Thermostabilization of a Model Viral-Vectored Oral Thin Film Vaccine using DoE Approach

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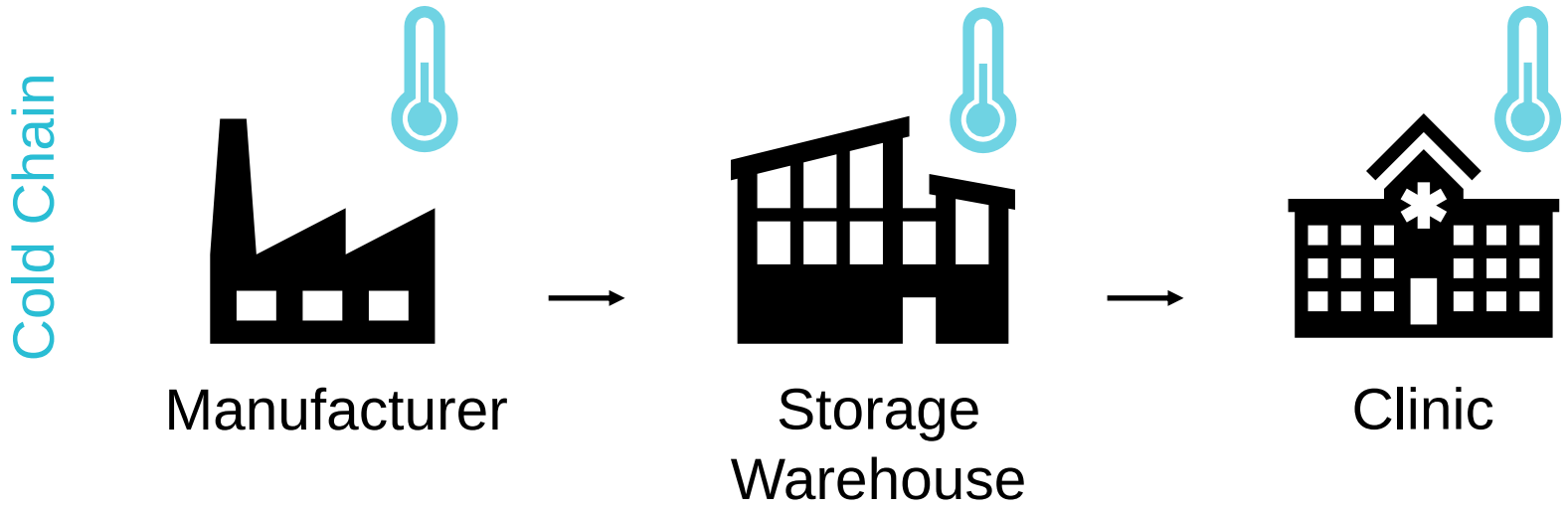
July 2025

CRS 2025 | Skin and Mucosal Delivery

Vaccines Require Consistent Cold Storage Infrastructure



50% of vaccines discarded annually[1]

Reduced accessibility of vaccines[2,3]

Vaccines can be thermostabilized by drying

Lyophilized vaccines (MMR vaccine): **RT stable for several months**

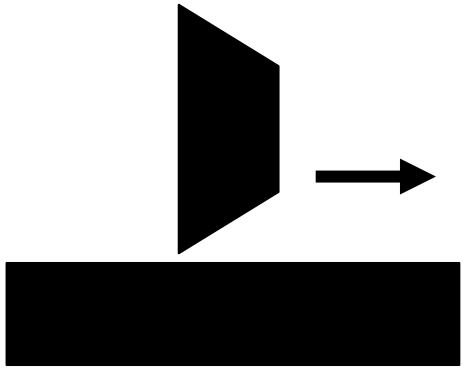
[1]*Adv. Drug Deliver. Rev.* **2021,** 2021.01.016
[2]Kartoglu, Umit. Monitoring Vaccine Wastage at the Country Level: Guidelines for Programme Managers. 1–63 https://iris.who.int/handle/10665/68463 (2005).
[3]*Health Policy.* **2021,** 2021.03.013

Solvent Casting as Preferred Alternative Vaccine Thermostabilization Method

Thermostabilization Methods

Spray-drying

Lyophilization



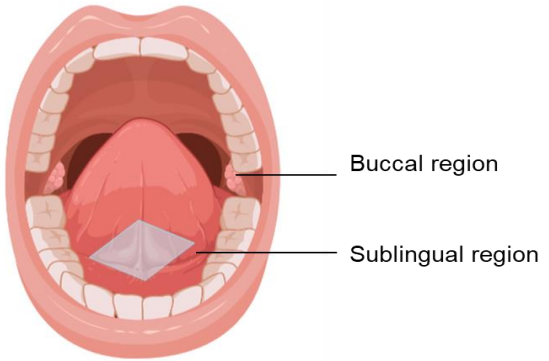
Solvent casting

Commercialized Oral Thin Films (OTFs):

Melatonin

THC

Olanzapine
(Schizophrenia)



<u>Model Vaccine:</u>
Adenovirus
(approved for use in COVID-19 vaccines)

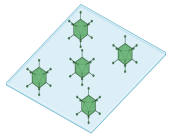
Biorender

Challenge

Existing solvent casting methods rely on an overnight ambient drying step and must be produced in batches.

Objective

Using a 1 h quick-drying step, produce an OTF formulation capable of stabilizing adenovirus-loaded OTFs at ambient temperature

