Formulation and Freezing Effects in Lyophilized Viral Vaccines

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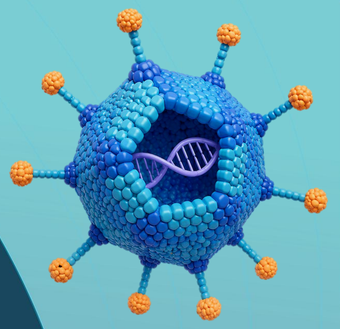


Background

Why lyophilize viral vaccines?

- Improves storage stability
- Reduces cold-chain dependence
- Increases global accessibility

Development challenges!

- Formulation and freezing influence on lyophilizate structure and virus stability remain poorly understood
- Biosafety restrictions complicate solid-state characterization of viral products due to open handling restrictions

HYPOTHESIS: The low mass fraction of the virus has negligible impact on key solid-state cake properties!

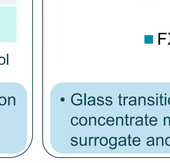
Study Scope?

- **Q1: How do formulation composition and freezing influence cake properties and infectivity?**
- **Q2: Can virus-free formulation surrogates accurately represent solid cake properties of virus containing formulations**

Study Design

Model system

Adeno viral vector:
ChAdOx1 nCoV-19
(AZD1222)
at 25 µg/ml



Formulation

Base: 10 mM TRIS, pH 7.5, 5 mg/mL P188

Formulation	Excipients	Rationale
F1	275 mM Mannitol 55 mM Sucrose	Semi-crystalline
F2	100 mM NaCl 200 mM Sucrose	NaCl as ionic excipient
F3	110 mM NaGlutamate 200 mM Sucrose	Charged Amino acid
F4	300 mM Glycine 150 mM Sucrose	Neutral Amino acid

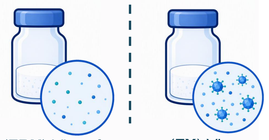
Freezing

Shelf-Ramp (**SR**)
1 ºC/min

Annealing (**A**)
-15 ºC, 3 h

Quench (**Q**)
pre-cooled
shelf at -45 °C

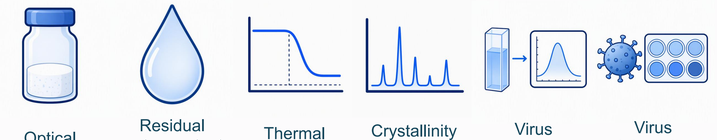
Comparison



(**FBX**) Virus-free "surrogate" Formulations

(**FX**) Virus containing Formulations

Analytical attributes



Optical solid appearance

Residual water content by Karl-Fischer Titration

Thermal events by DSC

Crystallinity by powder XRD

Virus concentration by UV

Virus infectivity by TCID50

Overview of Findings

Attributes	Q1 Effect of Formulation & Freezing	Q2 Surrogate suitability Virus-free vs Virus
Appearance	⚠ Formulation effects F2 displayed collapse	✓
Residual Water	⚠ Formulation effects	✓
$T_{g'}$ (DSC)	⚠ Formulation effects	✓
T_g and T_{eut} (DSC)	⚠ Formulation effects	⚠ Select differences in solid T_g observed
Crystallinity	✗ Clear effects of Formulation and Freezing	✓
Virus Concentration	✓	✗ Requires virus containing sample
Virus Infectivity	✗ Clear effects of Formulation and Freezing	✗ Requires virus containing sample

✓ No Form & Freezing effect Surrogate Suitable

⚠ Form OR Freezing effect Limitations to Surrogate

✗ Form AND Freezing effect Not a Surrogate

Appearance

Form.	(FX) Virus Containing	(FBX) Surrogate
F1_SR		
F1_Q		
F1_A		
F2_SR		
F3_SR		
F4_SR		

- Optical appearance is governed by the formulation and minorly by freezing.
- No impact of presence of virus.

