Manufacturing challenges and rational formulation development for AAV viral vectors

Arvind Srivastava, Ph.D., Technical Fellow, Avantor Inc.

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Agenda

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AAV structure

02

AAV manufacturing workflow

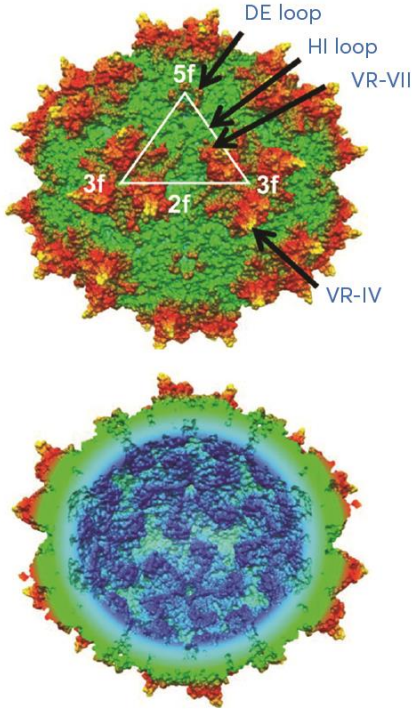
03

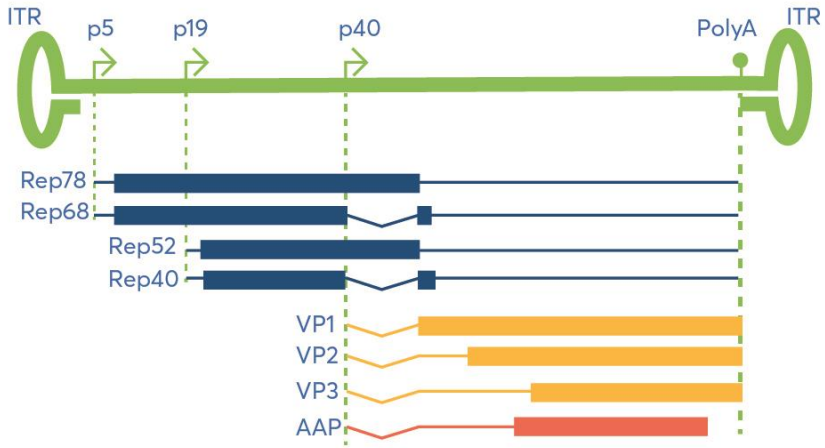
Challenges in AAV manufacturing unit operations

04

Rational formulation design

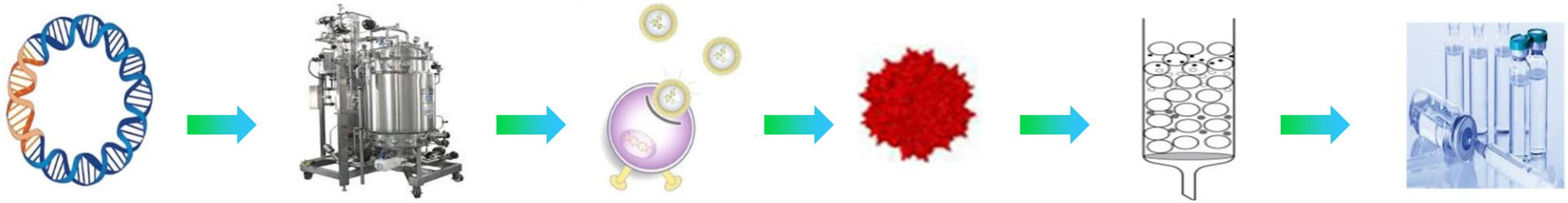
AAV structure





- **AVV viral vectors are delicate and complex molecule; therefore, requires careful manufacturing process design**

AAV viral vector manufacturing workflow



Plasmid development and production

Cell expansion

Plasmid transfection

Viral vector production

Viral vector purification

Viral vector formulation, fill/finish

Upstream

Downstream

Formulation, fill/finish

– **Manufacturing of AAVs is a complex process that requires several upstream, downstream, and fill/finish operations**

Types of capsids generated during AAV production

Full capsids

Partially-filled capsids

Empty capsids

Aggregates

Causes	Correct genome packaging	Packaging of partial vector or host cell DNA	No genome packaging	Low stability and solubility in matrix
Ration of all capsids	Harvest: < 30% Purified: < 70%	Harvest: < 10% Purified: < 1%	Harvest: < 70% Purified: < 30%	Small: < 2% Large: < 1 ppm
Therapeutic Effect	intended *in vivo* gene transfer	Increases risk of immunotoxicity, reduces transduction		

