STABILITY CHARACTERIZATION OF MULTIPLE SEROTYPES OF ADENO-ASSOCIATED VIRUS USING CHARGE DETECTION MASS SPECTROMETRY

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INTRODUCTION

Adeno-Associated Virus (AAV) (Figure 1) is a commonly used as a delivery vector for gene therapies. There are a variety of unique AAV serotypes, with every serotype possessing different chemical and physical properties that affect their relative stability, including tolerance of elevated temperatures [1]. Developing methodologies to gain a further understanding of the characteristics of the AAV serotypes is crucial for the successful utilization of AAV vectors in gene therapies. With the vulnerability AAV serotypes to temperature fluctuations, AAV samples need to be stored at -80 °C for long term storage and 4 °C for short term storage. During a long transport and extended sample preparation, the AAV samples might undergo fluctuating temperatures which can alter the ratios of full/empty capsids in a sample and therefore alter their potency. Charge Detection Mass Spectrometry (CDMS) has been demonstrated to be an effective way to obtain full/empty ratios. This technology has also been shown to successfully monitor not only full/empty ratios but also the relative abundance of the partially filled AAV capsids.

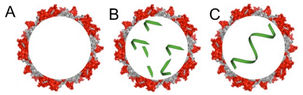

Figure 1: Illustration of an a) empty, b) partial, and c) full AAV capsid

METHODS

Sample Preparation

Full and empty AAV 5 and AAV 8 capsids (Virovek), each consisting of 2.0 x10^{13} vg/mL as determined by either qPCR or ELISA, were buffer exchanged into aqueous ammonium acetate (200 mM) with 0.01 % Pluronic™ F-68 (Gibco™) using Micro Bio-Spin™ P-6 tris columns (Bio-Rad). The AAV sample were split into four categories after thawing, 4 ° C, 22 °C, 35 °C, and 50 °C. 20 µL of the buffer exchanged full AAV samples were incubated at each temperature for 30 minutes using a thermomixer with no mixing or shaking for one cycle. 20 µL of each temperature treated sample was then placed into a 96-well plate and loaded into the Nanomate Triversa™ (Advion); additionally, 100 % empty AAV samples were directly loaded into the 96-well plate for analysis with no temperature treatment implemented.

Instrumentation

A prototype benchtop Charge Detection Mass Spectrometer (CDMS) that is a modified version of the system built by Megadalton Solutions (Figure 2) was used for all analyses [2-3]. The CDMS was coupled to the Nanomate Triversa™ equipped with a 5 µm ID nozzle and standard A chip. The charge domain of the CDMS was calibrated using the charge state envelope of glutamate dehydrogenase (GDH) that was buffer exchanged with aqueous ammonium acetate (200 mM); an example of the obtained GDH spectrum is shown in Figure 3a-c. Charge state and the *m/z* were verified by generating a calibration where the *m/z* peaks found in the GDH spectrum were plotted against the theoretical *m/z* values of GDH found in literature. 10 µL of each sample was aspirated by the Nanomate Triveris™ prior to be infusion and electrospray ionization with 1.77 kV applied to the nozzle. The spectra were collected until approximately 3000 ions were detected within the mass range of 2-6 MDa.

Data Processing

Signal processing and data visualization were performed using a prototype software developed in-house. Ions trapped for 100 ms were recorded and frequency information converted to the mass domain. Within the software, only trapped ions for the entire 100 ms are compiled and binned to generate the corresponding histograms. The *m/z*, mass, and charge were binned to obtain the average, sigma, and area of each peak. Full, partially filled, and empty capsid abundances were calculated for each AAV serotype at varying temperatures using this approach.

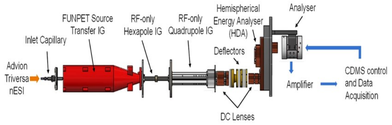

Figure 2: An instrument schematic [2-3] of the prototype benchtop CDMS. The system is a modified version built by Megadalton Solutions that includes components such as an inlet capillary, FUNPET, hexapole, quadruple, deflectors, hemispherical energy analyzer (HDA) and an analyzer (electrostatic linear ion trap).