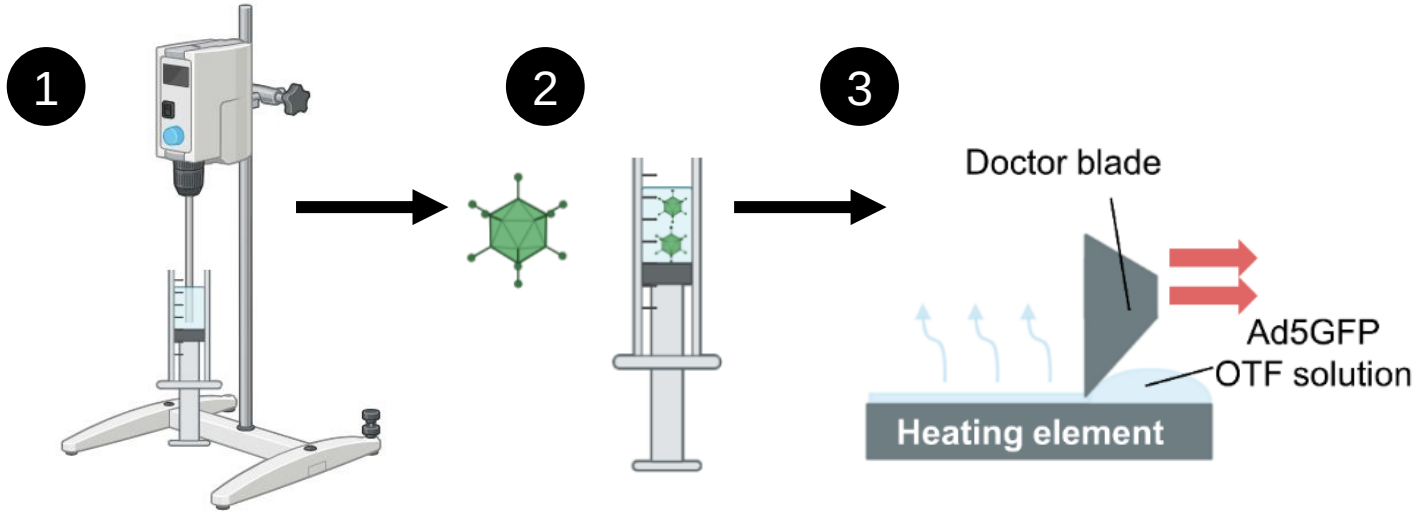


Adenovirus OTF

RT

Preparation of Adenovirus-loaded OTFs

Oral Thin Film (OTF) Formulation



Prepare blank *pullulan-based* OTF stock

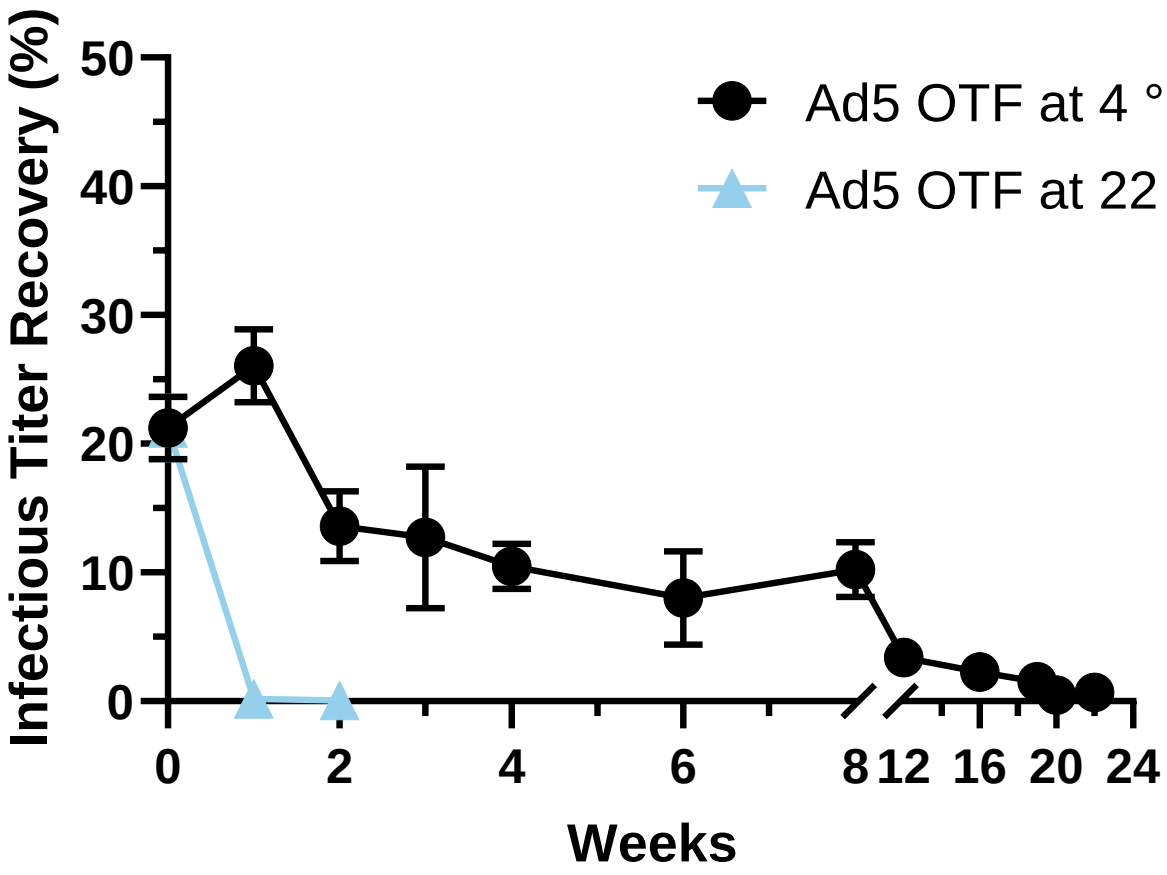
Add Ad5GFP solution

Cast OTFs 37 °C, 1 h

Virus Infectious Titer Recovery

Measurement of fluorescent reporter protein expression, GFP *in vitro* by flow cytometry

