1

Objectives

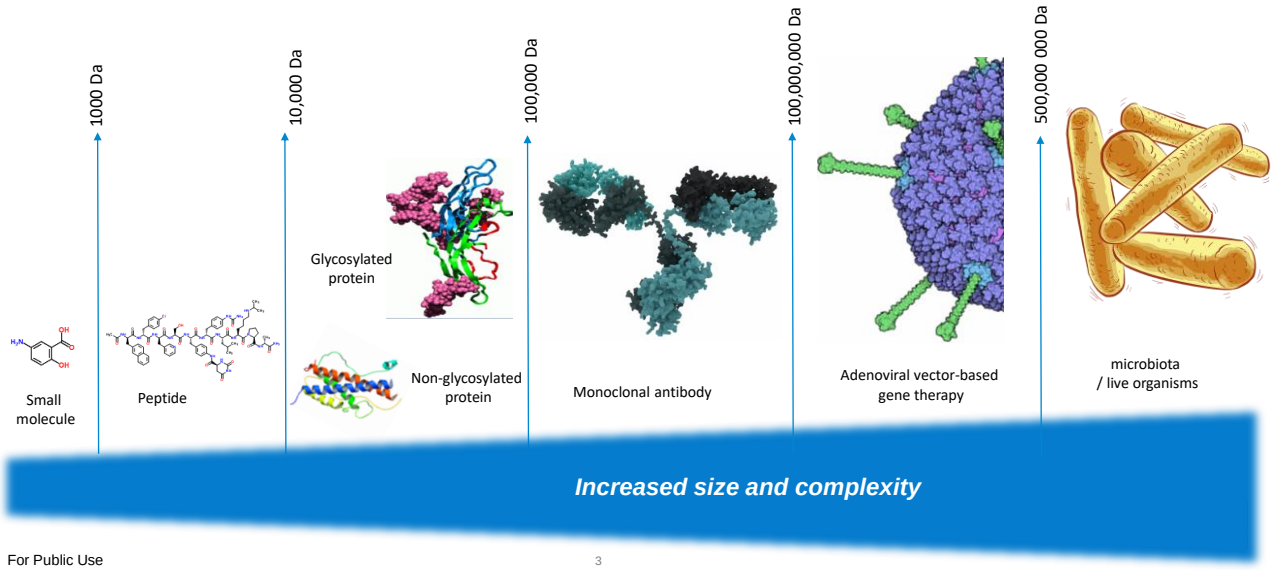
- ✓ Understanding the complexity of protein formulations
- ✓ Requirements for preserved multidose formulations (AET/PET)
- ✓ Extensive stability studies

Outline

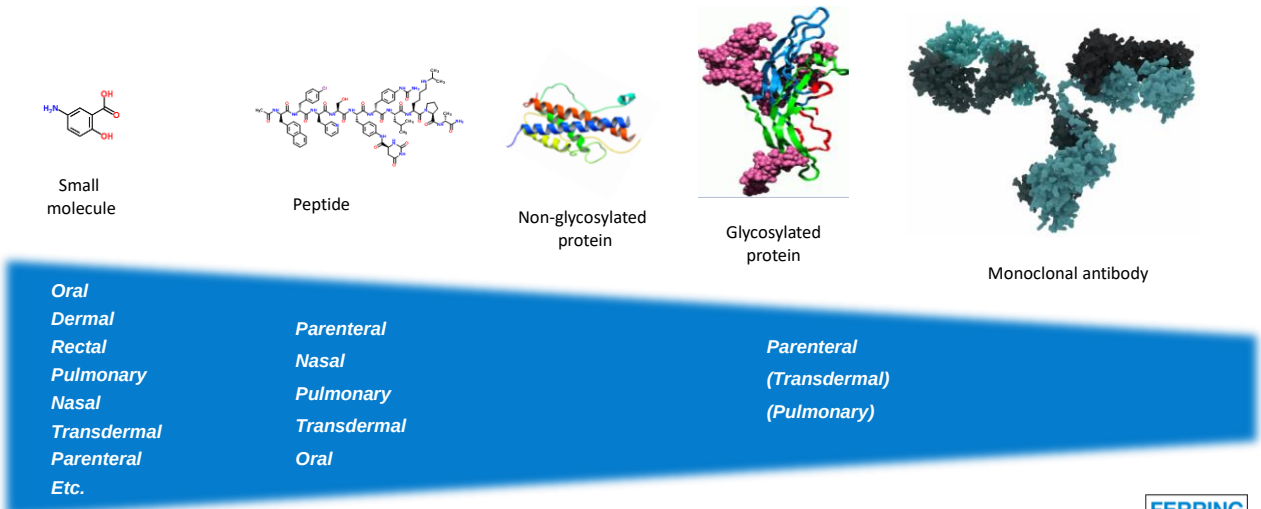
- ❑ *Introduction*
- ❑ *Delivery Routes*
- ❑ *Focus in Development*
- ❑ *Compatibility of excipients*
- ❑ *Preservation Efficacy*

