Simplifying Progress

Estimation of Empty | Full AAV10 Particle Ratio Using MALS Detector

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Introduction

Serotype 10 adeno-associated virus (AAVrh_10mCherry) was analyzed on the PATfix® HPLC system with the CIMac™ AAV full | empty analytical column to estimate the ratio of empty and full AAV particles based on the peak area of the chromatogram given with three different detectors. AAV included a protein capsid containing single-stranded DNA. CIMac™ AAV column consisted of a strong anion exchanger with QA chemistry (quaternary amine).

Methods

Detectors

- Absorbance (260 nm and 280 nm)
- Fluorescence (excitation 280 nm and emission 348 nm)
- MALS (multi angle light scattering) at 90° angle

Conditions

- Buffer A: 20 mM BTP + 2 mM $MgCl_2$ pH 9.0
- Buffer B: 20 mM BTP + 2 mM $MgCl_2$ + 500 mM NaCl pH 9.0

Figure 1: CIMac™ AAV full | empty

Table 1: Method E_F CIMac™ AAV

Time (min)	Buffer A (%)	Buffer B (%)	Flow (mL/min)
0	100	0	1
1	100	0	1
6	60	40	1
7	0	100	1
8	0	100	1
8.02	100	0	1
11	100	0	1

Results - LS showed better resolution between the E and F peaks compared to FLD

As the Beer-Lambert law states, the peak area of absorbance is a function of molar concentration and absolute molar extinction coefficient of the corresponding component at a specific wavelength. To determine the ratio of empty to full capsids, the absolute values of the molar extinction coefficients for each, at both 260 and 280 nm, would be required, and these would differ among transgene and AAV serotypes.

Similarly, for dilute solutions, fluorescence intensity can be described as a function of molar concentration, molar extinction coefficient (not the same as the molar extinction coefficient for absorbance), and quantum yield. It is speculated that the DNA insert of the AAV should not exhibit fluorescence and therefore, the absolute molar extinction coefficients could be the same for empty and full AAV particles, which indicates that concentration could be directly proportional to the peak area.

From the Rayleigh ratio, light scattering can be described as a function of mass concentration, molar mass, particle size, and shape. Empty and full AAV particles appear to have the same capsid diameter and shape, suggesting that only the mass concentration and molar mass should be a factor of the light scattering signal.

Empty and full AAV particles ratio was estimated with the shown equation. Since the absolute molar extinction coefficients were not known, the equation was simplified by assuming the same complete molar extinction coefficients for empty and full AAV particles at both 260 and 280 nm (relative full | empty molar extinction coefficients ratio of 1). This led to greatly overestimated values of full AAV particle percentage. In addition, absorbance at 260 nm and 280 nm gave very different ratios of empty and full AAV particles.

Fluorescence and light scattering showed similar ratios of empty and full AAV particles, since the chromatograms' peak areas appeared to be directly proportional only to the concentrations of empty and full AAV particles. The slight difference between the two signals could be attributed to the difference in resolution. Light scattering showed better resolution (0.87) between the empty and full peaks than fluorescence (0.74).

Using the estimated values of the empty and full AAV particle percentage given by the fluorescence and light scattering detectors, the relative full/empty molar extinction coefficients ratio at 260 nm and 280 nm was calculated, where it was estimated that full AAV particles had about 4.1 - 4.6 higher absorbance than empty AAV particles at 260 nm and about 2.0 - 2.2 higher absorbance at 280 nm for the same concentration.

Figure 2: PATfix®

Table 2: Peak area by signal

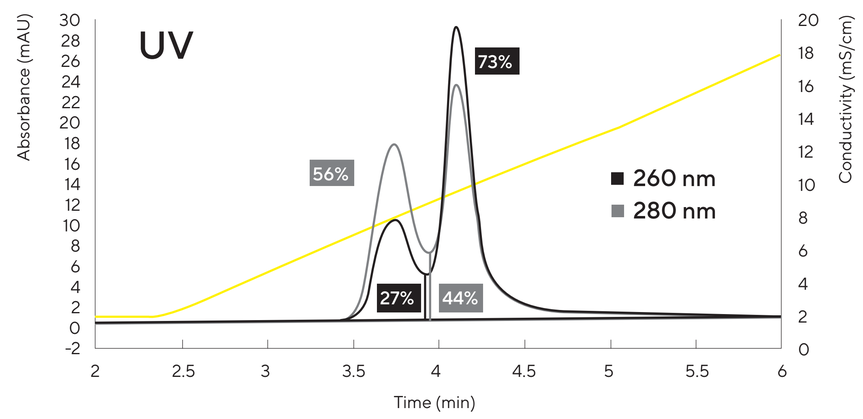
	Peak area (mVs or mAUs)		
Signal	**Empty**	**Full**	**Resolution**
Light scattering	196.5	127.7	0.87
Flourescence	1029.1	593	0.74
Absorbance (260 nm)	158.2	420.4	0.96
Absorbance (280 nm)	276.1	355.1	0.95

