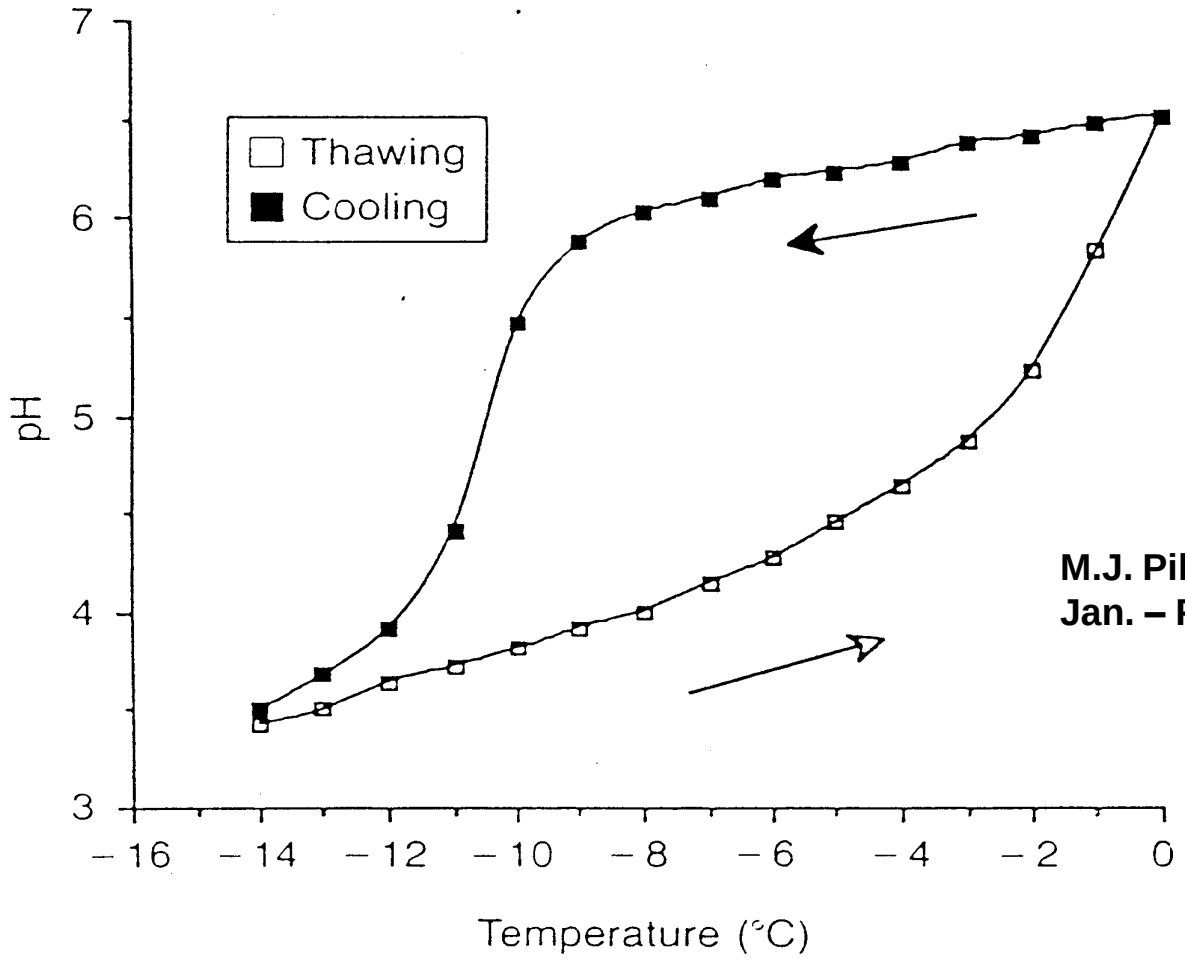
PERJETA Prescribing Information

- It is a HER2/neu receptor antagonist indicated for treatment of patients with HER2-positive metastatic breast cancer (MBC) (in combination with trastuzumab and docetaxel).
- Supplied as a sterile, clear to slightly opalescent, colorless to pale brown liquid for intravenous 456 infusion.
- Each single use vial contains 420 mg of pertuzumab at a concentration of 30 mg/mL in 457 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20.
- Preparation for Administration
  - Withdraw the appropriate volume of PERJETA solution from the vial(s).
  - Dilute into a 250 mL 0.9% sodium chloride **PVC or non-PVC polyolefin** infusion bag.
  - **Mix diluted solution by gentle inversion. Do not shake**.
  - Dilute with 0.9% Sodium Chloride injection only. **Do not use dextrose** (5%) solution.