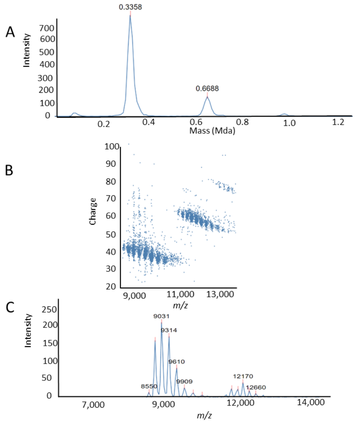

Figure 3: a) The mass spectrum of GDH collected for 15 minutes with 3000 ions trapped within the selected mass region of 0.2-1.2 MDa. b) The scatter plot reflecting the single ions trapped within the 100 ms trapping time generating a 2d *m/z* vs. charge diagram. c) The *m/z* spectrum obtained from the 15 minutes of sample acquisition. This spectrum was used to calibrate the CDMS shown in Figure 2, by collecting the experimental *m/z* values and plotting them against the theoretical GDH charge states found in literature.

RESULTS

AAV 5

The AAV5 serotype 100 % full standard as received was observed to feature mass distributions at 5.4 MDa, 4.7 MDa, and 3.8 MDa, corresponding to the full, partially filled, and empty capsids and illustrated in Figure 4a-b. With increasing temperature, the abundance of the full capsid decreased by 14 % and the abundance of the partial capsid decreased by 10 %. In addition, the empty capsid abundance increased by 24 % from 22 °C to 50 °C. Full/empty, full/partial, and empty/partial ratios were calculated from the mass and charge spectrum and shown in Figure 5.

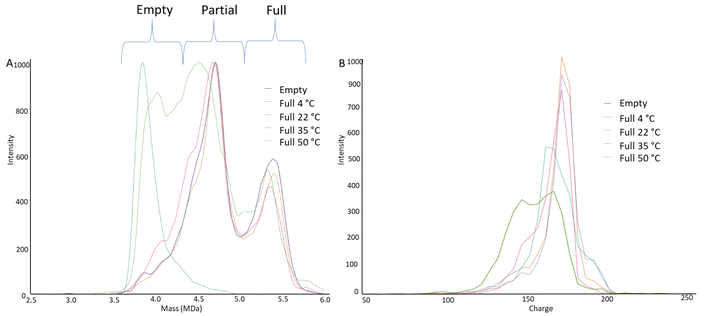

Figure 4: a) Overlayed mass spectrum of AAV5 zoomed into to the 2.5-6 MDa mass range with 100 % empty and full capsid treated with 30-minute incubations at 4, 22, 35 and 50 °C. b) Charge spectrum of AAV5 zoomed into the 2.5-6 MDa mass range with 100 % empty and full capsid treated with 30-minute incubations at 4, 22, 35 and 50 °C.

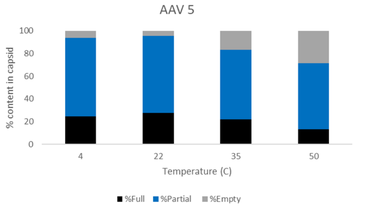

Figure 5: Relative abundance of full, partially filled, and empty capsid populations for AAV5 following incubation at 4, 22, 35, and 50 °C, generally illustrating an increase in the relative abundance of the empty capsid population. Additionally, the relative abundance of the full and partial capsid populations generally decreased from 4-50 °C.

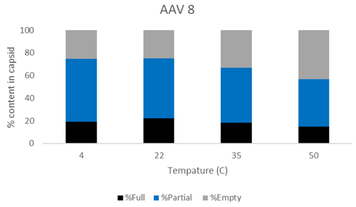

Figure 6: Relative abundance of full, partially filled, and empty capsid populations for AAV8 following incubation at 4, 22, 35, and 50 °C, illustrating an increase in the relative abundance of the empty capsid population. Additionally, the relative abundance of full and partial capsid populations generally decreased from 4-50 °C.

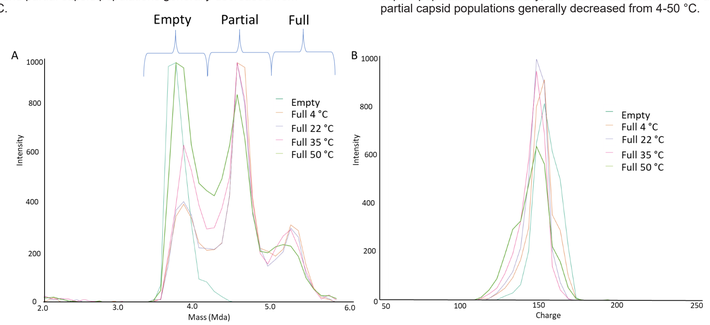

Figure 7: a) Overlayed mass spectrum of AAV8 zoomed into to the 2.5-6 MDa mass range with 100 % empty, and full capsid treated with 30-minute incubations at 4, 22, 35, and 50 °C. b) Charge spectrum of AAV8 zoomed into the 2.5-6 MDa mass range with 100 % empty, and full capsid treated with 30-minute incubations at 4, 22, 35 and 50 °C.

