

## Botulism Antitoxin Characterization with AFFF-MALS

Aggregation must be defined in biological products for human use, and without sufficient quantities the loss of approval looms. High-performance size-exclusion chromatography (HPSEC) is a traditional structural assay and was employed annually to monitor the integrity of antitoxin treatment for botulism, but recently health agencies have raised the concern of aggregation. Because HPSEC can only infer molecular size or mass, requiring reference to external standards and assumptions, we used light-scattering to measure product composition and integrity without calibration to extraneous standards.

Multi-Angle Light Scattering (MALS) of antitoxin separated by asymmetric field-flow fractionation (AFFF-MALS) was developed as orthogonal assays to our HPSEC. Isocratic separation followed by a membrane wash showed the expected molar masses for antitoxin components of IgG and fragments but no aggregate (Fig. 1, 12-23 min vs. later).

To enhance assay sensitivity for large analytes, a rapid gradient method of excess sample was applied using a cross flow of 3-0 ml/min over 12 minutes followed by an extended wash. This revealed some higher mass entity (Fig. 2, small light scattered peak at 33-37 min).

Although this high-mass component was observed by Rayleigh scattering, it had no measurable concentration by either UV absorption or differential refraction, so molar mass and molecular size could not be calculated. This established the minute presence of detectable yet immeasurable and undefined high-mass component.

Review of prior data with these known absolute molar masses confirmed that whole and near-whole immunoglobulin were previously misinterpreted as aggregate by HPSEC. This demonstrated that the antitoxin product is structurally stable even after a 10-year shelf life. Light-scattering assays, like AFFF-MALS, which avoid the assumptions and errors of traditional HPSEC, are proposed as new standard methods to monitor physical integrity of immunoglobulin mixtures such as botulism antitoxin.

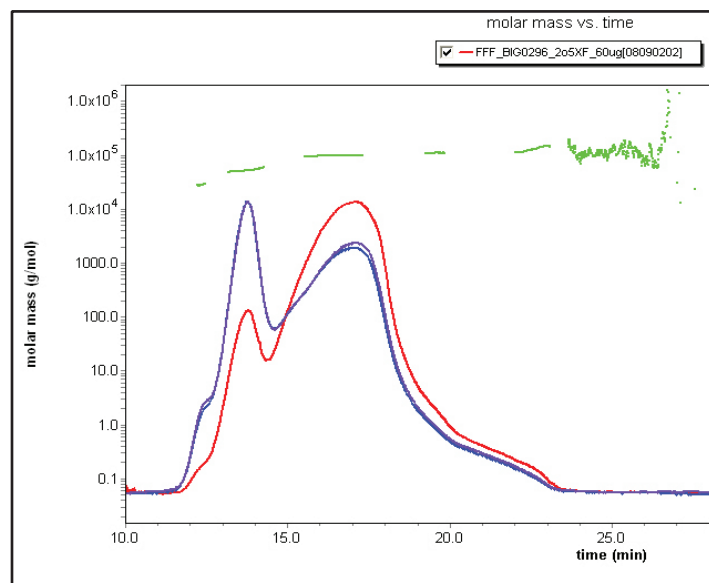


Figure 1. Isocratic AFFF-MALS shows antitoxin components of expected masses but no aggregate.

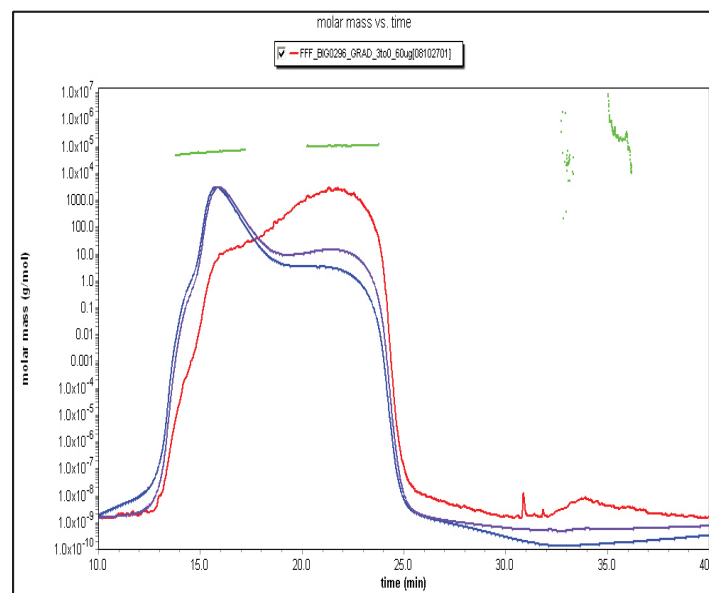
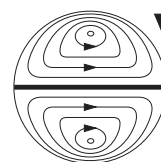


Figure 2. Gradient AFFF-MALS reveals sparse high-mass component having no detected concentration.

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