2

Different modalities – different challenges



Delivery routes



Protein delivery routes

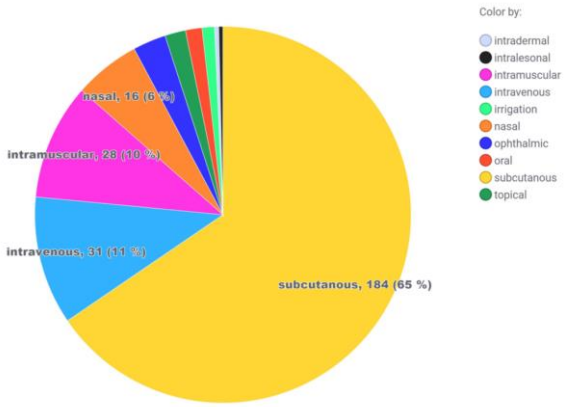
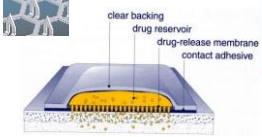


Figure 1. Number and percentage distribution of preservative-containing market products (protein and peptides) in relation to the application route. This figure includes products containing one preservative as well a preservative combinations and products with antibacterial water.

Stroppel, L. et al. *Pharmaceutics* 2023, 15, 563. https://doi.org/10.3390/pharmaceutics15020563.
Review: Antimicrobial Preservatives for Protein and Peptide Formulations: An Overview



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5

Multidose products – a patient friendly product design

Injection pens most common multidose device

- Contain preservatives (several injections from one container)
- Convenient for patients, better dosage management, and minimized product wastage
- Possibility for individualised dosing
- Less cost-intensive production process (fewer units)
- Less packaging waste (both economic advantages and sustainability aspects)
- In a few products preservatives are added as stabiliser (PEGASYS® and REBIF®)**

"When I use the pen, I know I'm getting the dose I dial every time."

Stroppel, L. et al. *Pharmaceutics* 2023, 15, 563.
https://doi.org/10.3390/pharmaceutics15020563

** Gervasi, V. et.al. *Eur J Pharm Biopharm* (2018)

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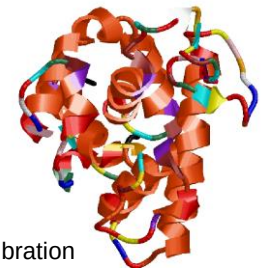
6

Proteins – a big challenge



Very large and unstable molecules

- Structure is held together by weak non-covalent forces
- Easily destroyed by relatively mild storage conditions
- Damage during shipping (e.g. temperature excursion, mechanical shock (dropping), vibration
- Easily destroyed/eliminated by the body
- Hard to obtain in large quantities
- High concentration– risk of aggregation and increased viscosity
- Low concentrations – risk of adsorption to solid-liquid and air-liquid interphases
- Surface-induced denaturation
- Sensitive to stress during manufacturing, shipping and handling (physical and chemical degradation)

