Orthogonal characterization of AAV samples by simultaneous monitoring of multiple analytical parameters during chromatography

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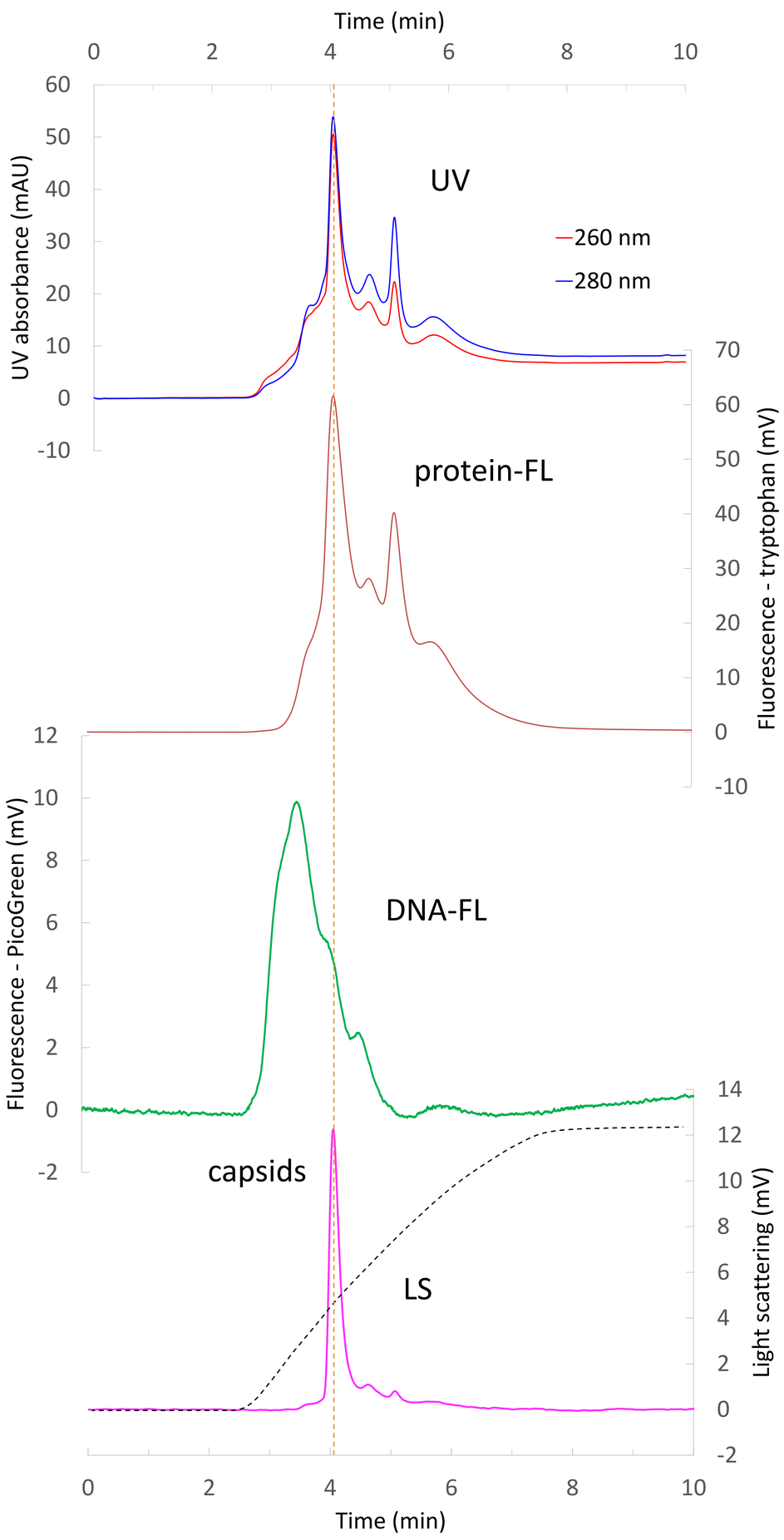
INTRODUCTION

Fast, accurate, and meaningful characterization of cell culture harvests and in-process samples is critical for both process development and for documentation of in-process control. UV analysis of chromatography profiles has been a valuable tool for decades, but it has major limitations with respect to sensitivity and its ability to discriminate the product of interest from particular contaminant classes.

In this study we use filtered lysate containing AAV 8 to demonstrate the ability of a strong cation exchange monolith (CIMac™ SO3-0.1) coupled with multiple monitors to enable high sensitivity detection of AAV capsids while characterizing the relative distributions of DNA and protein contaminants. This approach can be used to evaluate cell culture methods, influence of harvest time, lysis methods, and effectivity of purification methods across a process.

RESULTS



UV detection is used widely to detect DNA, RNA, and proteins. Analysis of wavelength ratios (260/280) can also identify areas that are enriched in nucleic acids or proteins, respectively. Purified proteins are generally 280-dominant with 260/280 ratios in the range of 0.3 to 0.6. Purified nucleic acids are 260-dominant with 260/280 ratios close to 2.0. Intermediate ratios indicate mixtures, as shown in the figure. Another shortcoming of UV detection is that it does not indicate which peak in a multipeak profile represents AAV.

Tryptophan fluorescence (brown line) is associated exclusively with proteins and it is not influenced by nucleic acids. It is more sensitive than UV but shares the limitation of not being able to indicate which peak in a multipeak profile is the desired AAV.

PicoGreen® added to a sample in advance of chromatography intercalates into DNA and becomes fluorescent. This enables specific detection of DNA (green line) without influence by proteins. Preliminary data indicate it detects only DNA outside the capsids.

Light scattering (magenta line) discriminates among eluting species according to their size. Since AAV capsids are much larger than contaminating host proteins and DNA, they scatter light much more effectively. This enables definite identification of the AAV peak from smaller contaminants (orange marker just after 4 min).

Beyond providing a convenient fingerprint, composite profiles such as this can provide valuable insights. For example, purified DNA does not bind to cation exchangers. It binds here only because it is complexed to protein contaminants and AAV.

Chromatographic conditions:

- Loading buffer: 50 mM CH_3COONa pH 4.0
- Elution buffer: 50 mm CH_3COONa + 3 M NaCl pH 4.0

Detectors:

- UV absorbance (260 nm and 280 nm)
- Fluorescence tryptophan
  (excitation 280 nm, emission 348 nm)
- Fluorescence PicoGreen®
  (excitation 485 nm, emission 520 nm)
- Light scattering at 90° angle
- Conductivity and pH

CONCLUSIONS

- Host-protein content, host-DNA content, and AAV content was characterized in a single rapid 15 min analysis with multiple detectors, each providing unique assay information.
- Light scattering showed great potential for in-process control of AAV characterization in crude harvest material, since the AAV capsids peak was clearly visible from all other contaminants.
- Additionally, progress across the stages of downstream purification can also be monitored, which can provide valuable insight into the effectivity of the purification process.