Source: Gimpel et. al. *Mol Ther Methods Clin Dev.* (2021),20:740-754

Challenges during upstream process

Plasmid development	• Lot-to-lot variability in yield and purity • Cost of plasmid DNA
Cell expansion	• Adherent cell culture is difficult to scale up and to monitor cell conditions • Suspension cell cultures provide scalability; however, produce lower cell densities compared with adherent cells
Plasmid transfection	• Three-plasmid transfection system can be inefficient, as not all cells receive optimal ratios of the plasmids required for efficient packaging • The most widely used polyethyleneimine (PEI) is toxic to producing cells and its performance is sensitive to changes in pH

Challenges during downstream process

Cell lysis	• Virus particle aggregation and precipitation during cell lysis • Triton X-100 is listed for "substance of very high concern list" by the European Chemicals Agency under REACH regulations.
Filtration	• Cell lysis generates significant amount of cell debris, which is difficult to pass through filters • Virus particles may aggregate/lose functionality as a result of shear stress during the filtration process
Platform purification	• Low virus yield due to the lack of robust purification processes • Serotype dependent strategy to achieve optimal yield while maintaining product potency and integrity
Separation of empty from full capsids	• CsCl gradient method is difficult to scale-up and intolerant of operator errors • Virus particle damage due to extreme elution condition and low yield due to overlap between empty and full capsid peaks in IEC chromatogram

Challenges during formulation and fill/finish

Product degradation

Physical degradation

- Aggregate/fragments
- Particulate/precipitates
- Unfolding
- Surface adsorption

