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Designing and screening formulations to improve manufacturability and distribution of AAV gene therapies

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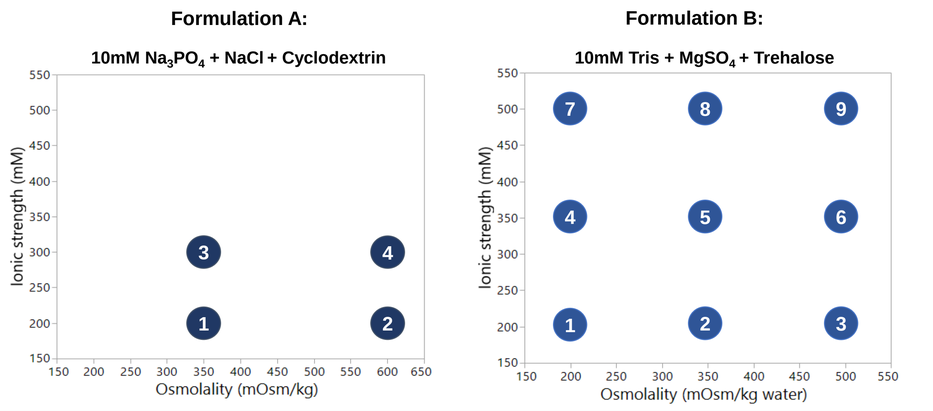
Introduction

AAV gene therapies in a liquid formulation are rarely considered stable under refrigeration and are typically stabilized through freezing at -80°C. However, the process of freezing and thawing may compromise product quality, while transportation and storage of frozen materials is often complicated and expensive. Furthermore, the use of -80°C reduces the number of available drug delivery device options, e.g., pre-filled syringes, which can hinder accessibility of the product.

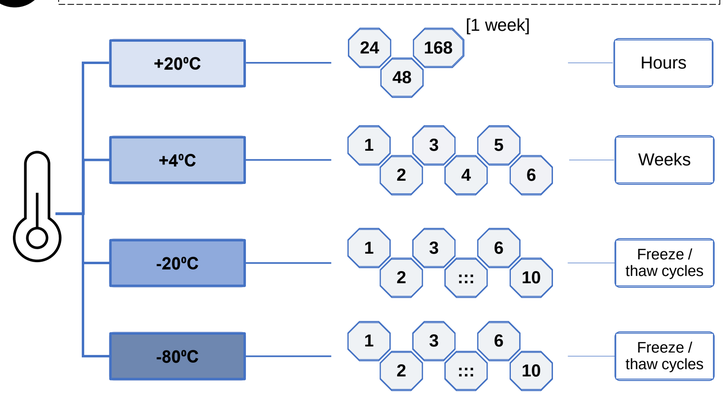
The aim of this work was to design, screen and select a formulation that would maintain AAV2-AQP1 product quality at various storage and freeze / thaw conditions over time.

Methods

1 Formulations A and B with different buffering components, tonicity agents (salts), cryoprotectants (sugars) and a surfactant to achieve different ionic strength and osmolality.