Adenovirus can be incorporated into OTF using 37 °C, 1h solvent casting process



Ad5 retained **21 ± 2% infectious titer upon incorporation into an OTF.**

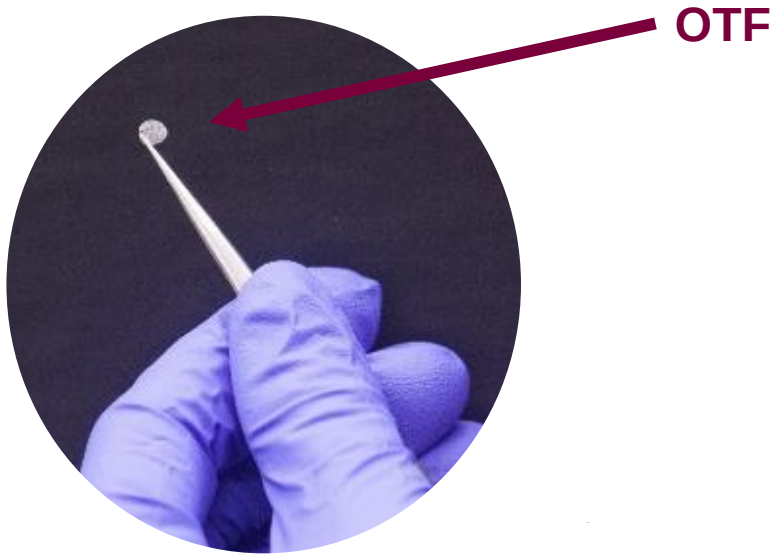
Initial Storage Stability Assessment

4°C

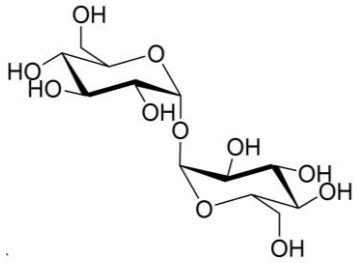
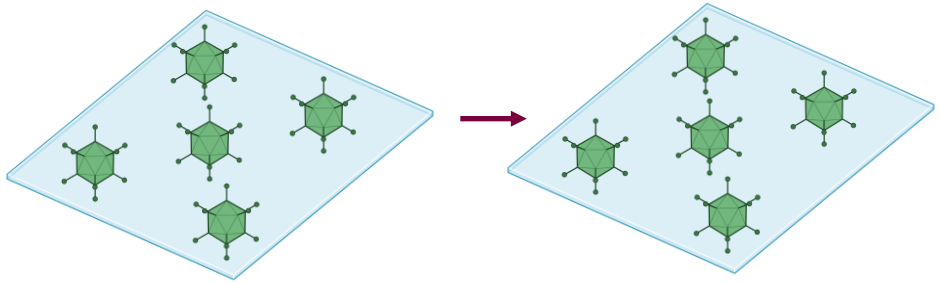
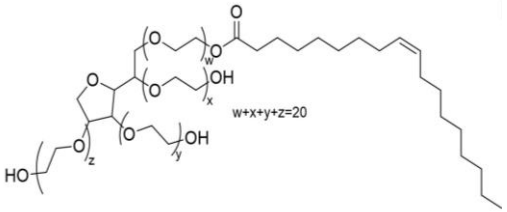
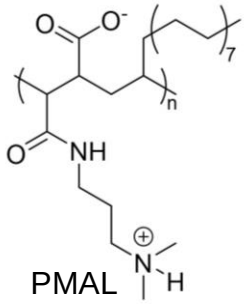
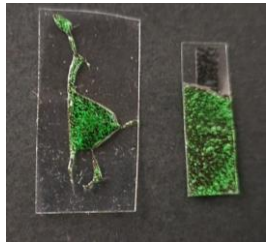
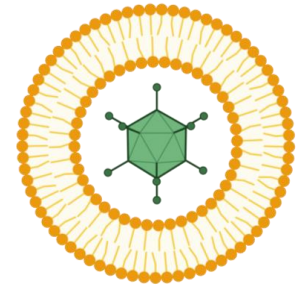
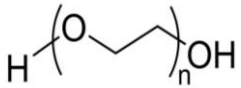
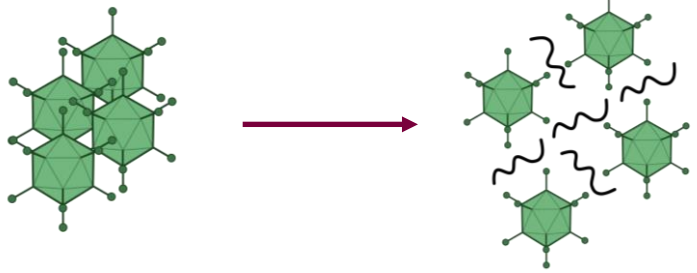
Ad5 OTFs maintained over **50% of original infectious titer at 8 weeks**