Formulation strategies

1. Choice of dosage form (liquid, frozen, freeze-dried, etc)
2. Selection of excipients (including pH)
3. Defining process used to assemble the drug product formulation at the proper protein concentration
4. Choosing the appropriate packaging and storage conditions

STORAGE

- Room temperature ☺
- Refrigerated 😐
- Frozen ☹

Manning et al, Review article, Pharmaceutical Research (2024) 41:1301-1367, Stability of Protein Pharmaceuticals. Recent Advances

Typical content of a multidose protein formulation

Protein (the active ingredient)

Preservative (prevents microbial growth)

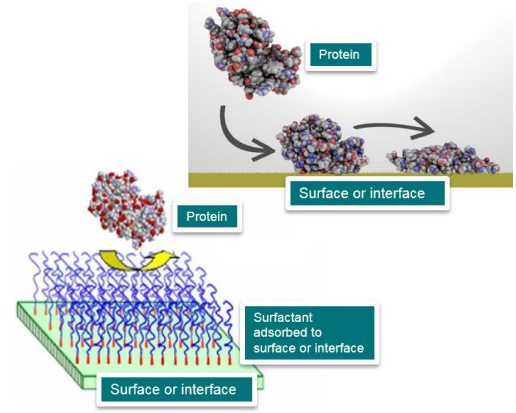
Buffer (secure stable pH)

Surfactant (prevent surface adsorption)

Antioxidant

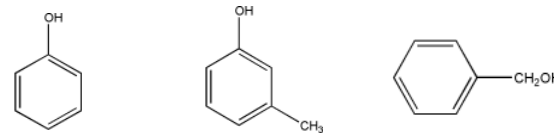
Stabiliser/tonicity agent

Bulking agent, cryoprotectant (freeze dried formulations only)



Challenges with preservatives

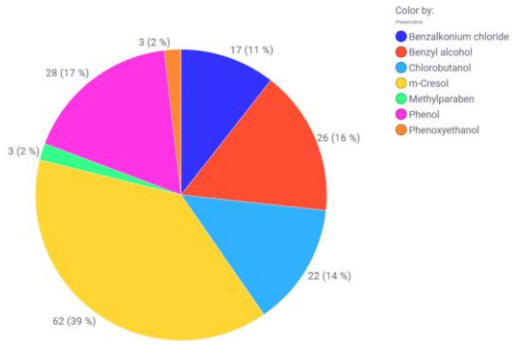
- Few preservatives approved for s.c. delivery
- Affect stability of biologicals
- React or incompatible with other excipients
- Efficacy affected by excipients and drug
- Degradation mechanism poorly understood



Preservative	pH Range	pH Range in Licensed Protein formulations
Phenol	<9	4.2-7.8
Metacresol	<9	3.8-7.8
Benzyl alcohol	<8	6.0-8.0

Stroppel, L. et al. *Pharmaceutics* 2023, 15, 563. https://doi.org/10.3390/pharmaceutics15020563.
Review: Antimicrobial Preservatives for Protein and Peptide Formulations: An Overview

Preservative distribution in peptide/protein DP

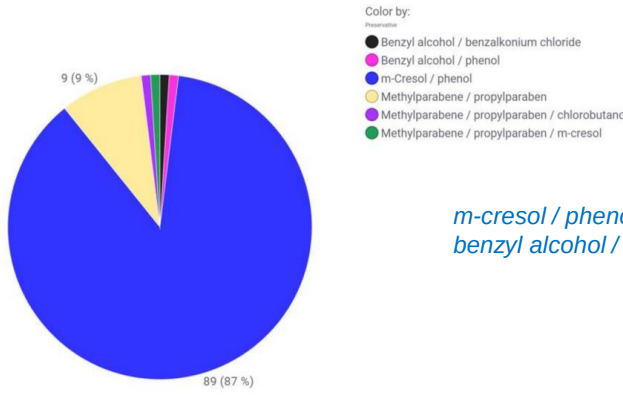


m-cresol *39% (yellow)*
phenol *17% (pink)*
benzyl alcohol *16% (red)*

Stroppel, L. et al. *Pharmaceutics* 2023, 15, 563. https://doi.org/10.3390/pharmaceutics15020563.
Review: Antimicrobial Preservatives for Protein and Peptide Formulations: An Overview