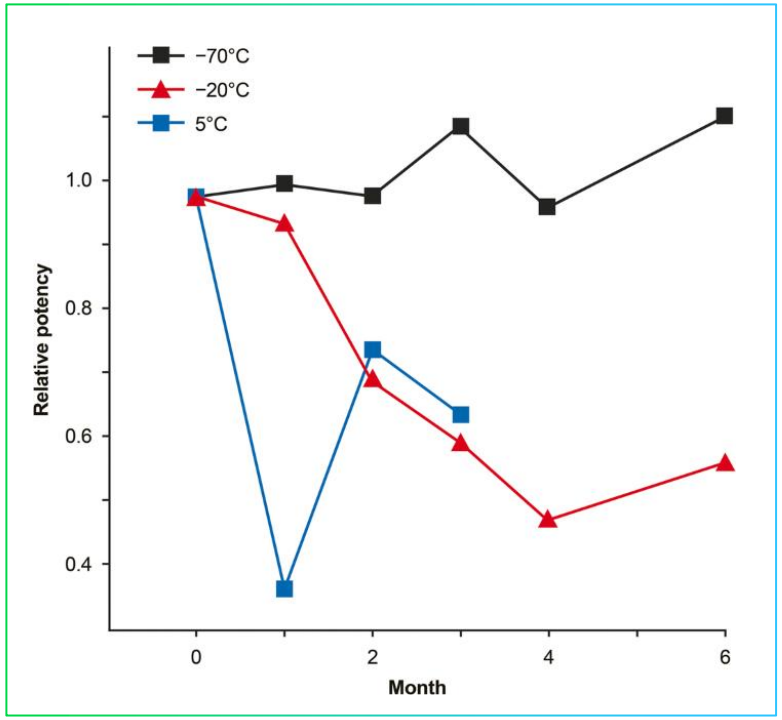
Chemical degradation

- Oxidation
- Deamidation
- Disulfide formation/exchange
- Isomerization

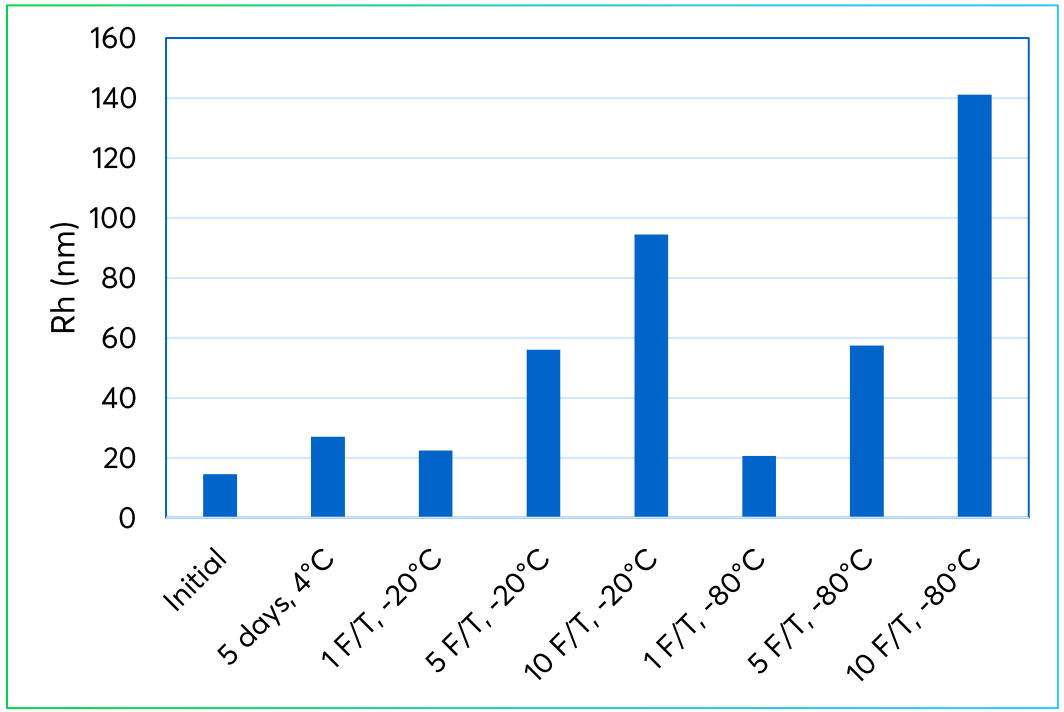
Fill/finish

- Virus particle aggregation and particulate formation as a result of shear stress during fill/finish operations.
- Filling facilities commonly used for manufacturing small volume gene therapy products are semi-automated, raising sterility concerns due to crimping and sealing errors

Viral vector stability



Source: Rodrigues, et. al. Pham. Res (2019),2:1-20

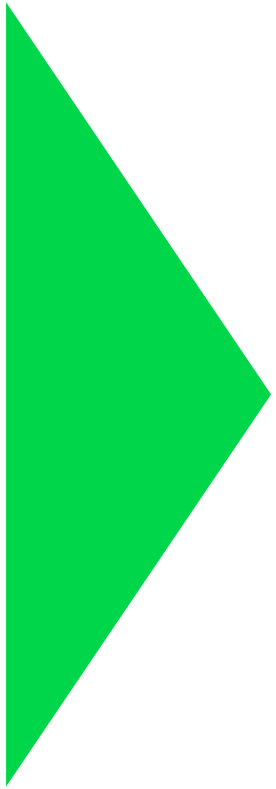


Source: Wright et. al. *Mol Ther.* (2005),12:171-178

- **AVV are unstable products; therefore, it must be formulated carefully for an optimal performance**

Factors that could affect viral vector stability

- **Thermal stress**
  - Heat
  - Freezing
- **Freeze-thaw stress**
- **Mechanical stress**
  - Shearing
  - Shaking
- **Light**
- **pH**
- **Oxygen**
- **Surface contact**