22°C

Ad5 OTFs retained only **0.2 ± 0.1% infectious titer for 1 week**

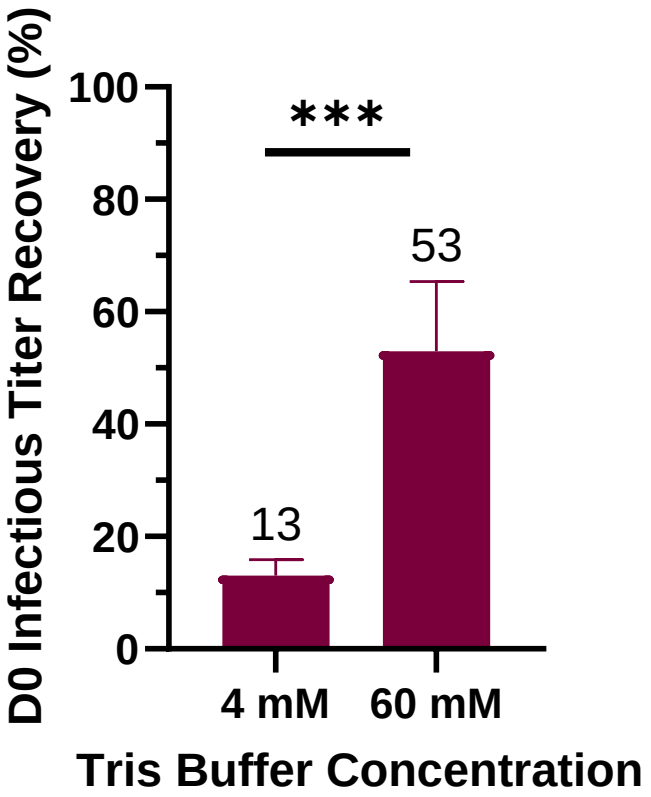


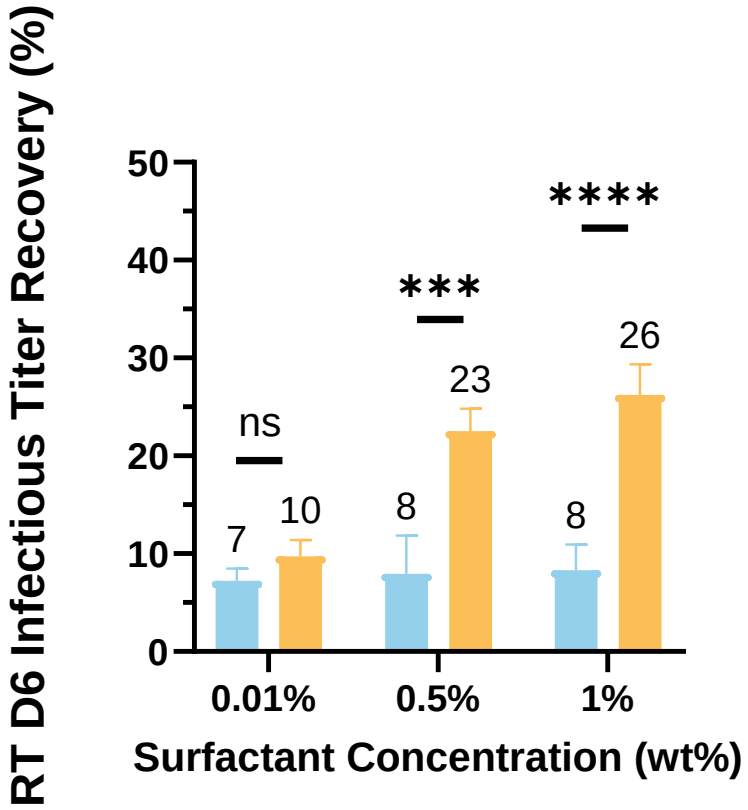
Choice of Oral Thin Film Excipients to Optimize Thermostability

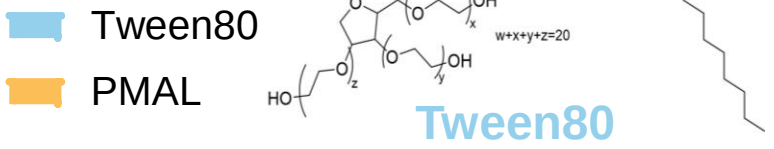
Excipient	Chemical Structure	Stabilizing Mechanism
Trehalose[7]		
Tween80 OR PMAL[8,*] ***Poly (Maleic Anhydride-Alt-1-Octadecene) substituted with 3-(Dimethylamino) Propylamine**	 Tween80  PMAL	 No Tween80 Tween80  PMAL
Poly(ethylene) glycol[9] 8000 Da		

[7]*Sci. Rep.* **2019,** s41598-019-44020-w.
[8]*Sci. Adv*. **2020**, aau4819.
[9]*Nat. Commun.* **2016,** 13520.

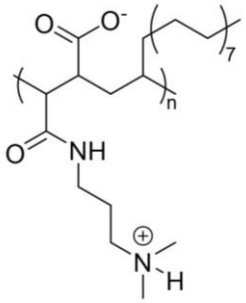
Pre-Screening Studies Identify Buffer and Surfactant Excipients for Thermostability







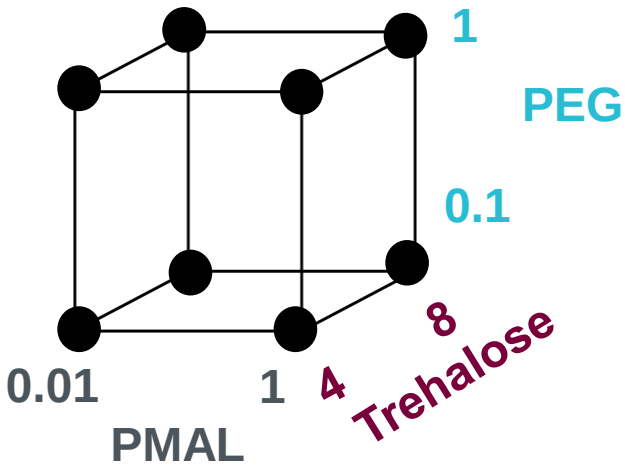
Tween80



PMAL

Design of Experiment: Full Factorial Model Design

Variable	Factor	Levels (Formulation Composition wt%) -	+
A	Trehalose	4	8
B	PMAL	0.01	1.0
C	PEG 8000 Da	0.1	1