Distribution of preservative combination

In peptide/protein DP



m-cresol / phenol *87% (blue)*
benzyl alcohol / phenol *low % (pink)*

Stroppel, L. et al. *Pharmaceutics* 2023, 15, 563. https://doi.org/10.3390/pharmaceutics15020563.
Review: Antimicrobial Preservatives for Protein and Peptide Formulations: An Overview

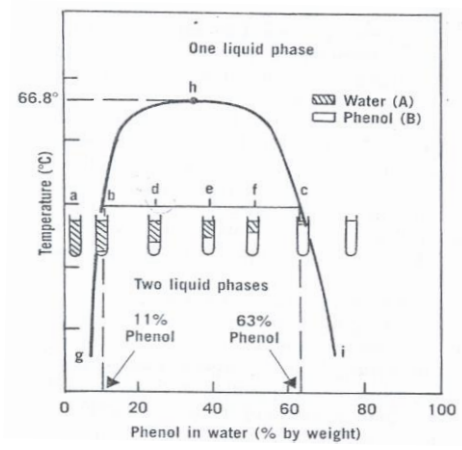
Stability of preserved formulation

1. Minimal effective preservative concentration needed to prevent microbial growth
2. Compatibility of preservative and packaging materials
3. Incompatibilities of preservatives with other excipients
4. Possible adverse effects of preservatives in patient

Manning et al, Review article, Pharmaceutical Research (2024) 41:1301-1367, Stability of Protein Pharmaceuticals. Recent Advances

Phenolic preservatives – solubility gap

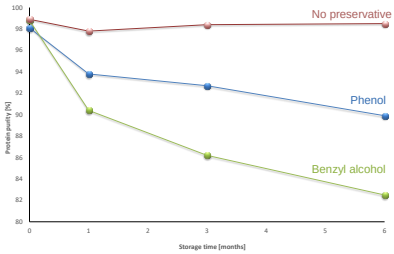
Kinetics important during product manufacturing



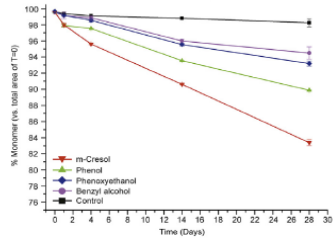
Campbell, A.N. et al, *J.Am.Chem.Soc.* (1937)

Protein stability decreased by preservatives

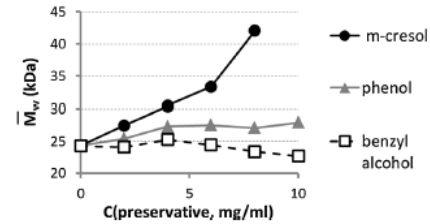
Most proteins destabilised by preservatives