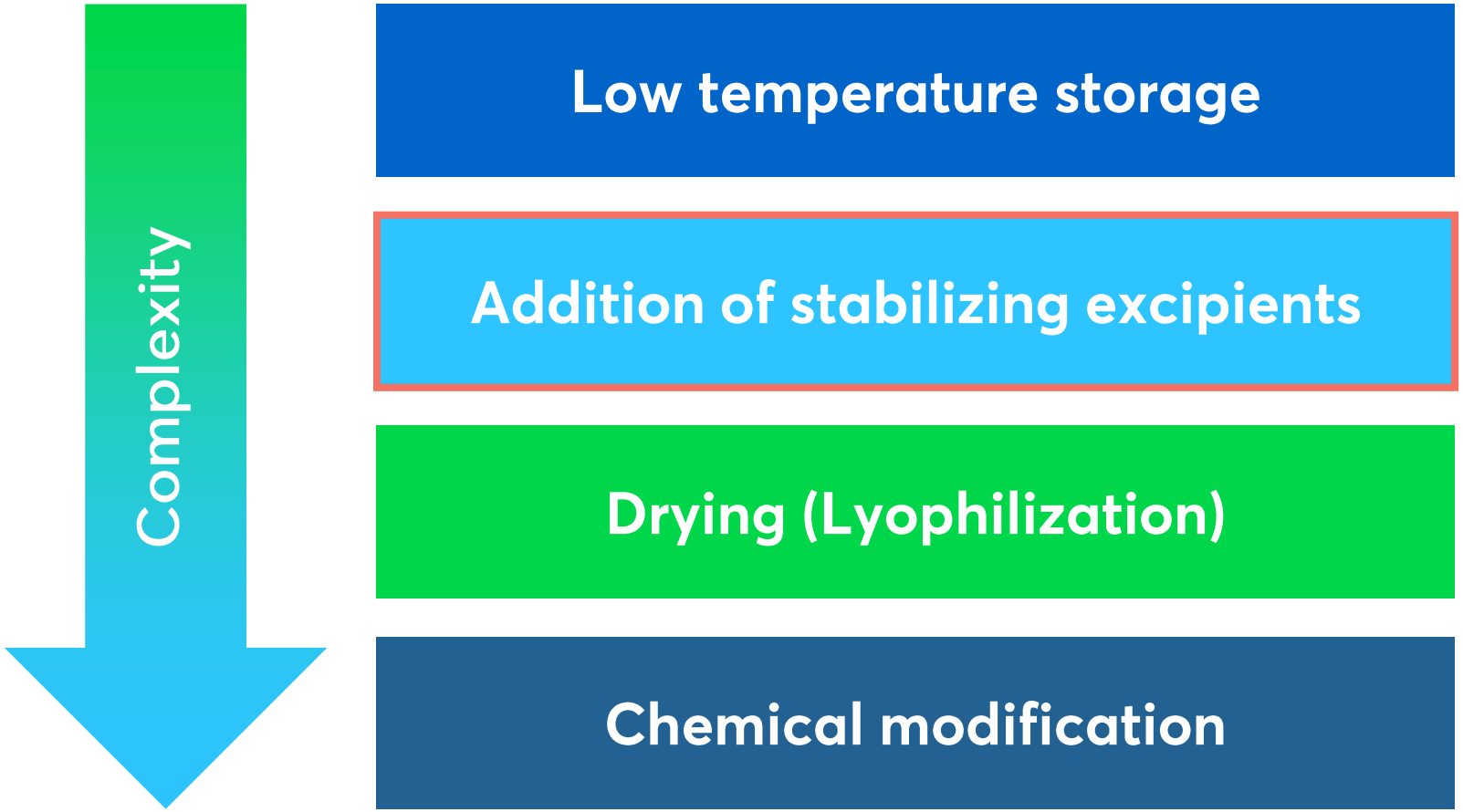
Loss of infectivity

Genome leak

Loss of particle integrity

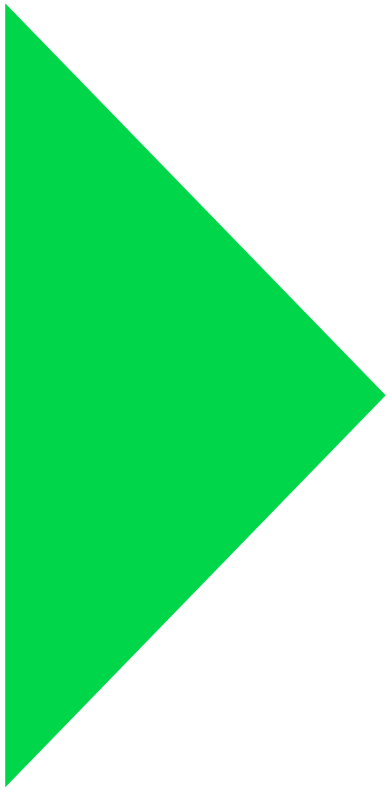
Immunogenicity

Approaches to viral vector stabilization



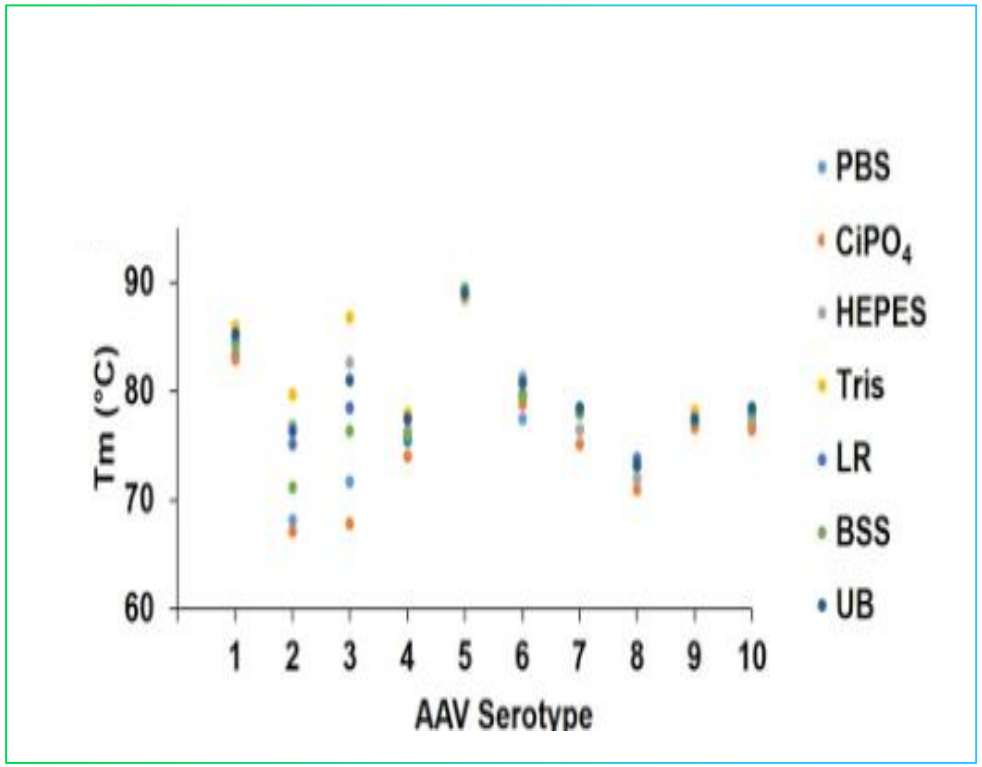
Rational formulation design using excipients