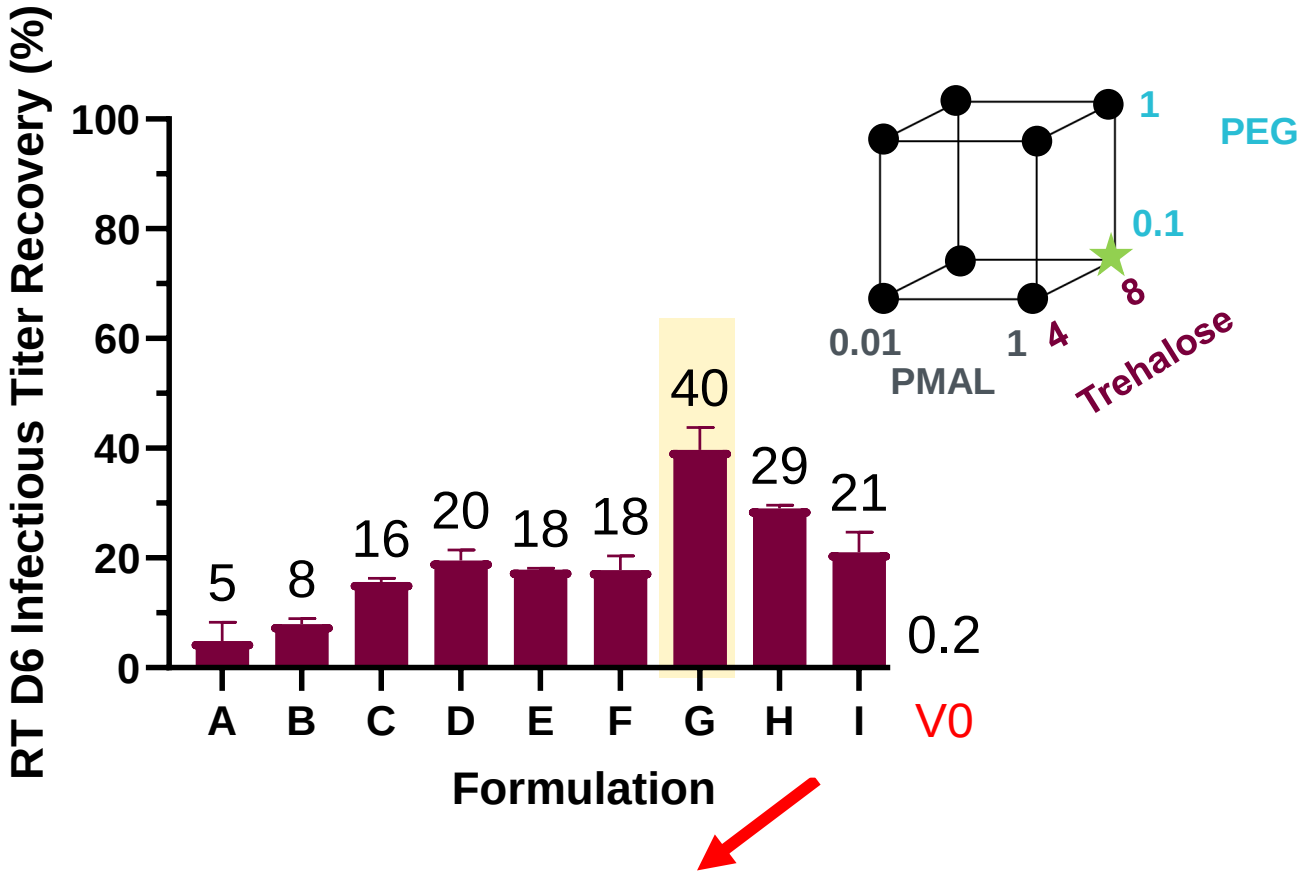


Final Model: $D6\ RT\ Infectious\ Titer\ Recovery$

$$= b_0 + b_1 x_{PMAL} + b_2 x_{trehalose} + b_3 x_{PEG} + b_4 x_{PMAL} x_{trehalose} + b_5 x_{trehalose} x_{PEG} + b_6 x_{PMAL} x_{PEG}$$

2^3 Full Factorial DoE Identifies Optimum Ad5 OTF Formulation for RT Thermostability

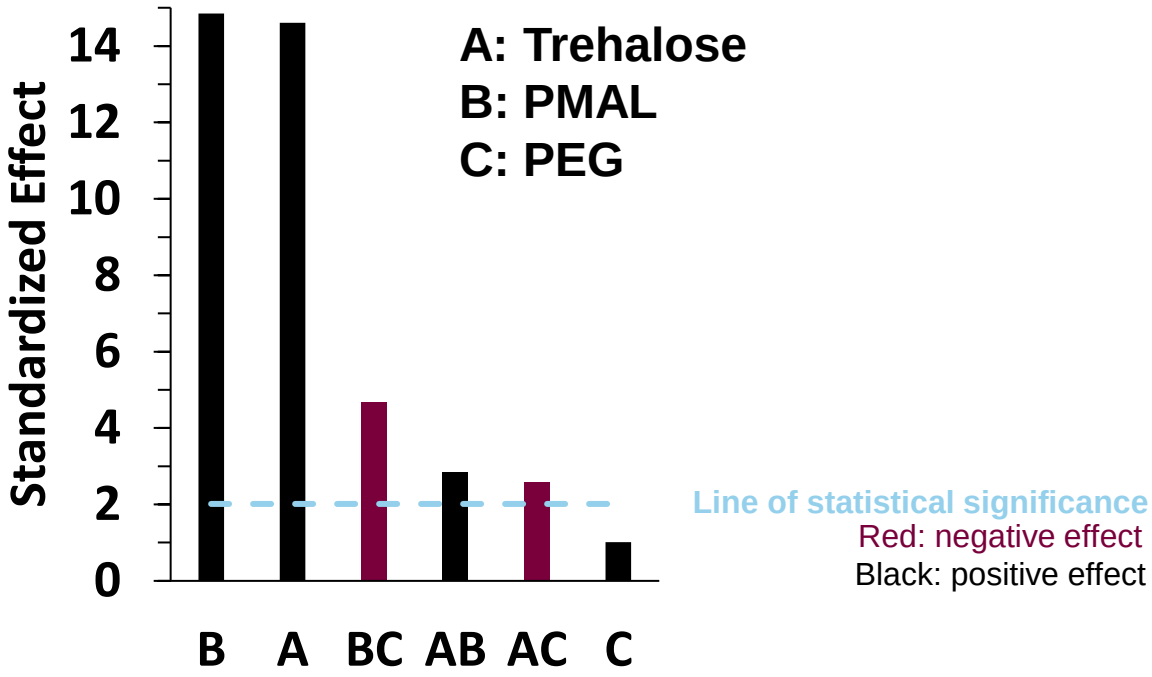
Formulation	PMAL (wt %)	Trehalose (wt %)	PEG (wt %)
A	0.01	4.00	0.10
B	0.01	4.00	1.00
C	0.01	8.00	0.10
D	0.01	8.00	1.00
E	1.00	4.00	0.10
F	1.00	4.00	1.00
G	1.00	8.00	0.10
H	1.00	8.00	1.00
I	0.50	6.00	0.55



200-fold improvement from initial formulation

Ad dose retention is **potent enough for preclinical murine studies (10^7 PFU/OTF disc)**

2^3 Full Factorial DoE Identifies Optimum Ad5 OTF Formulation for RT Thermostability