Ferring data, not published

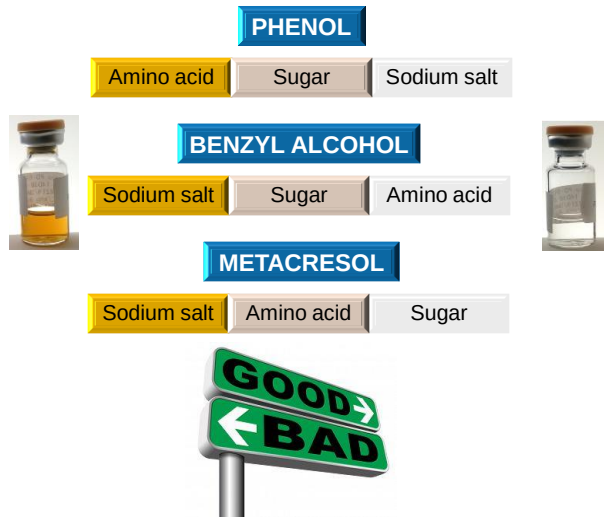


J. Arora et al,
J.Pharm.Sci. **2017**, 1508-1518



P. Heljo et al,
Pharm.Res. **2015**, 3201-3212

Preservative + excipients → unwanted colouration



Preservatives lower surfactant cloud point

Can cause unwanted phase separation

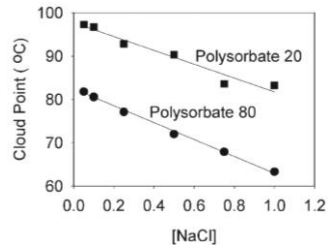
All oxyethylene based surfactants show **temperature dependent solubility**

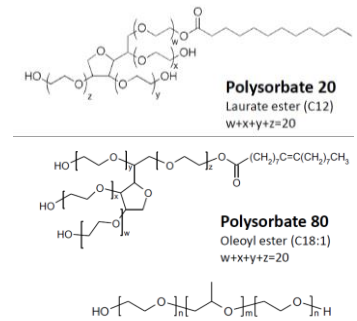
Increasing temperatures give progressive **dehydration of ethylene oxide groups**

Increased temperature → decreased solubility → turbidity (due to phase separation) = **cloud point**

Cloud point **affected by additives**

Addition of **preservatives** give surprisingly large, **reduction of cloud point**

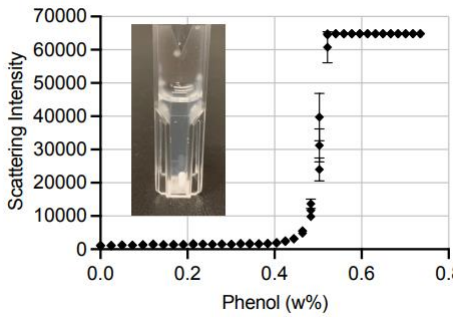


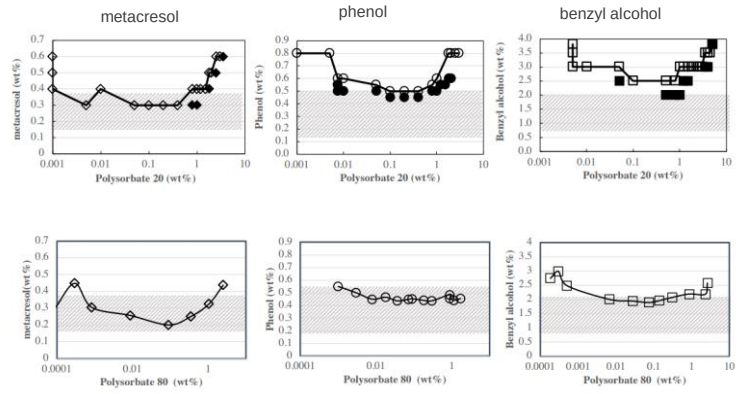


Kerwin, B.A. *J.Pharm.Sci.* (2008)

Cloud point for different preservative-surfactant systems

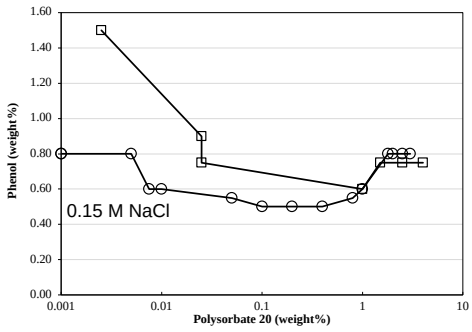
Cloud point determined with light scattering*





Johanna Hjalte, Marie Wahlgren et.al., manuscript

Cloud point depression – synergistic effects



Formulation	Phenol concentration at cloud point
Polysorbate 80	4.9 mg/mL
Polysorbate 80 + 0.15 M NaCl	4.3 mg/mL
Polysorbate 80 + 0.15 M $NaNO_3$	4.6 mg/mL
Polysorbate 80 + 0.15 M Na_2SO_4	4.3 mg/mL
Polysorbate 80 + 0.3 M glucose	4.5 mg/mL
Polysorbate 80 + 0.3 M sucrose	4.4 mg/mL
Polysorbate 80 + 0.3 M mannitol	4.3 mg/mL
Polysorbate 80 + 0.6 M mannitol	3.5 mg/mL
Polysorbate 80 + 0.3 M glucose + 0.3 M mannitol	3.0 mg/mL