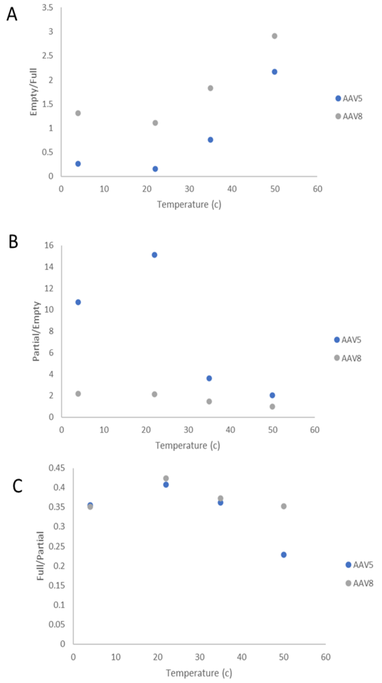

Figure 8: a) The full/empty ratios of AAV5 and AAV8 at 4, 22, 35 and 50 °C. b) The partial/empty ratios of AAV5 and AAV8 at 4, 22, 35 and 50 °C. c) The full/partial ratios of AAV5 and AAV8 at 4, 22, 35 and 50 °C.

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RESULTS

AAV8

The distribution of the relative abundance of AAV8 capsid population following incubation at varying temperatures is illustrated in Figure 6. The AAV8 serotype exhibited mass distributions at 5.4 MDa, 4.7 MDa, and 3.8 MDa, corresponding to the full, partially filled, and empty capsids and illustrated in Figure 7 a) and b). With increasing temperature, the abundance of the full capsid decreased by 8 %, and the abundance of the partial capsid decreased by 10 %. Additionally, the empty capsid abundance increased by 18 % from 22 °C to 50 °C. Full/empty, full/partial, and empty/partial ratios were calculated from the mass and charge spectrum in Figure 7 a) and b).

Comparison AAV Serotypes

The relative abundance of the full capsid population was observed to drop with increasing temperature for both serotypes. Above 22 °C, the relative abundance of the partial and empty capsid distributions was observed to increase, resulting in a linear decrease in the full/empty ratios of both AAV serotypes (Figure 8a). Additionally, the ratios of empty/partial and full/partial are shown in Figure 8 b) and c). This figure illustrates the trends of each ratio for both serotypes.

DISCUSSION

Two full AAV serotypes were temperature treated and the capsid content monitored by CDMS. The serotypes showed similar reductions in nucleic acid content as a function of increasing temperature. With the monitoring of the relative abundance of AAV capsid content the selected AAV serotypes were found to demonstrate a ~10 % decrease in full AAV capsid content from room temperature (22 °C) to 50 °C. The change of ~10 % in full capsid content is overall a minimal change. However, this ~10 % fluctuation after only 30 minutes of heating and extended exposure would result in great degradation and affect integrity and potency of the capsid. As temperature increases, the empty capsid distribution linearly increases in relative abundance. Three different charge state distributions were observed and the distribution transitioned into a lower charge and presumably less structured capsid. Together these data support the gradual loss of nucleic acid content as a function of the reduced integrity of the AAV capsid with increasing temperature. The temperature conditions were selected to limit the chance of capsid collapse. The absence of a sharp change in the empty/full ratio suggests that the AAV capsids were not fully releasing genetic content or fully collapsing over the studied temperature range.

CONCLUSIONS

- **A prototype CDMS was used to characterize the full, empty, and partial capsid distributions from two AAV serotypes.**
- **Changes observed in the charge distribution suggest alterations in the capsid stability**
- **An increase of ~3 % of the relative abundance of full AAV capsid population as temperature is increased from 4-22 °C.**
- **Full/empty ratios for two AAV serotypes showed a linear decrease as incubation temperature increased.**
- **A ~10 % loss in the relative abundance of AAV capsid population was observed as temperature increases from 22-50 °C.**

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

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