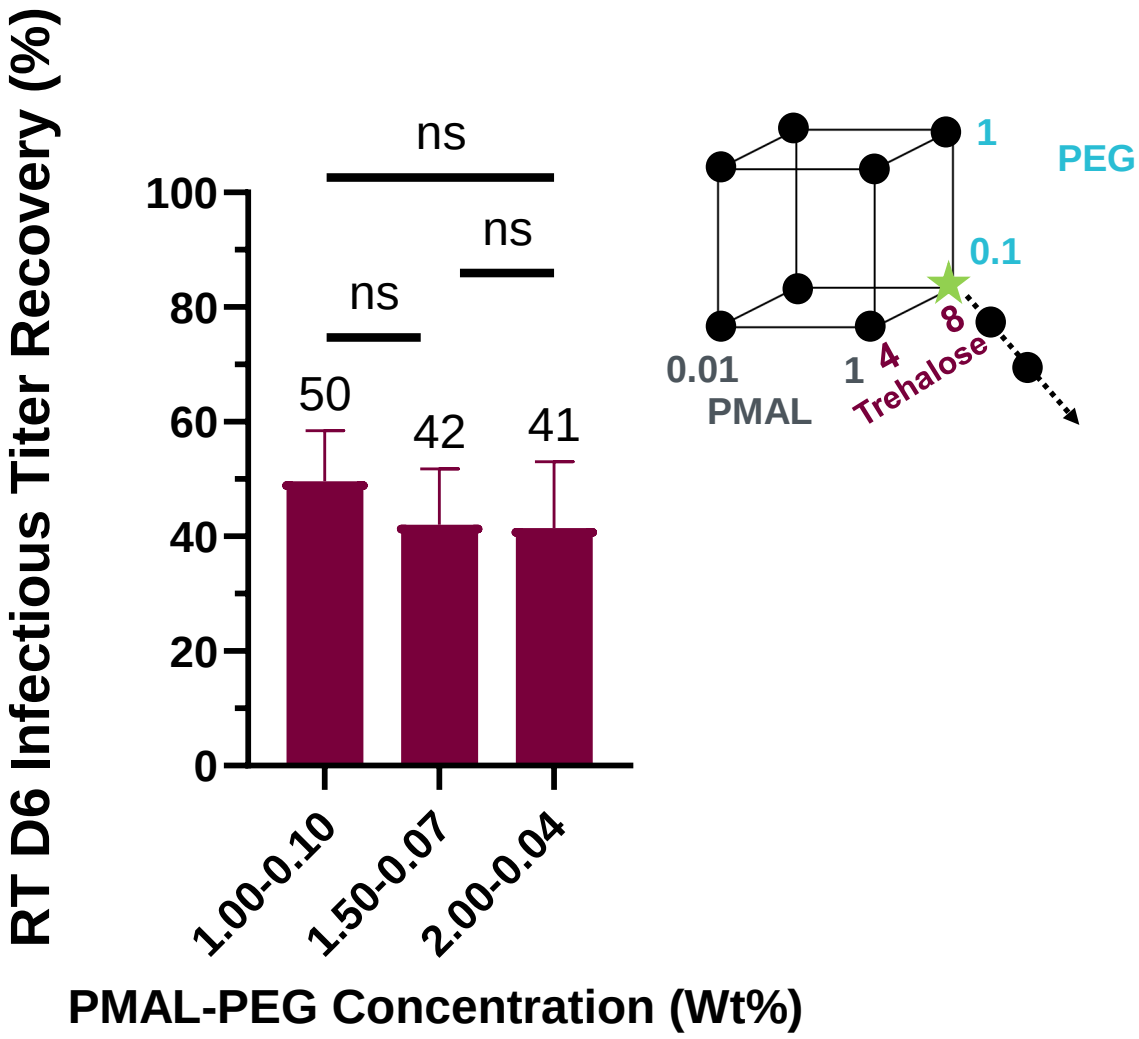
↑ trehalose and ↑ PMAL had significant individual impacts on Ad5 OTF thermostability

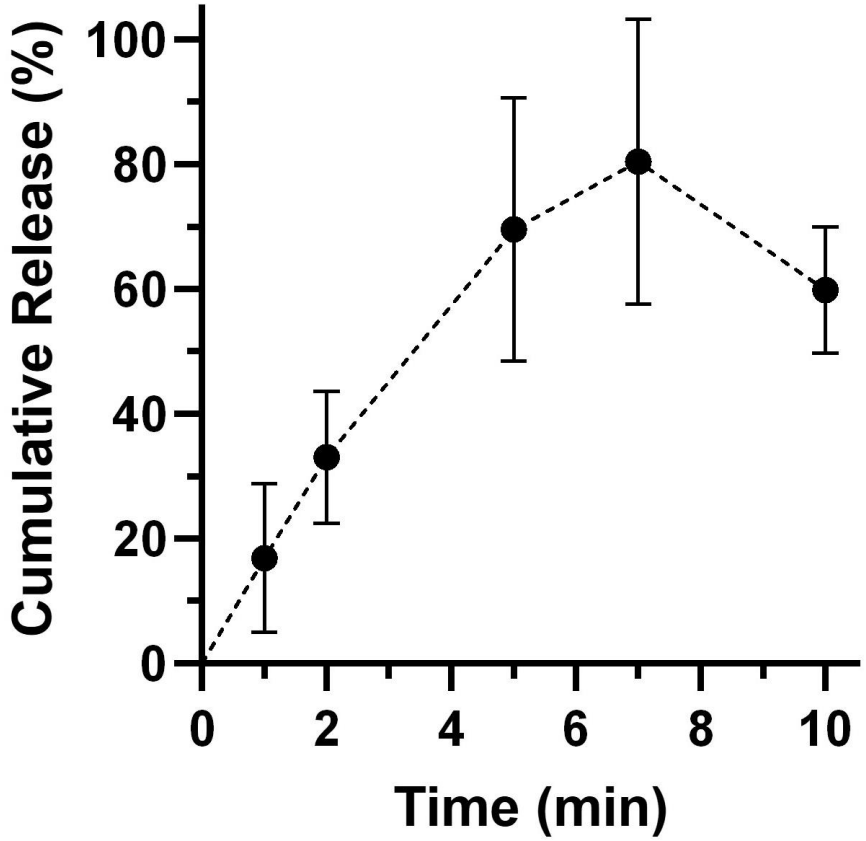
D6 Infectious Titer Recovery

$$= 19.3 + 7x_A + 7x_B - 0.5x_C + 1.4x_Ax_B - 1.2x_Ax_C - 2.2x_Bx_C$$

R^2 (adjusted): 90%
R^2 (predicted): 88%

Expanding the Design Space Confirms Optimum Formulation



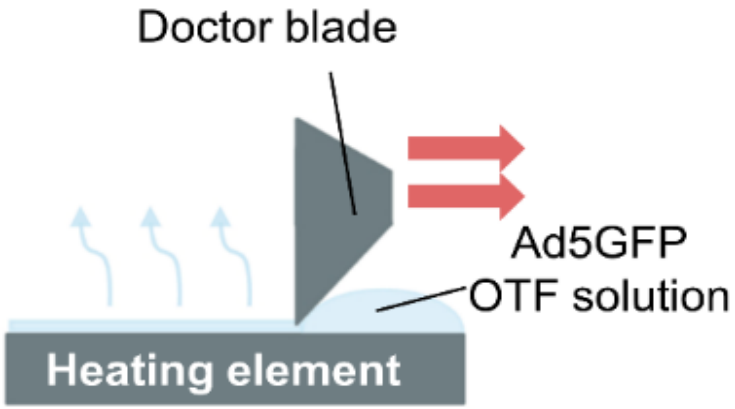


↑ PMAL and ↓ PEG, along direction of steepest ascent, results in no improvement in infectious titer recovery