Johanna Hjalte, Marie Wahlgren et.al., submitted manuscript

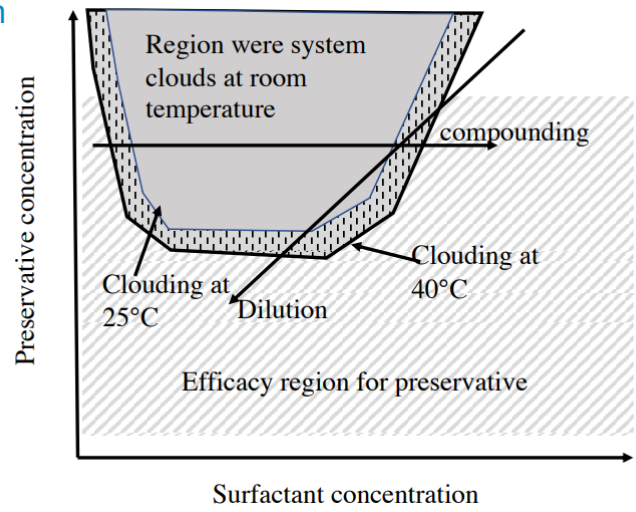
Importance of clouding

Clouding can cause problem

Drug product compounding

Stability studies

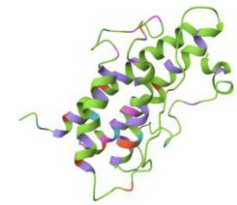
Temperature excursions

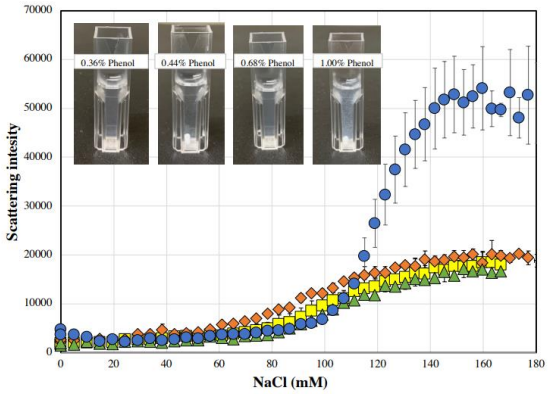


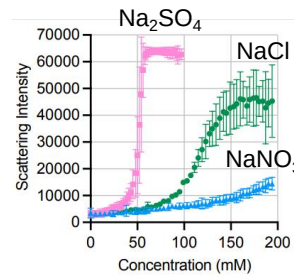
Johanna Hjalte, Marie Wahlgren et.al., submitted manuscript

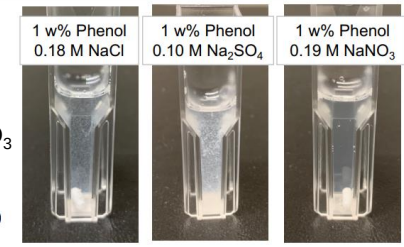
“Clouding” in surfactant free formulations

Preservatives can trigger precipitation in protein formulations









Johanna Hjalte, Marie Wahlgren et.al., manuscript

The preservative should protect the product against microbial proliferation without compromising the product performance, meaning:

- Exert a wide spectrum of antimicrobial activity at low inclusion levels.
- Maintain activity throughout product manufacture, shelf life and usage.
- Not compromise the quality or performance of product, pack or delivery system.
- Not adversely affect patient safety or tolerance of the product.