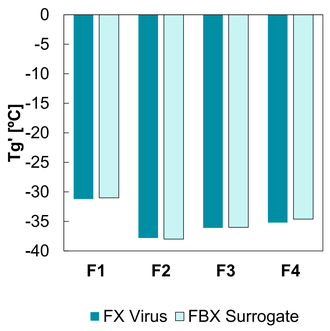
Solid-state cake properties

Form.	Residual water (%w/w)	T_g (ºC)	T_{eut} (ºC)
F1_SR	0.7	n.d.	52.7*; 157.5#
FB1_SR	0.8	n.d.	52.6*; 157.8#
F2_SR	1.4	**43.3**	n.d.
FB2_SR	1.1	**51.3**	n.d.
F3_SR	1.3	**67.9**	52.6*
FB3_SR	1.5	**69.7**	51.5*
F4_SR	0.6	n.d.	51.4*
FB4_SR	0.6	**57.5**	52.1*

n.d. not determined; * eutectic melt P188; # eutectic melt Mannitol

- Solid-state cake properties are governed by the formulation
- Minor negative impact of Virus on glass transition temp.
- No virus effect on residual water and eutectic temp.

Freeze-concentrate



- Glass transition temperature of freeze-concentrate matches very well between surrogate and virus containing formulation.

Mannitol Crystallinity

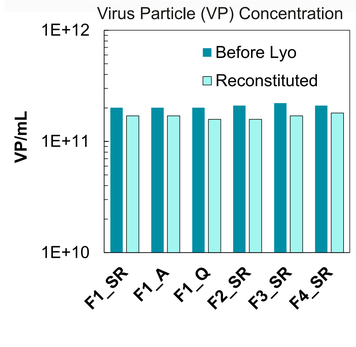
Form.	Hydrate	Delta	Beta	Alpha	Amorph
F1_SR					57.7 %
FB1_SR					45.0 %
F1_Q					62.9 %
FB1_Q					59.0 %
F1_A	14.8 %	85.2 %			31.4 %
FB1_A	18.5 %	81.5 %			32.7 %

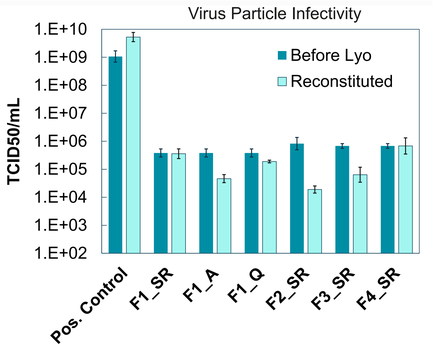
Polymorph status:

Absent	Suspected	Trace	Minor	Major

- Mannitol crystallization is dictated by both formulation and freezing effects.
- Surrogate formulations captures same trends as virus.

Virus Concentration & Infectivity





- A reduction in virus concentration is observed after lyophilization. No formulation or freezing effects.
- Infectivity is generally preserved but strongly depends on formulation and freezing protocols. Formulations F1 and F4 upon shelf freezing most successful.
- Annealing seems to greatly reduce the virus infectivity.

TAKE AWAY Q1: Formulation composition strongly affected cake structure, resulting in amorphous and partially crystalline cakes.

- Freezing protocol influenced solid-state properties, particularly mannitol crystallization and hemihydrate formation in mannitol-containing formulations.
- Virus infectivity after lyophilization depended on both formulation and freezing conditions, with shelf-ramped freezing generally outperforming annealing.
- Formulations F1 (containing Mannitol & Sucrose) and F4 (containing Glycine & Sucrose) after shelf-ramp freezing showing the best overall quality.

TAKE AWAY Q2: Virus-free surrogates (FBX) closely matched virus-containing (FX) formulations in cake appearance, residual moisture, and crystallinity.

- No meaningful effect of the virus was observed on freeze concentrate (Tg'), residual water results, or powder XRD-based solid-state characterization.
- Surrogates cannot replace measurements of virus-specific attributes such as infectivity and may not fully capture all thermal behavior.
- Virus-free formulations can therefore serve as practical surrogates for selected physicochemical characterization, reducing biosafety-related analytical complexity.

Arsiccio et al. Lyophilization of a viral vaccine: Strategies for surrogate use in analytical characterization of cake properties and considerations on virus infectivity
J Pharm Sci. 2026 Aug; 115(8): 104336. doi: 10.1016/j.xphs.2026.104336.

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