Ad5 cumulative release peaks at **80 ± 23** % **after 7 min**

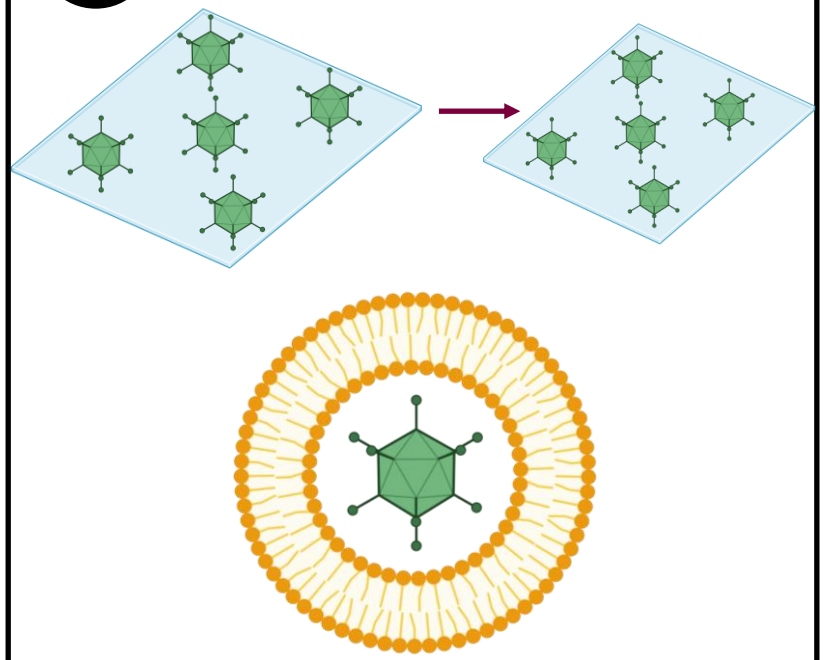
Summary

1



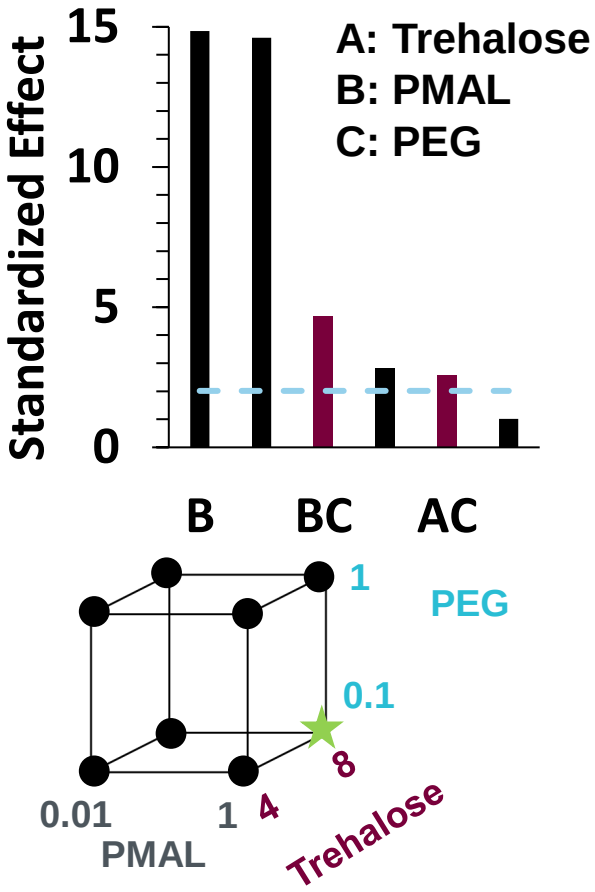
Ad OTFs retain
53% of initial infectious titer
with short solvent casting method

2



↑ trehalose and **↑ PMAL** improved OTF thermostability by **200-fold**

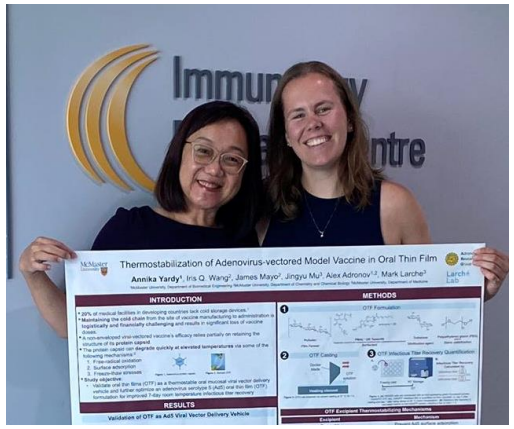
3



R^2 of 90%
PMAL and trehalose = significant contributors to thermostability

Acknowledgements

- Adronov Group
  - **Dr. Jim Mayo**
  - **Yva Rasco**
  - **Grace Lenihan**
  - Dr. Sara Salimi
- Larché Group
  - **Dr. Iris Wang**
  - Dr. Tom Mu
  - Sudeshna Dhar
  - Mackenzie Thorpe