KEYTRUDA Prescribing Information

- It is a human programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of patients with unresectable or metastatic melanoma.
- Supplied as 50 mg lyophilized powder in a single-use vial for reconstitution.
- Each 2 mL of reconstituted solution contains 50 mg of pembrolizumab and is formulated in L-histidine (3.1 mg), polysorbate-80 (0.4 mg), sucrose (140 mg). May contain hydrochloric acid/sodium hydroxide to adjust pH to 5.5.
- Preparation and Administration
  - Add 2.3 mL of Sterile Water for Injection, USP by injecting the water **along the walls of the vial and not directly on the lyophilized powder** (resulting concentration 25 mg/mL).
  - **Slowly swirl the vial**. Allow up to 5 minutes for the bubbles to clear. **Do not shake the vial.**

Effect of Freezing on the pH of a Citric Acid-Disodium Phosphate Buffer System



M.J. Pikal, Pharm. Tech. Int., Jan. – Feb. 1991

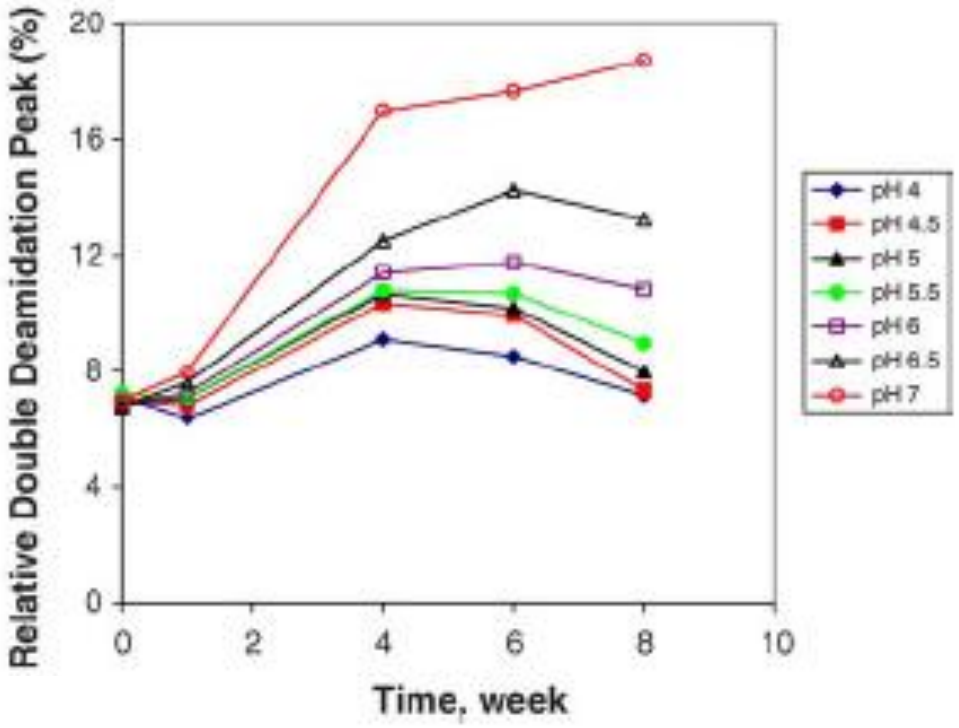
Buffer System

- Complex physico-chemical behavior: can be amorphous, partially amorphous or crystalline depending on formulation and process conditions
- Pay attention to freezing-induced pH shifts!

- Phosphate buffer: selective crystallization of the less soluble component [$Na_2HPO_4 \cdot H_2O$ (T_e – 9.7°C)], NaH_2PO_4 remains in solution, but pH decreases
- Na and K salts behave different
- Tartrates and Succinates similar to phosphates
- TRIS, His, Citrate can be good options

Buffer	Initial pH	pH after freezing	△ pH
Sodium phosphate 100 mM	7.5	4.1	- 3.4
Sodium phosphate 8 mM	7.5	5.1	- 2.4
Potassium phosphate 100 mM	7.0	8.7	+ 1.7
Potassium phosphate 100 mM	5.5	8.6	+ 3.1
Potassium phosphate 10 mM	5.5	6.6	+ 1.1

Influence of pH on Deamidation



- Fully human MAb very prone to deamidation
- "Double" heavy chain deamidation in solution (0.5 mg/mL in 10 mM Citrate buffer at 25°C)

The higher the pH (from 4 to 7), the higher the deamidation

Int. J. Pharm., 308 (2006) 46

Influence of pH and Buffer Species on Deamidation

pH	Buffer	"Double" deamidation rate constant at 25°C
4.0	Citrate	0.56
4.5	Citrate	0.86
5.0	Citrate	1.16
6.0	Citrate	2.61
6.5	Citrate	4.07
7.0	Citrate	6.90
4.0	Tartrate	0.60
4.5	Tartrate	1.24
5.0	Succinate	1.33
6.0	Succinate	2.79
6.5	Phosphate	7.80
7.0	Phosphate	9.26