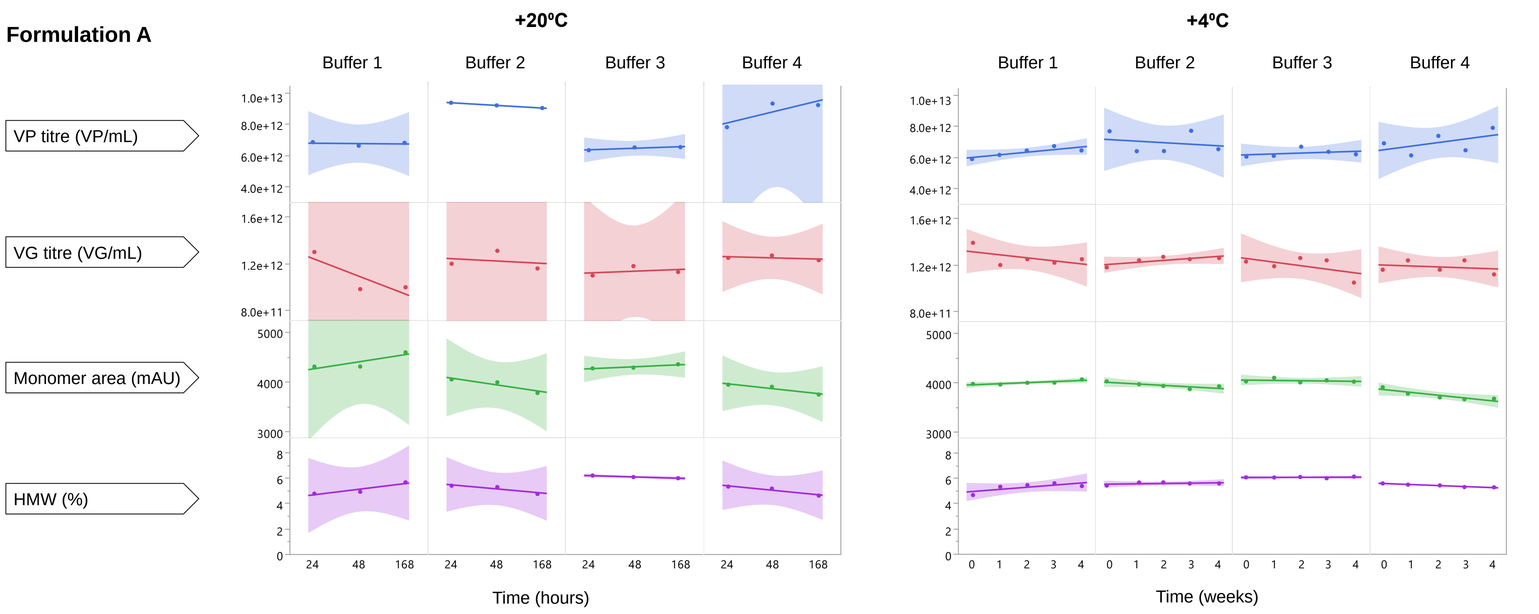


2 Sample storage conditions, timelines and freeze and thaw cycles.



Results

1 **Objective:** Analyse multiple formulations for changes in VP titre, VG titre, monomer area and HMW.
Results: AAV2 stability was affected by the different buffer compositions with Buffer 3 seemingly being the less varied.