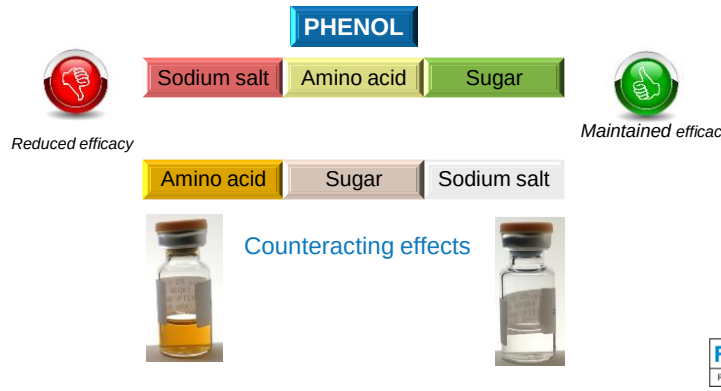
Antimicrobial Preservatives Part One: Choosing a Preservative System, American Pharmaceutical Review, January 2012, David P. Elder and Patrick J. Crowley
https://www.americanpharmaceuticalreview.com/Featured-Articles/38886-Antimicrobial-Preservatives-Part-One-Choosing-a-Preservative-System/

Loss of preservation efficacy

Preservation efficacy reduced by

- pH
- surfactants
- buffers
- stabilisers

Effect varies with preservative

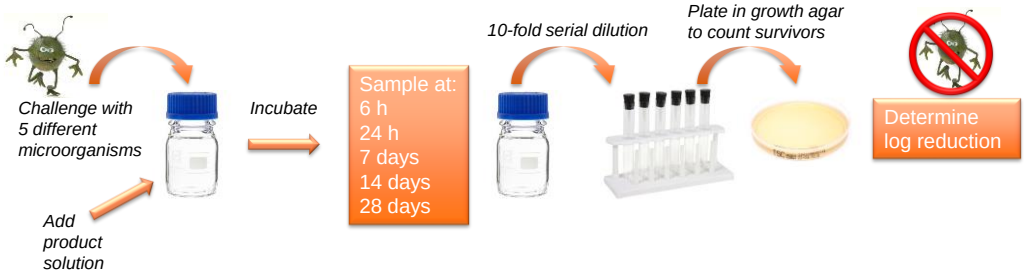


Multidose products must prevent microbial growth

Preservative efficacy test according to pharmacopoeia

"Antimicrobial effectiveness must be demonstrated for all injections packaged in multiple-dose containers." USP <51>

Similar test method and requirements in Ph.Eur., USP, JP and Ch.P.



AET/PET tests for sterile parenteral product

- ❑ EP (5.1.3) Efficacy of antimicrobial preservatives
- ❑ USP <51> Antimicrobial effectiveness testing (category 1)
- ❑ JP <19> Preservative effectiveness testing(category IA)

NR: no recovery
NI: no increase

Test organism	type	6 h	24 h	7 d	14 d	28 d
S. aureus	Gram + coccus	EP A: 2	EP A: 3 EP B: 1	EP B: 3 USP/JP:1.0	USP/JP:3.0	EP A: NR EP B: NI USP/JP: NI
P. aeruginosa	Gram - bacillus	EP A: 2	EP A: 3 EP B: 1	EP B: 3 USP/JP:1.0	USP/JP:3.0	EP A: NR EP B: NI USP/JP: NI
E. coli	Gram - bacillus			USP/JP:1.0	USP/JP:3.0	USP/JP: NI
C. albicans	yeast			EP A: 2 USP/JP: NI	EP B: 1 USP/JP: NI	EP A/B: NI USP/JP: NI
A. brasiliensis	mold			EP A: 2 USP/JP: NI	EP B: 1 USP/JP: NI	EP A/B: NI USP/JP: NI

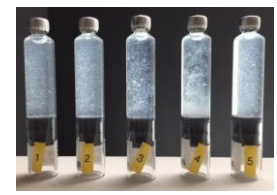
Stability studies for a multidose formulation

Early development

- Formulation screening
- Preservative screening
- Primary packaging materials screening
- Freeze/thaw stability studies
- Antimicrobial effectiveness test