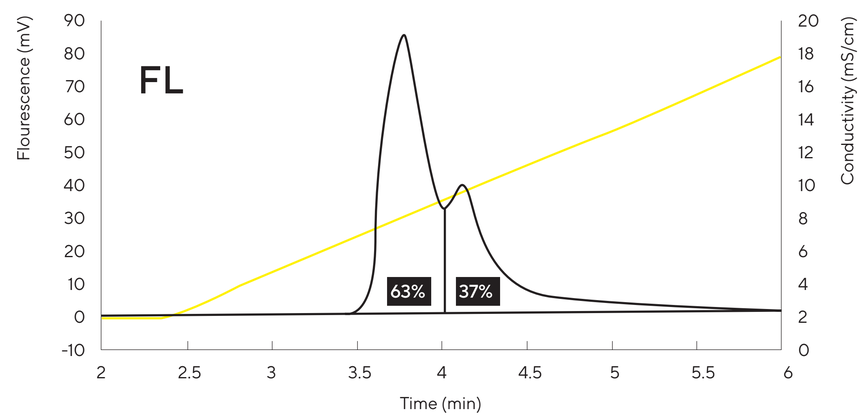
Calculation of particle ratio:

$$\text{Full (\%)} = \frac{\text{Area}_{\text{Full}}}{\text{Area}_{\text{Full}} + \text{Area}_{\text{Empty}} \cdot \frac{\varepsilon_{\text{Full}}}{\varepsilon_{\text{Empty}}}}$$

Table 3: Molar extinction coefficient

	Flourescence		Light scattering	
Calculated	**260 nm**	**280 nm**	**260 nm**	**280 nm**
$\varepsilon_{Full}/\varepsilon_{Empty}$	4.6	2.2	4.1	2.0





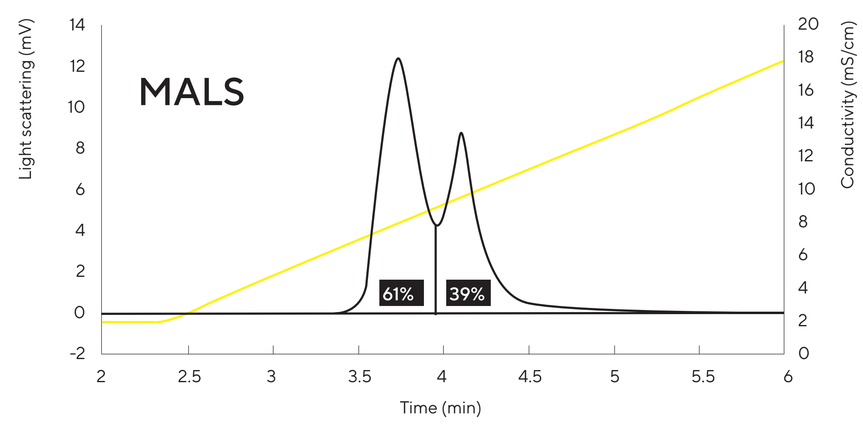


Figure 3: Chromatograms per signals

Conclusion

- Empty and full AAV particle ratios were estimated using the absorbance, fluorescence and light scattering detectors.
- Estimation of empty and full AAV particle ratio with the absorbance signal was not accurate without knowing the exact values of the molar extinction coefficients.
- Assuming the relative full | empty molar extinction coefficients ratio of 1 greatly overestimated the amount of full AAV particles with the absorbance signal Absorbance at 260 nm and 280 nm also showed very different ratios of empty and full particles.
- Fluorescence and light scattering signals showed similar ratios of empty and full particles.
- Using the estimated values of the empty and full AAV particle ratio given by the fluorescence and light scattering signals, the relative full | empty molar extinction coefficients ratio at 260 and 280 nm was calculated.
- Full AAV particles had between 4.1 - 4.6 absorbance than empty AAV particle at 260 nm and between 2.0 and 2.2 at 280 nm.