

Protein Characterization with QELS

As illustrated in Application Note #13 in the category of Synthetic Polymers, on-line quasi-elastic light scattering (QELS) together with MALS detection provides insight into the structure of polymers when both hydrodynamic and root-mean square radii are measured. In this application note, we report how the on-line WyattQELS detector helps to evaluate the quality of the SEC separation and estimate the shape of proteins.

Figure 1 shows the molar masses of Protein X and BSA as a function of elution volume. These measurements were made by a miniDAWN Tristar detector under identical SEC conditions. Protein X is approximately 57 kD, which is very close to 67 kD value for the BSA monomer. Protein X, however, eluted much *later* than the BSA monomer did. The late elution is most likely due to either a much more compact structure of Protein X than that of BSA, or interaction between Protein X and column packing material.

At the same time that the molar mass was being measured by the miniDAWN Tristar, the Wyatt QELS detector was used to measure the hydrodynamic radii (R_h) of these proteins. The results are shown in Figure 2. Protein X has an R_h of 3.0 nm, comparable to an R_h of 3.4 nm for the BSA monomer. This is expected from a globular structure. We conclude that the unexpectedly *late* elution of Protein X is definitely due to column interaction rather than a more compact structure.

In summary, the separation mechanism of SEC can be assessed by combining information of hydrodynamic radius and elution volume, which then helps to develop a better SEC method. Further, the shape of proteins can be estimated by comparing the ratio of the molar mass and hydrodynamic radius to those of proteins with known structure.

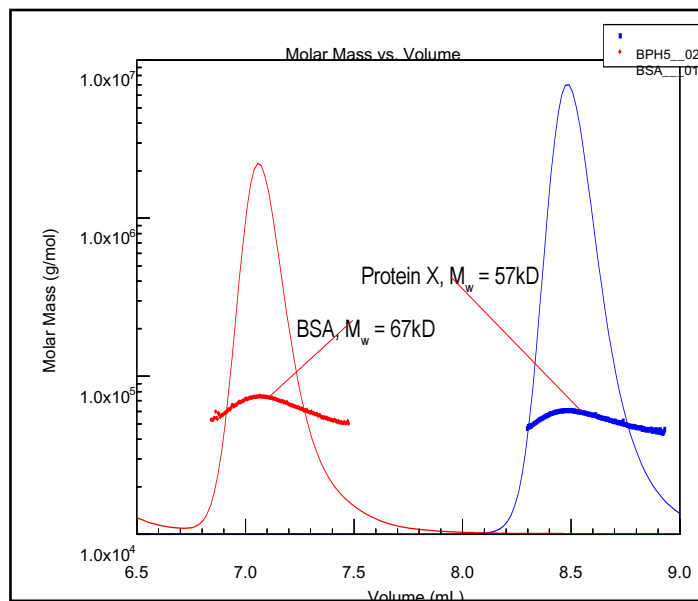


Figure 1. Molar mass versus elution volume plots of BSA monomer and Protein X.

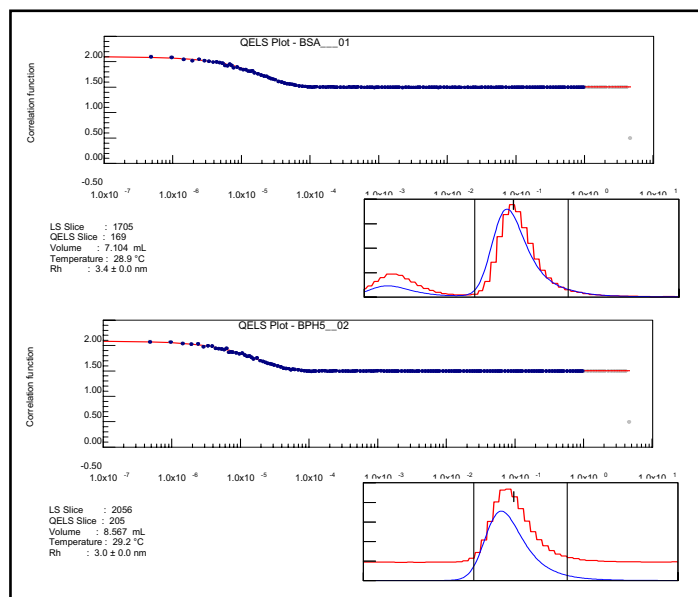
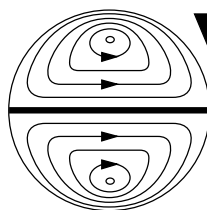


Figure 2. Correlation functions of BSA monomer (top) and Protein X, from which R_h is calculated.



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