

## Peptide Hormone Aggregation by AFFF

Peptides and small proteins comprise one major class of hormones, ranging in molecular weight from 632 (TRH) up to 29,000 (LH). Many of these hormones aggregate, form fibrils and eventually gel when isolated *in vivo*, resulting in limited storage stability. In this study, a proprietary peptide hormone ( $M_w \sim 3500$ ), was evaluated for its aggregation behavior using the Eclipse AFFF.

Size exclusion chromatography did *not* show evidence of large aggregates, even though viscosity and batch light scattering experiments indicated their presence. The SEC results are likely biased by the physical filtering of the aggregates on entering the frit of the SEC column, as evidenced by increased back-pressure *after* injection.

The hormone was dissolved in filtered 0.01 N HCl and separations were performed using an Eclipse AFFF system (1 kDa MWCO membrane, 250  $\mu$ L injection, 0.01 N HCl mobile phase). The solution was injected immediately after dissolving in acid, and then incubated at 22°C for 22 hours, resulting in a partially gelled solution which was injected *without* filtration. The detectors were a DAWN EOS and Shimadzu UV and RI detectors.

The initial sample shows mainly monomeric peptide (Figure 1). The  $M_w$  of aggregates grows from 369,200 g/mol to  $2.337 \times 10^6$  g/mol after 22 hrs of incubation (Figure 2).

The results of this experiment *verify* that large particles present in this hormone solution are aggregates which grow over time as the solution gels. AFFF is an appropriate separation method for analyzing these viscous peptide aggregates which are *not* detected by SEC and can cause increased back-pressure in SEC systems. AFFF is a useful alternative to SEC for understanding the aggregation of peptide hormones *in vivo* and can play an important role in improving our understanding of the aggregation pathways and kinetics.

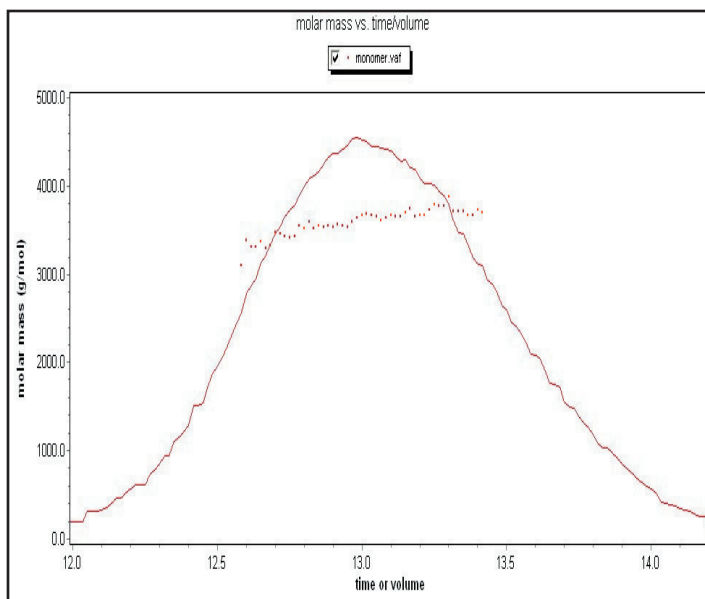


Figure 1. Molar mass of the peptide monomer as obtained from MALS. Weight average molar mass is 3587 g/mol. Rayleigh ratio fractogram is overlaid.

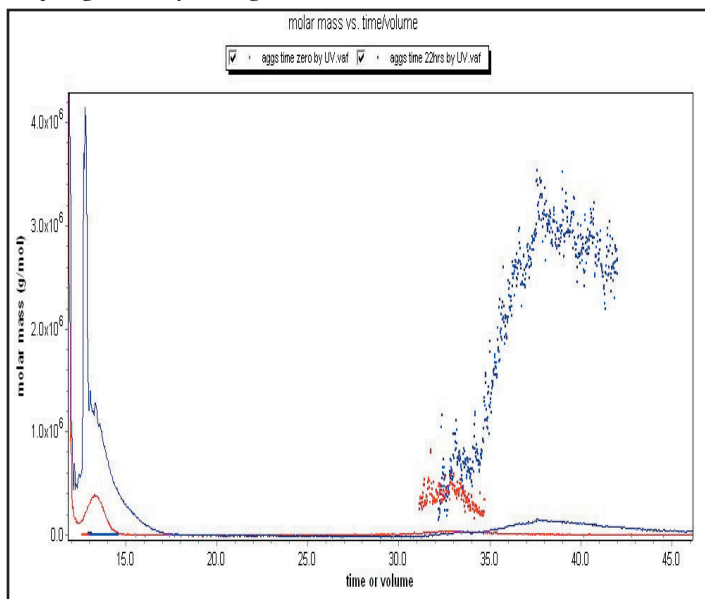
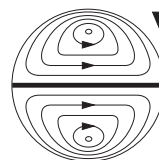


Figure 2. Molar mass of peptide aggregates as obtained from MALS. Weight average molar mass at time zero (red dots) is 369,200 g/mol, and after 22 hours (blue dots) is  $2.337 \times 10^6$  g/mol. Rayleigh ratio fractogram is overlaid.

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