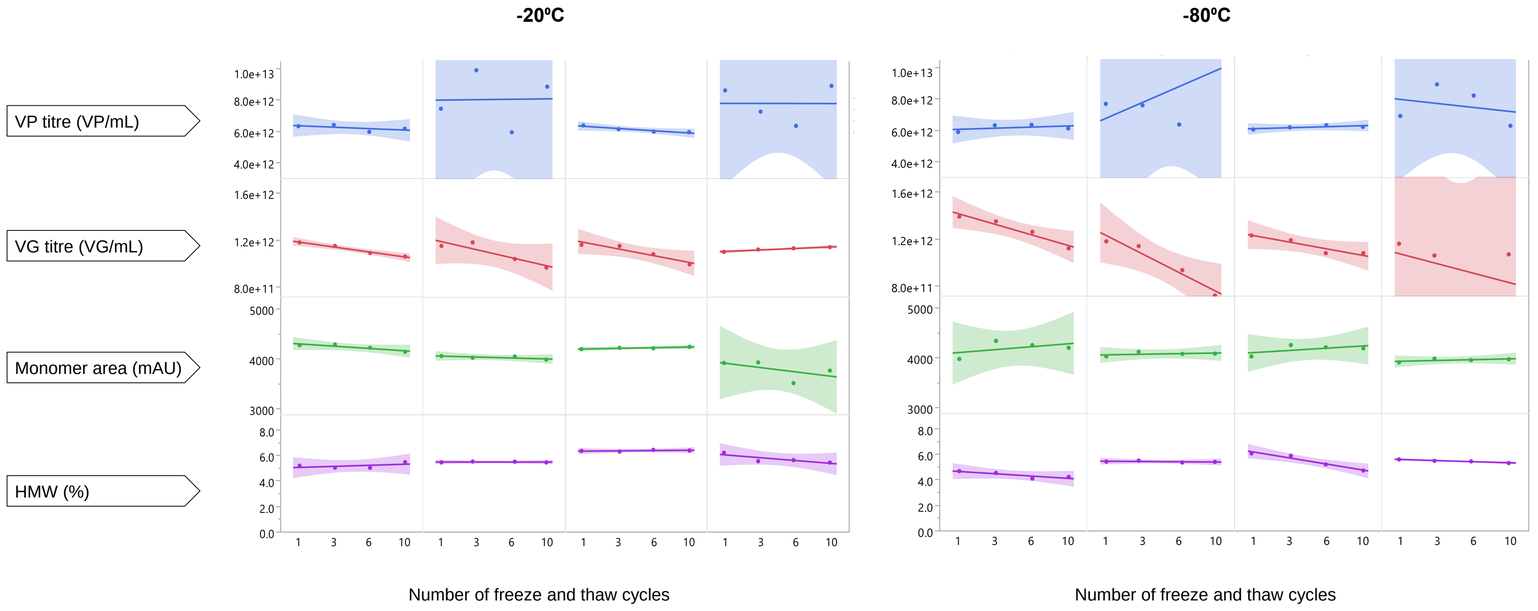


Figure 1. AAV2 stability in different buffers from Formulation A and storage conditions over time. Changes to VP titre, VG titre, monomer area and higher molecular weight species (HMW) were monitored. Samples were thawed at the same time for at least 1 hour at room temperature. Analysis that was performed consisted of the following techniques or instruments: Gyrolab for VP titre, qPCR for VG titre, HPLC-SEC for both monomer area and HMW content. Variation amongst replicates for VP titre was below 5%, whilst that for VG titre was below 20%. The starting AAV2 VG titre was 1.2 × 10^{12} VG/mL for all buffers. The graphs contain a confidence region for the fitted line that illustrates the predicted trend of the data over time.

2 **Objective:** Summarise data to enable an easy selection of the best formulation.
Results: An increase in osmolality led to high VP titre variation, whilst changes to HMW presence were positively impacted by high ionic strength.

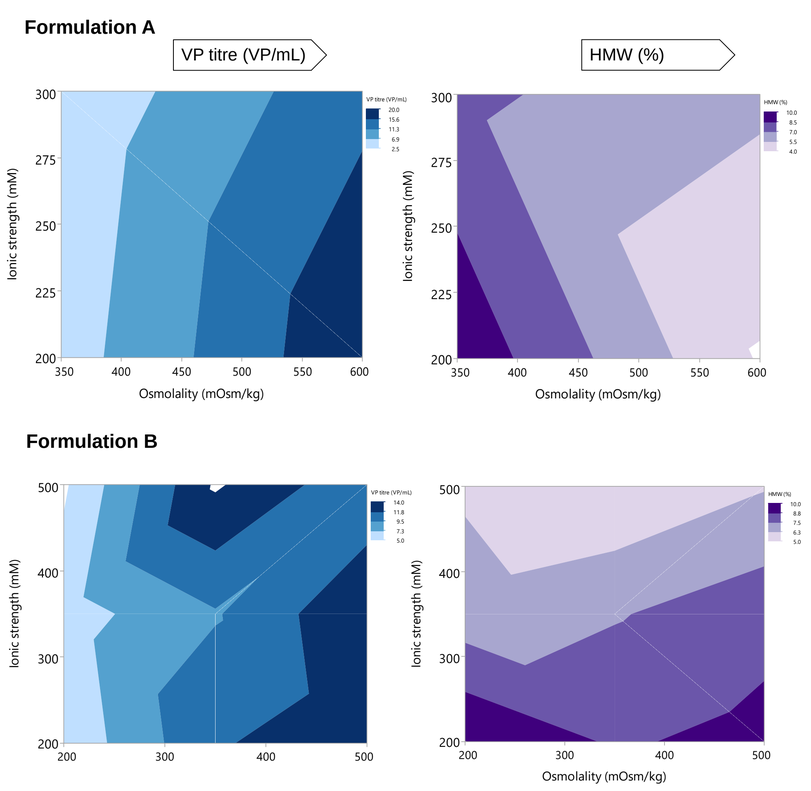


Figure 2. Relative standard deviation (RSD) of the total VP and HMW data for the two formulations. All storage conditions were used to calculate RSD value in percentage.

3 **Objective:** Repeat the study with the two chosen formulations with a 10-fold lower product concentration.
Results:
- Formulation A buffer containing Na_3PO_4 and NaCl provided the lowest RSD across the conditions.
- Virus infectivity was unaffected by 10 freeze / thaw cycles whilst stored at -80ºC.