Late development

- Long term and accelerated stability studies
- Transportation study
- Photostability studies
- In-use stability studies
- Antimicrobial effectiveness test
- Leachable studies
- Shipping study

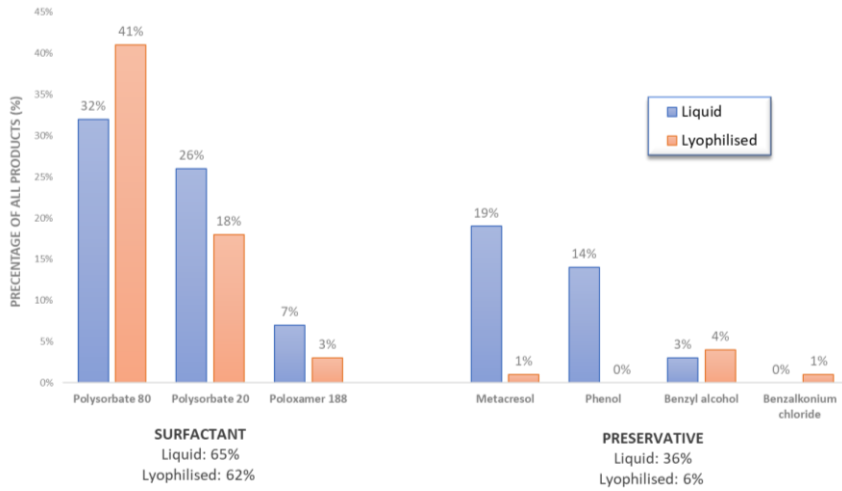


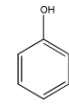
Do not freeze!

Protect from light

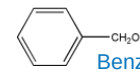
Parenteral protein products approved in EU 1995-2018

Products approved in EU from 1995*

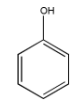




Metacresol, 1.5-3.2 mg/mL



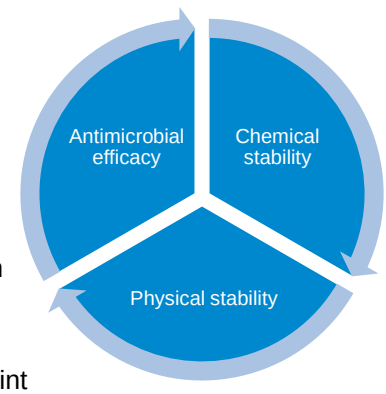
Benzyl alcohol, 9-10 mg/mL



Phenol, 0.8-5.7 mg/mL

*Gervasi, V. et.al. *Eur J Pharm Biopharm* (2018)

Conclusions



- 65% of all liquid parenteral protein products contain surfactant
- 36% of all liquid parenteral protein products contain preservative
- Multidose products must be preserved to prevent microbial growth
- Preservatives often destabilise proteins
- Presence of preservative can drastically lower surfactant cloud point
- Cloud point lowering effects are synergistic (e.g. salts and sugars further lower cloud point)
- Protein can precipitate in presence of preservatives, especially in presence of salt

Acknowledgement

NextBioForm

Centre for formulation and development of next generation biologics

6-year competence centre funded by Sweden's innovation agency (Vinnova) and Swedish Research Council (Vetenskapsrådet)

Focus on proteins and live biotherapeutic products

https://www.ri.se/en/nextbioform

Johanna Hjalte, Lund University

Marie Wahlgren, Lund University

Adrian Sanches-Fernandez, Universidade de Santiago de Compostela

Carl Diehl, SARomics biostructures

Stefan Ulvenlund , ENZA Biotech

Helen Sjögren, Ferring

Academia: RISE, Lund University, Malmö University, Uppsala Academic Hospital, MAX IV

Pharma: SOBI, Ferring Pharmaceuticals, Nanexa, AstraZeneca, BioGaia, Ilya Pharma, Spiber, Regenics

CRO: Vironova, Biogardia, ProPharma, CR competence, ENZA Biotech, SARomics Biostructures, Solve

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