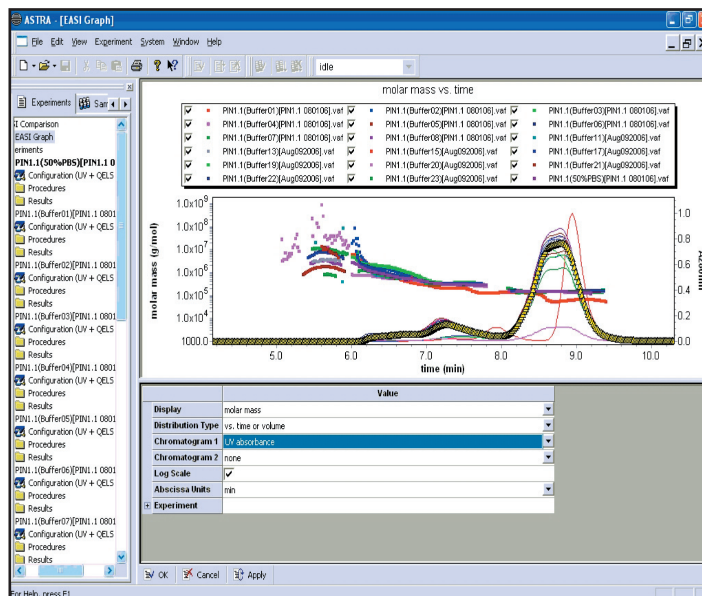
Protein Solutions™

Protein Aggregation States

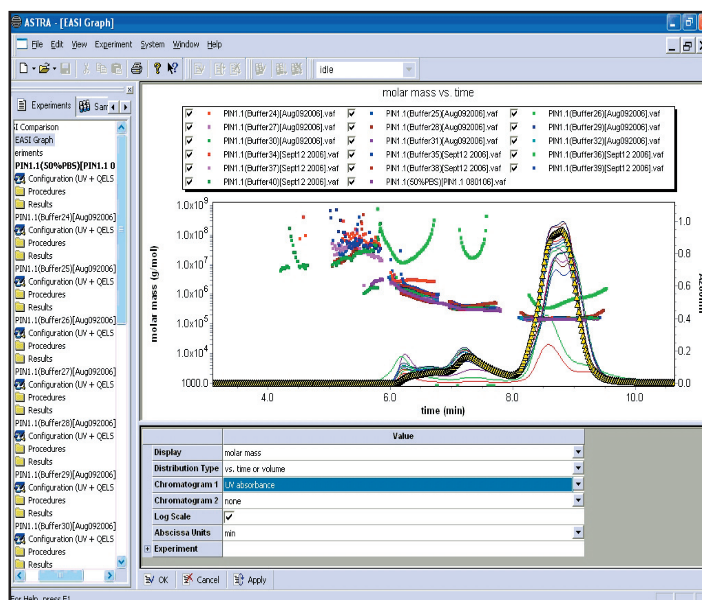
In order to monitor protein aggregation states in different buffers, we employed Wyatt Technology's DAWN HELEOS MALS instrument coupled with a UV detector and an Optilab rEX detector. We hypothesized that using these detectors we could track changes in the protein as the salt concnetrations and buffer solutions were modified, thereby giving us greater insight into the behavior and predictability of the proteins of interest.

The HPLC conditions were as follows: TSK-gel G3000SW column from Tosoh Bioscience, PBS isocratic mobile phase, 1ml/min flow rate, 30min run time. Prior to loading onto the HPLC column, the protein of interest was thoroughly dialyzed into various buffers containing different concentrations of salt spanning 150mM to 500mM as well as different buffer systems with pH values ranging from 5 to 8.5.

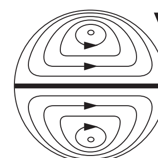


The curves with the yellow triangle labels in the two pictures display the status of the untreated protein. This experiment demonstrates several buffers significantly change the aggregation states of the protein.

The different states of the protein were separated into multiple peaks through SEC-HPLC and the molecular weights for these peaks were determined by MALS simultaneously. The molecular weights detected by MALS correlated very well with those expected for monomer, dimer, trimer and higher oligomers of this protein. As expected, in most of the buffer systems, the protein behaved similarly as the untreated control. However, unexpectedly, the MALS found that in a couple of buffers, the molecular weights were lower or higher than those expected, suggesting salt/pH dependent dissociation or association of the protein subunits. This experiment demonstrates that several buffers significantly change the aggregation states of the protein (the curves with the yellow triangle labels in the two pictures display the status of the untreated protein). Moreover, in-line molecular weight determination by MALS will assist researchers in accurate interpretation of the SEC results.



This note graciously submitted by Zhong-Fu Huang, Boehringer-Ingelheim Pharmaceutical, Inc., Ridgefield, CT 06877



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