WYATT TECHNOLOGY

APPLICATION NOTE

AN4006: Studying the performance of surfactants as chaotropic agents in protein formulation by DLS

Introduction

Surfactants are routinely incorporated into protein formulations in order to solubilize hydrophobic proteins or to act as chaotropic agents against protein aggregation. The chaotropic effect is a result of entropically favorable protein-surfactant binding, as opposed to inter-protein association or aggregation.

The sample examined in this study is a partially unfolded derivative of a parent compound that was in clinical trials as a treatment for neurological disorders. Versions of this derivative have been successfully used in the past to combat both neurological and venereal viruses. Because of its partial denaturation, the derivative has a tendency to aggregate in aqueous solution. This aggregation can be minimized by the inclusion of surfactants in the drug formulation.

Materials and Methods

The impact of a detergent on the aggregation behavior of the derivative was examined using a DynaPro® dynamic/static light scattering (DLS/SLS) instrument. The DynaPro provides hydrodynamic size and size distributions via its avalanche photodiode DLS detector, and simultaneously apparent molar mass (K*c/R) via its separate, dedicated SLS detector. As little as 2 µL per sample are required for each condition, making the NanoStar particularly attractive for early-stage studies. Data were analyzed by DYNAMICS® DLS software.

Results and Discussion

Figure 1 shows the distribution of radii for the parent compound, the derivative, and the derivative with detergent. In contrast to the high polydispersity observed for the derivative alone, Figure 1 indicated that the derivative-detergent sample is monodisperse. On the other hand, the results also indicate that the derivative-detergent sample is larger than the parent compound.

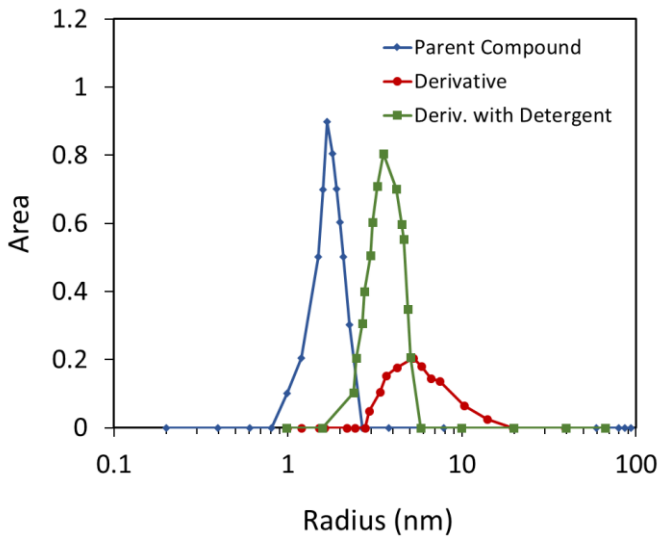

Figure 1: Distribution of radii for parent compound, derivative, and derivative with detergent.

The total intensity results for the derivative and the derivative-detergent are shown in Figure 2. For particles much smaller than the wavelength of the incident light, the molecular weight (M) and concentration (c) dependence of the scattering intensity I at low particle concentration can be described as shown in the equation below, where K^* is a constant.

$$I = K^* cM$$

As evident in Figure 2, the intensity for both samples is linear with concentration, implying that the molecular weight is independent of dilution. These results suggest that static light scattering could be used to determine the

molecular weight of the samples. The static results for the derivative-detergent sample are shown in Figure 3.

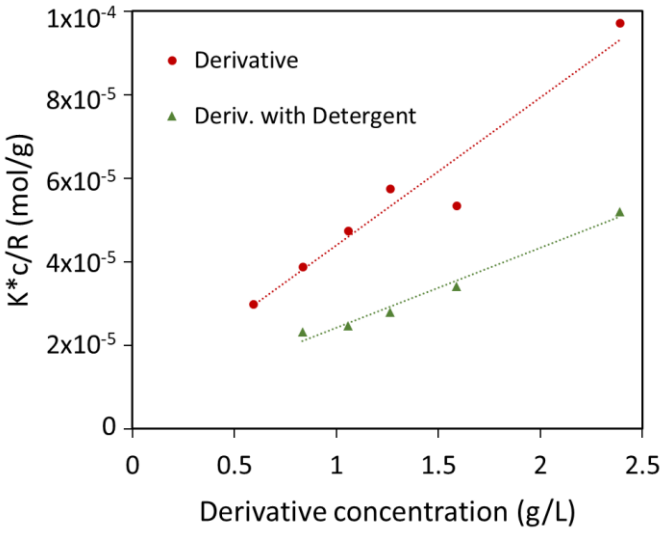

Figure 2. Concentration dependent intensity data for derivative (red circles) and derivative-detergent (green triangles).

The static results indicate a MW of 28 kDa for the derivative-detergent sample. This MW is consistent with gel electrophoresis data which indicates that the derivative is a trimer of 7.8 kDa parent protein in the presence of the detergent.

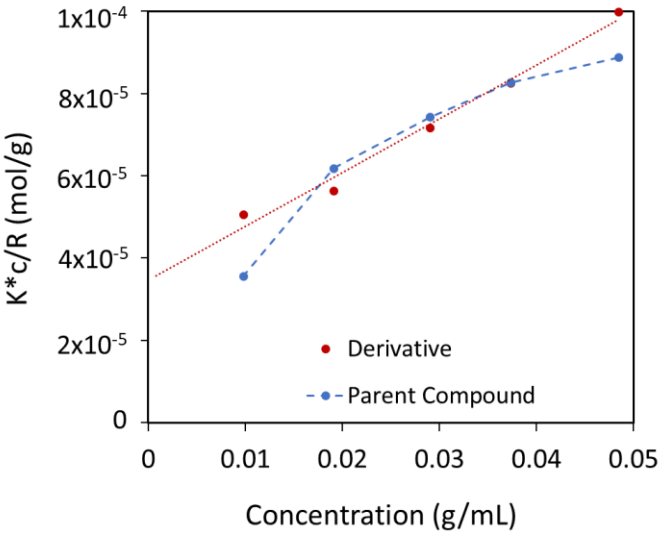

Figure 3. DYNAMICS static-MW model results for the derivative-detergent sample.

Conclusions

DLS/SLS studies using Wyatt's cuvette-based DynaPro instrument provide key supporting data to confirm the beneficial chaotropic effect of a surfactant for formulating a therapeutic protein derivative. The ability to do so with very small sample quantities and without complex preparations or reagents is an added bonus for biopharmaceutical formulators.

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