- **Buffer/pH**
- **Salts**
- **Surfactants**
- **Amino acid**
- **Sugars**
- **Other additives**

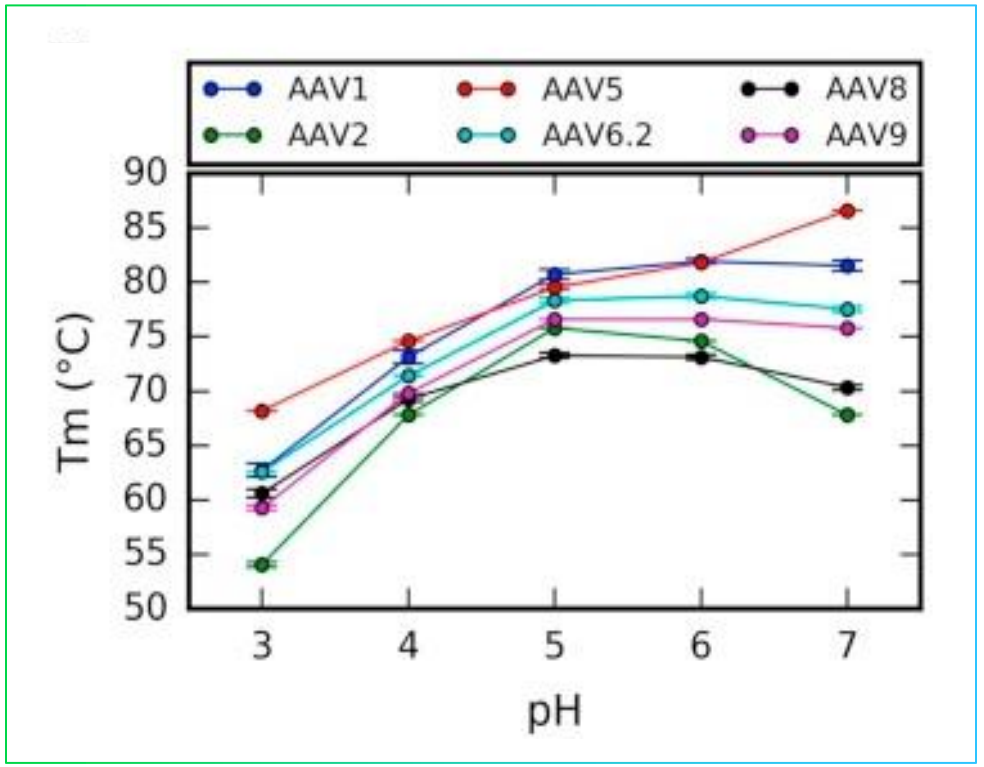


Optimized critical (CQAs) including biological activity under recommended storage and stressed conditions