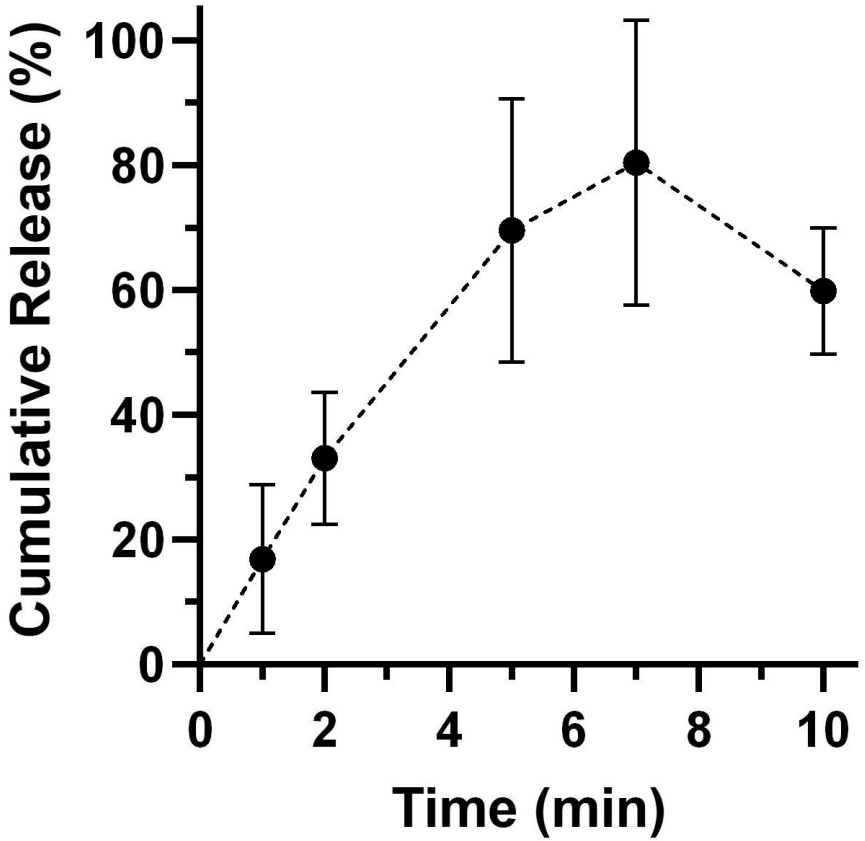
I. J. Pharm. **2024**, 125662

Funding Sources:

NSERC CRSNG

RDT RAPID DOSE THERAPEUTICS

Ad5 OTF Formulation Releases Ad5 Rapidly



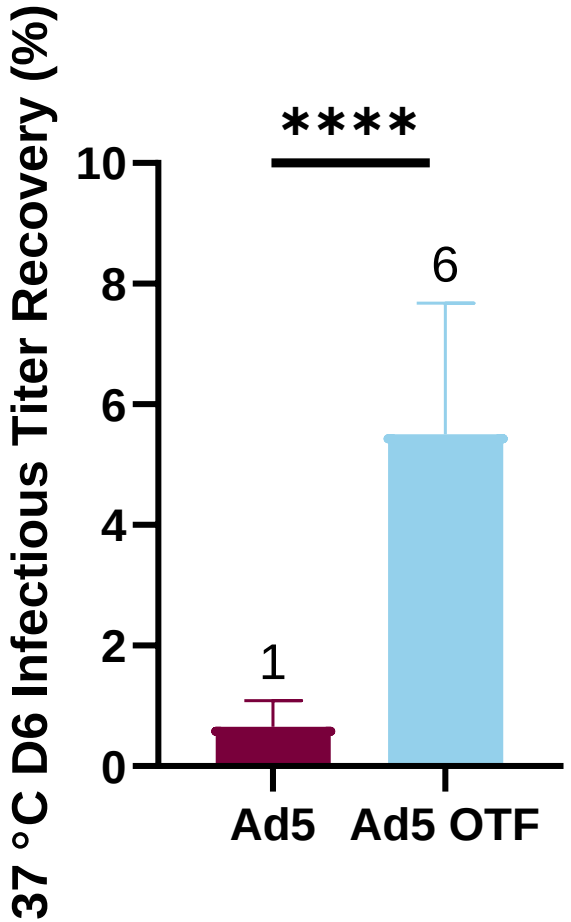
Ad5 cumulative release peaks at **80 ± 23** % **after 7 min**

Comparable to HPMC-based OTFs cumulative release profile release in literature[8]

HPMC: hydroxypropyl methylcellulose

[8] *Sci. Adv*. **2020**, aau4819.

Ad5 OTF out-performs liquid Ad5 control at 37 C



Solvent Casting as Preferred Alternative Vaccine Thermostabilization Method

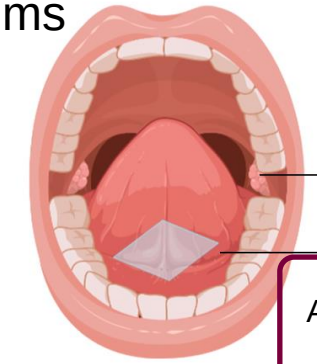
Commercialized Oral Thin Films (OTFs):

RDT
RAPID DOSE THERAPEUTICS

Melatonin

THC

Olanzapine
(Schizophrenia)



Solvent Casting

Aqueous film formulation → Film casting

OTFs formulated in our lab

- Batch-based
- Overnight ambient drying step to produce oral thin film

Challenge:

Can we improve upon the solvent casting method to dry films in 1 h for a semi-continous manufacturing method?

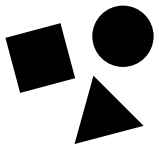
Adenovirus as Model Virus-Vectored Vaccine

Adenovirus

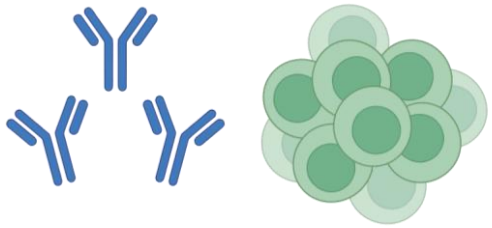
- Protein capsid containing dsDNA
- Thermally unstable
- Approved for use in COVID-19 vaccines

Properties

Safe



Adaptable to antigen of interest



Immunogenic

Biorender

[3]*Health Policy.* **2021,** 2021.03.013
[4]*Curr. Opin. Immunol.* **2023,** 102370
[5]*Expert Opin. Biol. Th.* **2010,** 519332

Lyophilized Vaccine Limitations and Alternative Vaccine Drying Methods

Lyophilization
(Commercial Gold Standard)

Freezing → Primary drying → Secondary drying

- Multi-day batch cycles
- Short shelf-life (hours to days)
- Stresses:

Spray-Drying

Liquid feed atomization → Droplet drying → Powder collection → Formulation processing

- Continuous process
- Specialized equipment
- Dry powder inhaler
- Stresses:

Solvent Casting

Aqueous film formulation → Film casting

- Batch-based
- Overnight ambient drying step to produce oral thin film