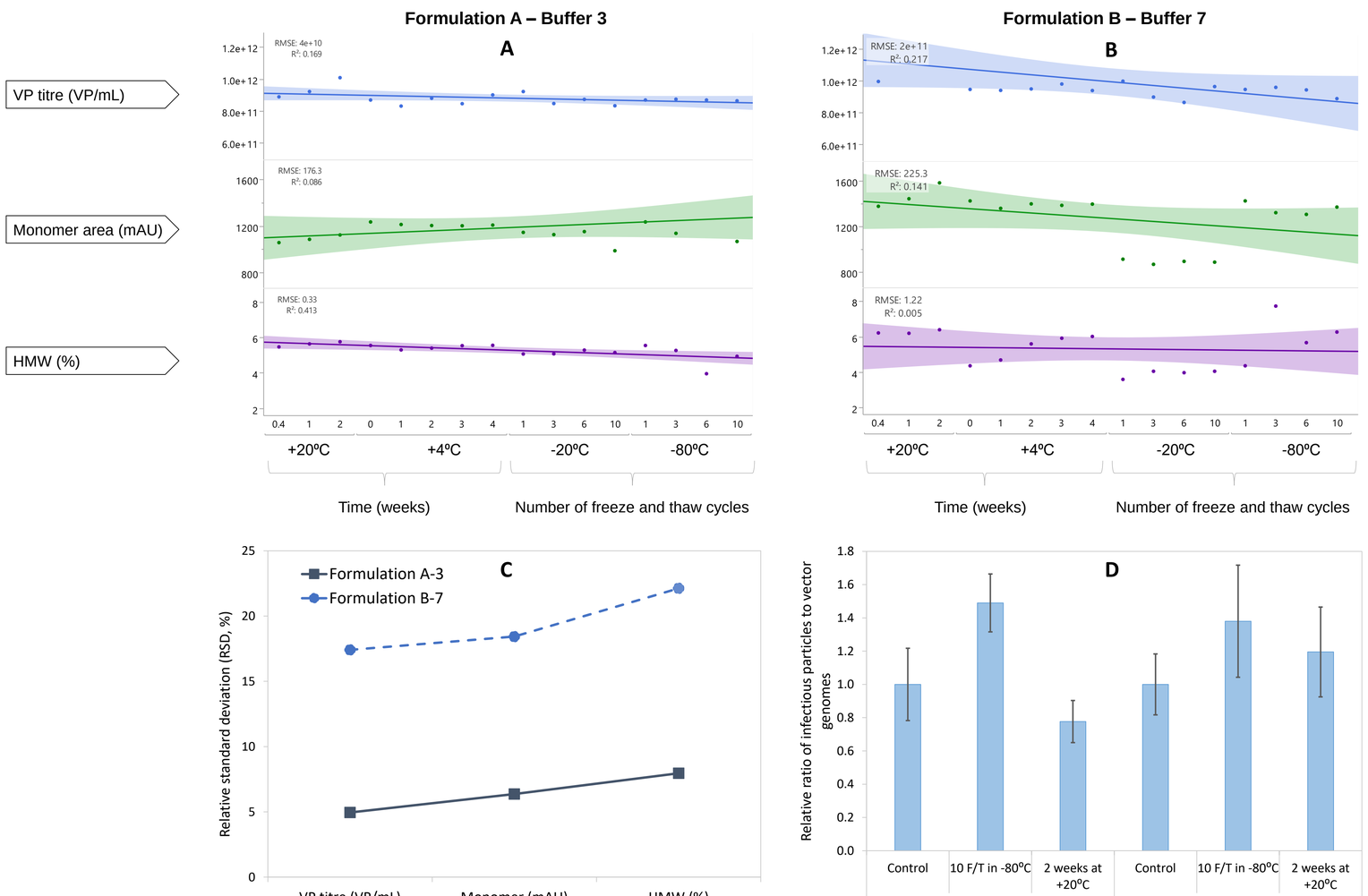


Figure 3. AAV2 stability in the chosen buffers with 10-fold lower starting product concentration. A-B – changes to VP titre, monomer area and higher molecular weight species (HMW) for the two formulations were monitored. Samples were thawed at the same time for at least 1 hour at room temperature. Analysis that was performed consisted of the following techniques or instruments: Gyrolab for VP titre, HPLC-SEC for both monomer area and HMW content. Variation amongst replicates for VP titre was below 5%. The starting AAV2 VG titre was 1.0 × 10^{11} VG/mL for all buffers. The graphs contain a confidence region for the fitted line that illustrates the predicted trend of the data over time. **C** – summary of the earlier data using relative standard deviation. **D** – infectivity assay data normalised to control. The infected cells were lysed, treated, and measured using a qPCR. The result is an average of three biological replicates, each replicate being an average of the three assay replicates. The error bar represents ±1 SD.

Conclusions

- Sodium phosphate and sodium chloride-based formulation buffers generally provided a more stable environment for the AAV2-AQP1.
- Buffer ionic strength of 300-500mM and osmolality below 400mOsm/kg water were found to be the most beneficial for AAV2 stability providing the least amount of variation to VP titre, VG titre, monomer area and HMW.
- The use of relative standard deviation helped to deconvolute complex data sets.
- Virus stability could be affected by differences in its concentration.