Effect of buffer type and pH on thermal melting of AAVs



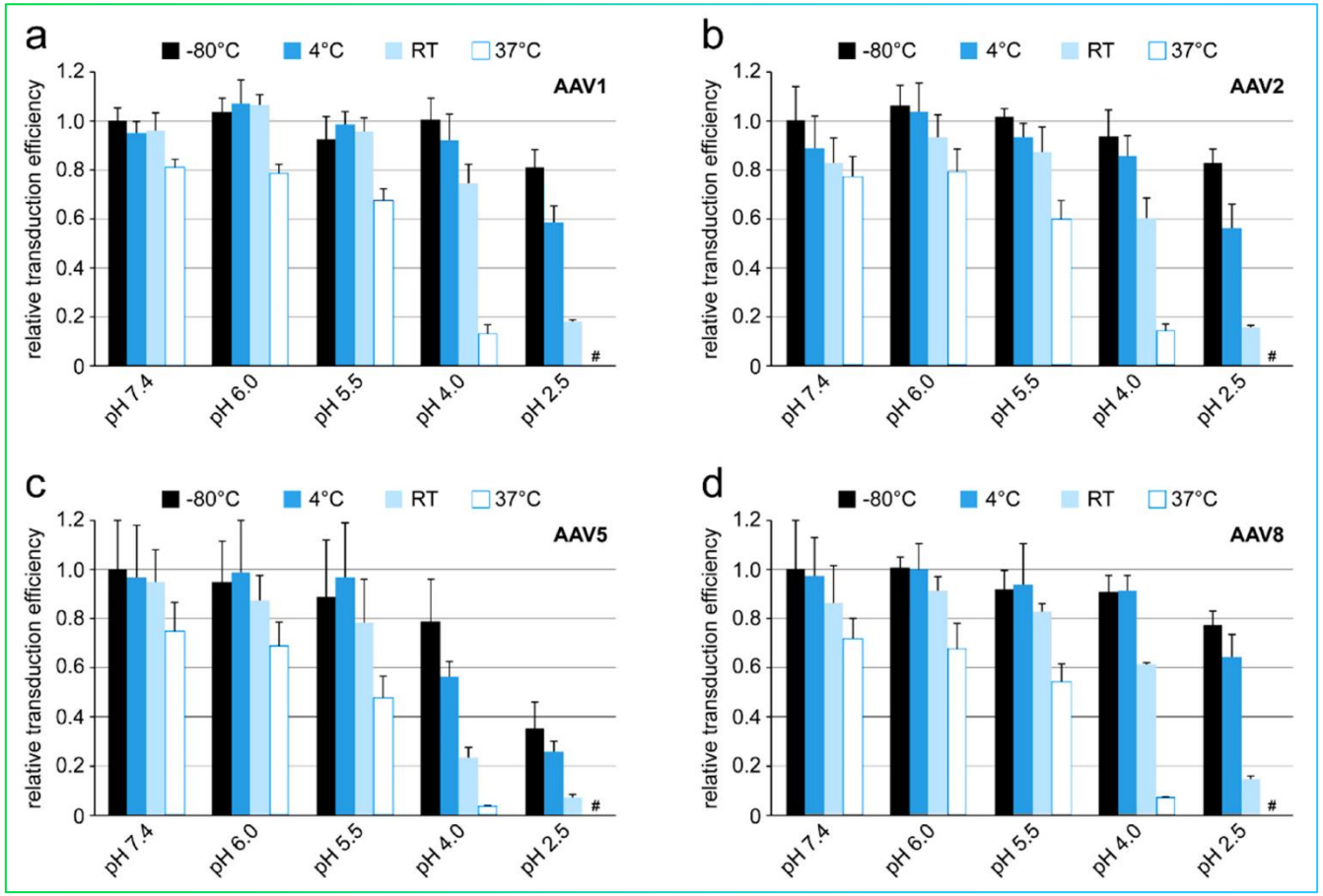
Source: Bennett et. al. *Mol Ther - Methods Clin Dev.* (2017),6:171-182



Source: Pacouret et. al. *Mol Ther.* (2017),25):1375-1386

- **Thermal melting temperature (Tm) depends on AAV serotype**
- **No single buffer condition or pH is optimal for all serotype**

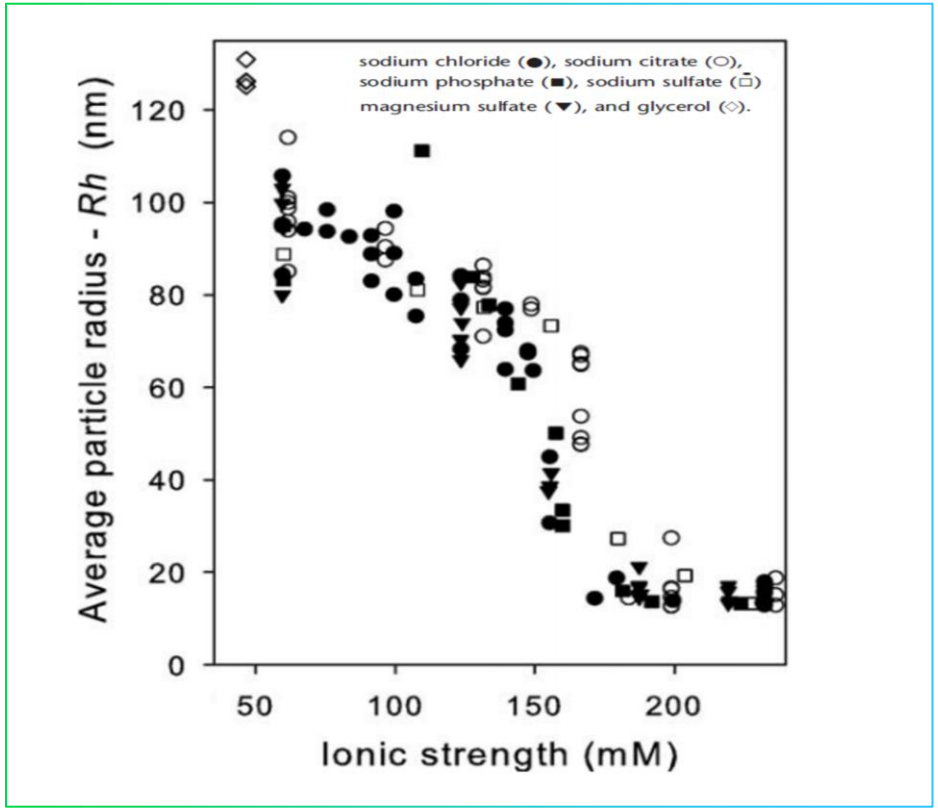
Effect of pH and temperature on transduction of AAVs