- In Tartrate or Succinate buffers, the deamidation rates were similar to those in Citrate buffers
- However, in Phosphate buffer at pH 6.5 and 7.0 the deamidation rates were significantly higher

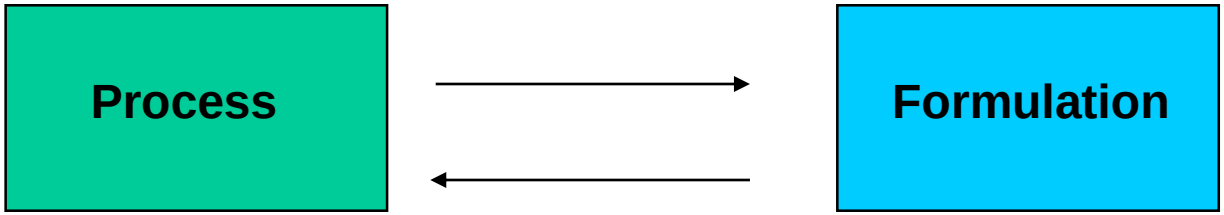
Cryo- Vs. Lyo-Protectants

- **Cryoprotectant**
  - Compounds that stabilize proteins in solution and also protect them from denaturation during freezing and freeze-thawing
- **Lyoprotectant**
  - Compounds that stabilize proteins during lyophilization and subsequent storage

Mechanisms of Stabilization

- **Thermodynamic Stabilization**
  - During freezing:
    - Addition of cryoprotectants: the "**preferentially excluded solute**" mechanism
    - Optimization of freezing rate in order to avoid the formation of a large ice surface area
    - Addition of **surface active agents** to reduce aggregation at the ice/protein interface
  - During freeze-drying and subsequent storage
    - Addition of a lyoprotectant: the "**water replacement**" hypothesis
- **Kinetic Stabilization**
  - The amorphous phase: the "**vitrification**" hypothesis

Formulation and Process



- **Formulation Determines Process**
  - T_g' and Collapse
    - Low T_g' means low temperature and long process
  - Product Resistance to mass transfer
    - High solids content means long process
- **Process may Determine Formulation Properties (i.e., T_g' and T_g)**
  - Crystallization may depend on freezing process
    - Incomplete crystallization of bulking agent and/or salts depress T_g'

“Glass Dynamics” and Stability

MAb/Sucrose	% Aggregates (SEC) after 2 months			H_2O	T_g
	5°C	40°C	60°C		
5 mg/Sucrose	0.4	0.5	6.0	1.6 %	60°C
5 mg/Trehalose	0.7	0.6	1.1	1.7 %	81°C
50 mg/Sucrose	0.6	1.0	2.2	1.3%	89°C
50 mg/Trehalose	0.8	1.1	2.3	1.4%	100°C

- Over a period of 2 months, the "low strength" (LS) Sucrose formulation is as stable as the LS Trehalose formulation at 40°C. However, at 60°C, the LS Sucrose formulation was less stable than the LS Trehalose formulation.
- If stored at 60°C, the LS Sucrose formulation is near its T_g, while the LS Trehalose formulation is ~ 20°C below its T_g. The enhanced mobility in the Sucrose formulation near its T_g may explain the aggregation at 60°C.
- If the aggregation is not related to the T_g of the formulation, the aggregation in Sucrose formulation at 60°C would be expected to increase with increasing MAb concentration. Instead, the High Strength (HS) Sucrose formulation is more stable than the LS formulation and as stable as the HS Trehalose formulation (both formulations are below their T_g!)
- The selection for the LS Mab formulation should be based on the stability data generated at 40°C, but not on data generated at 60°C!

Pharm. Res., 14 (1997) 591

Excipients and Primary Drying

- During the sublimation phase the product temperature should not exceed the collapse temperature (T_c), otherwise collapse of the freeze-dried cake may occur
- The water content of the remaining plasticizing water in the amorphous phase at its T_g' is the water content which cannot be removed by sublimation (rather by diffusion): it is referred to as W_g'

