

Characterization of Spongiform Encephalopathies

Neurodegenerative diseases such as Parkinson's, Alzheimer's, and the transmissible spongiform encephalopathies (TSEs or prion diseases) are often marked by abnormal protein deposits in the brains of afflicted individuals.

Successful size-based separation of these aggregates—which can range from small protein oligomers to large amyloid fibrils—has been a challenge for traditional methods such as size exclusion chromatography (SEC), polyacrylamide gel electrophoresis (PAGE), and ultracentrifugation. In both SEC and PAGE, large aggregates may be caught up in and/or subjected to shearing forces by the stationary phase, while ultracentrifugation techniques provide relatively limited resolution.

The Eclipse separation system, based on field flow fractionation, overcomes the limitations of these techniques.

The Eclipse was used to separate a collection of prion protein aggregates, which were analyzed on line by the 18-angle DAWN EOS, Optilab and WyattQELS instruments to obtain weight-average molar mass (M_w), radii of gyration (r_g), and hydrodynamic radii (r_h). Particle shape information (ρ value) was also obtained by comparing the ratio r_g/r_h .

The results obtained from the MALS and QELS measurements of the separation are shown in Figure 1. The fractionated aggregates contained a wide range of M_w values (less than 10^5 to greater than 10^7 Da), r_g values ranging from under 10 nm to approximately 300 nm, and r_h values from roughly 5 nm to more than 50 nm. As seen in Figure 2, values for ρ indicated that the aggregates in the early eluting fractions were fairly compact, while structures at the end of the elution were highly elongated.

With the ability to fractionate and characterize the molar mass, radii, and shape of a wide range of particle sizes, the FFF-MALS-QELS approach is an extremely valuable tool for the study of protein aggregates associated with neurodegenerative diseases.

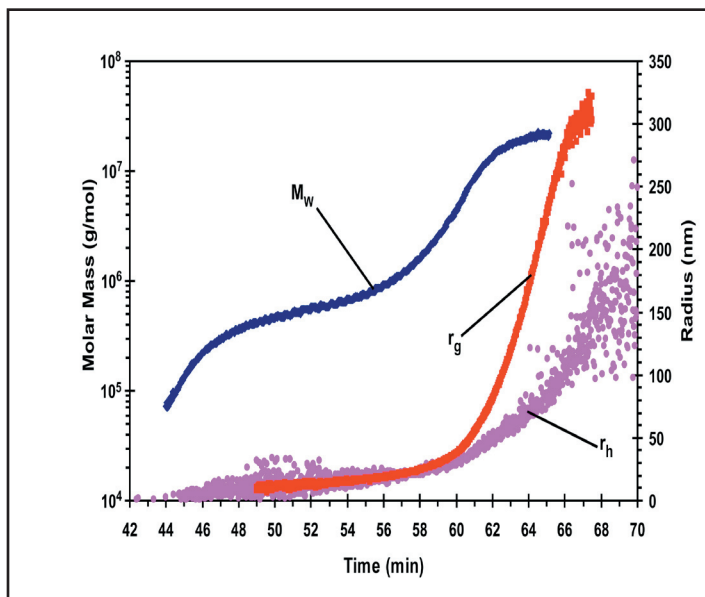


Figure 1: Mass and size analyses of Eclipse-fractionated prion protein aggregates based on data from DAWN EOS, Optilab and WyattQELS instruments.

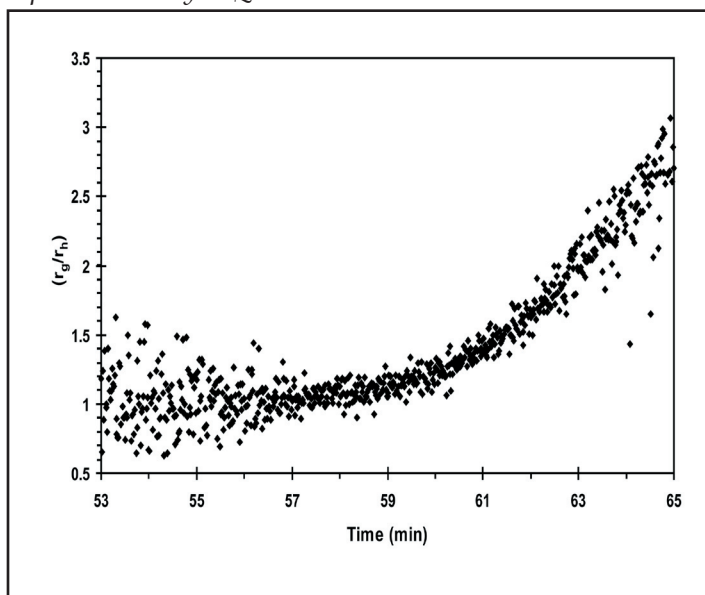


Figure 2: Elution time vs. ρ value (r_g/r_h) calculated from the data shown in figure 1, where ρ values in the range of 0.8 are indicative of compact spherical particles and ρ values >2.0 are typical for extended, rod-shaped particles.