- Highest transduction efficiency occurs between pH 7.4 and 6.0
- rAAV5 was the most susceptible to low pH storage conditions

Source: Lins-Austin et. al. *Viruses*. (2020),12(6):1-18.

Effect of salt type/concentration of AAV aggregation

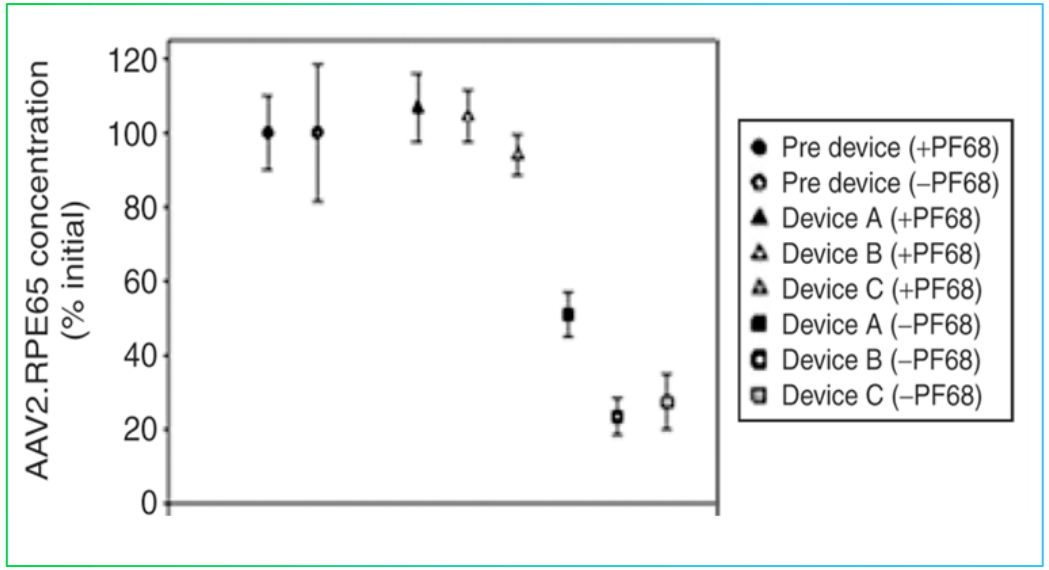


Source: Wright et. al. *Mol Ther.* (2005),12:171-178

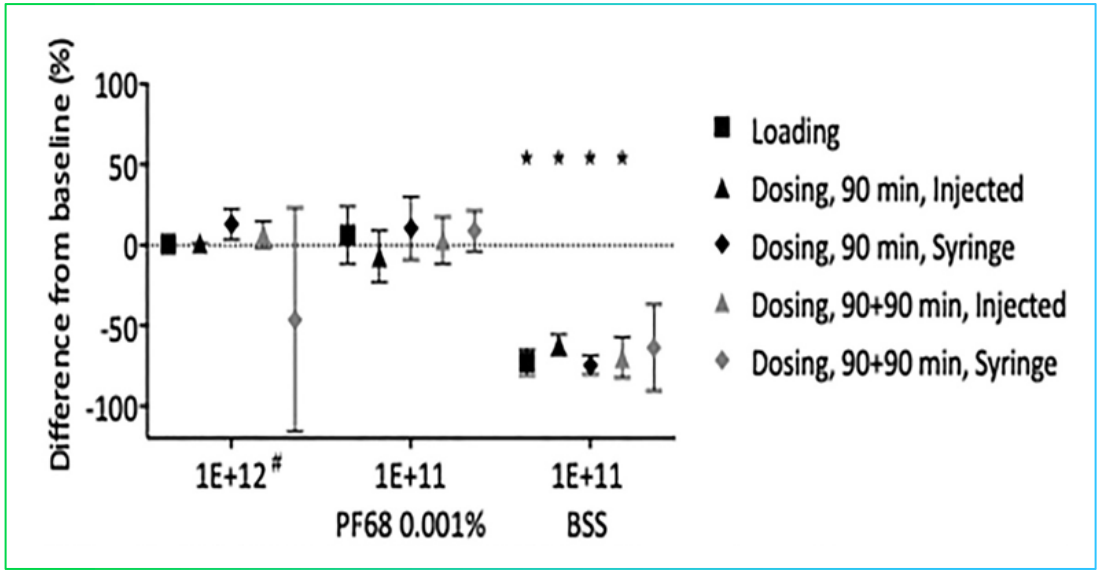
- Salt reduces particulate size, suggesting aggregate inhibition due to ionic strength
- Aggregation is mediated by electrostatic interactions
- Multivalent salts ($MgCl_2$) are more effective than monovalent (NaCl)