Excipients and Primary Drying

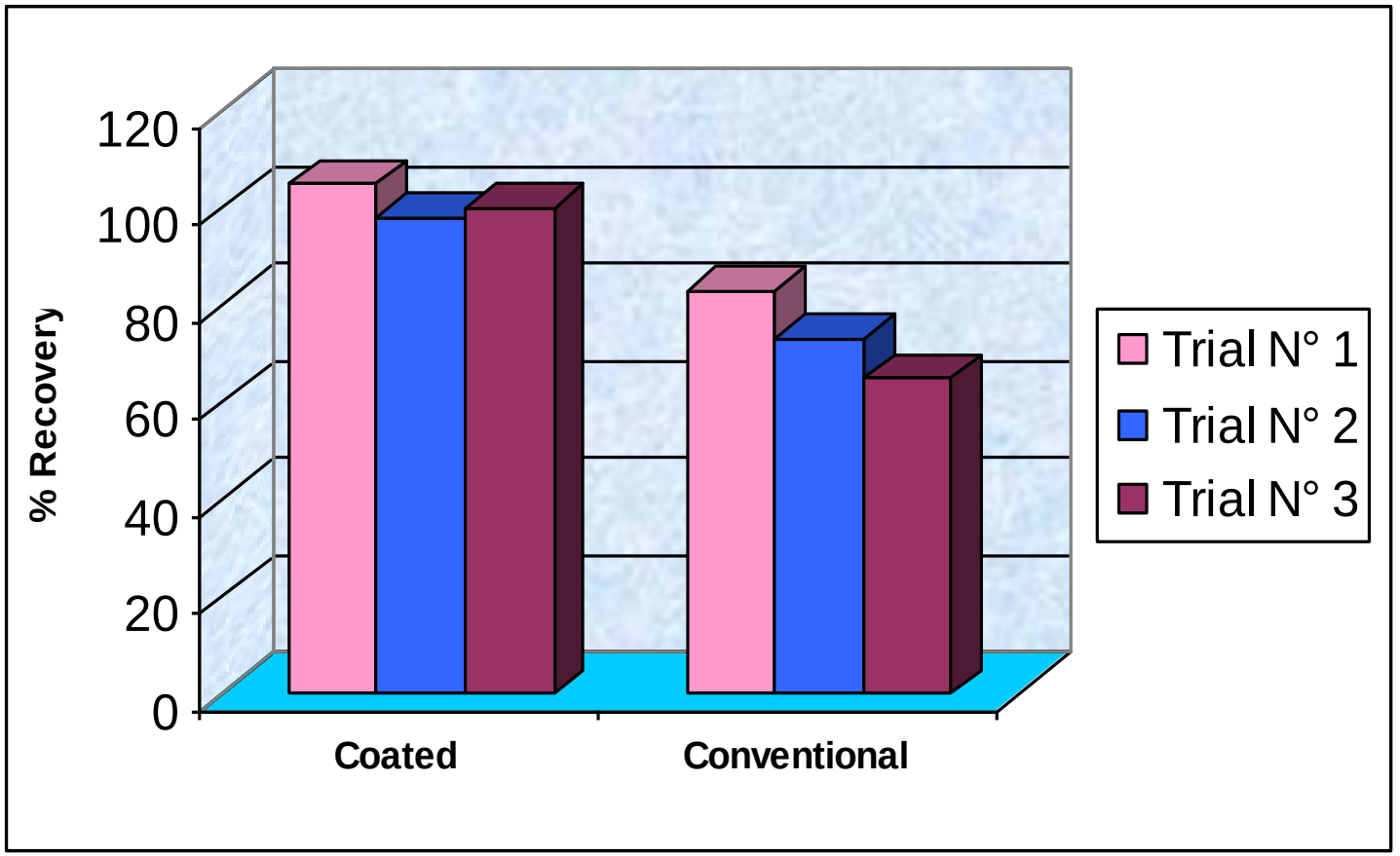
Compound	T_g' (°C)	W_g' (%)
Sucrose	- 32	35.9
Lactose	- 28	40.8
Trehalose	- 30	16.7
Sorbitol	- 43	18.7
Glucose	- 43	29.1
Glycerol	- 65	---
PEG	- 13	---
Dextran	- 9	---
HPβCD	- 8	---
Na Citrate/Citric Acid	- 40	---
Na_2HPO_4/KH_2PO_4 (1:1)	- 80	---

A.T.P. Skrabanja et al., PDA J. Pharm. Sci. & Technol., 48, 311 (1994)

- The composite **T_g'** of a multicomponent product to be freeze-dried should be **HIGH**, whereas its **W_g'** should be **LOW** (which can be achieved by incorporating a cryoprotectant)
- The shift to higher T_g' and lower W_g' increases the effectiveness (by lowering the probability of "collapse") and efficiency (e.g., lower energy requirements, shorter secondary drying times) of the drying process and the subsequent stability of the freeze-dried product

Protein Recovery After Reconstitution (10 μg/mL):

Teflon-coated Vs. Halo-butyl Rubber Stoppers



Thank you for your attention!

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