Effect of surfactant on the mechanical stability

and surface absorption of AAV particles



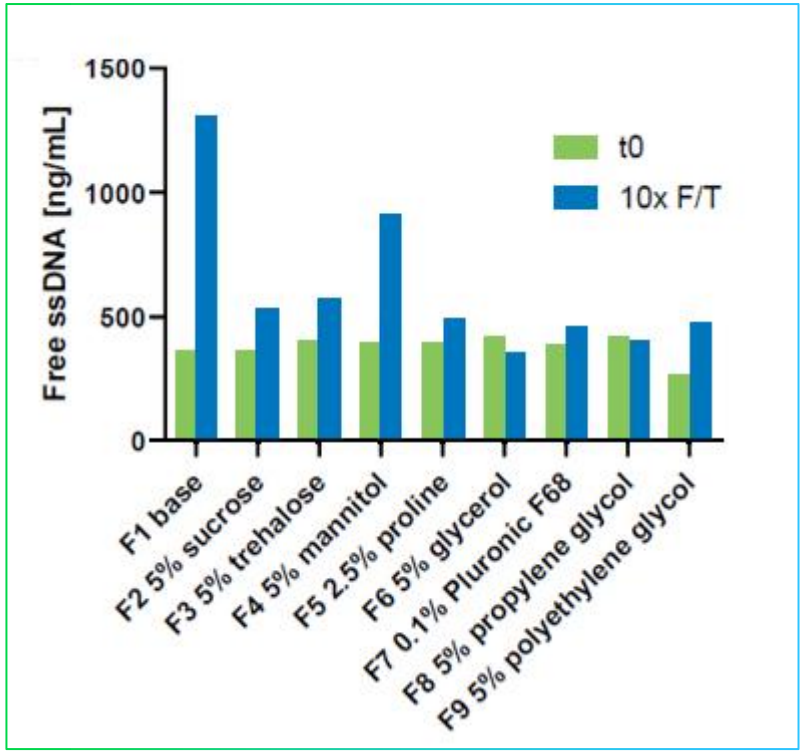
Source: Bennicelli et. al. *Mol Ther.* (2008),16:458-465

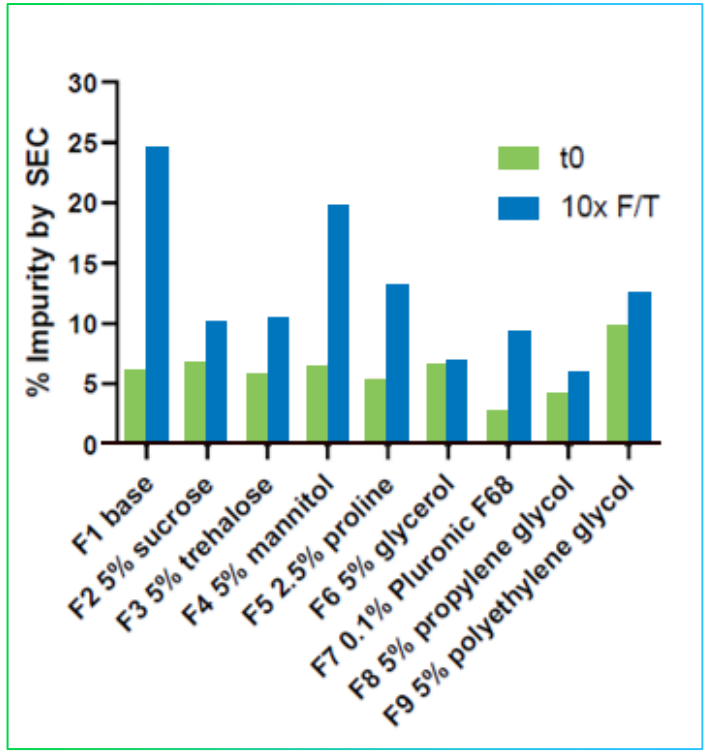


Source: Patrício et. al. *Mol Ther Methods Clin Dev.* (2020),17:99-106

– **Surfactant prevents shear induced aggregation/ precipitation and surface absorption of AAV particles**

Effect of osmolytes on AAV stability





Source: Xu et. al. Int. J. Pharm. (2022),615: 121464

– **Polyols, amino acids and surfactants can stabilize AAVs**

Thank you

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