Hemoglobin Characterization

Hemoglobin is the primary protein in the red blood cells of mammals. Its essential role causes it to transport oxygen from the lungs to any place in the body where it is needed. When oxygen is released, carbon dioxide binds to hemoglobin to be transported out of the body. Human hemoglobin contains four polypeptides, in which the tetramer equilibrates with a dimer form, depending on the conditions.

Using size-exclusion chromatography (SEC) in combination with multi-angle light scattering (MALS), a miniDAWN detector and Optilab DSP interferometric refractometer were used for the characterization. With this set-up, absolute molar masses and molar mass distributions can readily be obtained.

Figure 1 shows the hemoglobin molar mass *versus* elution time plot. The molar mass across the peak is *not* constant, which is likely due to an equilibrium shift from mostly tetramer to more dimer that took place during the course of chromatography run. With conventional column calibration it would have been difficult—if not impossible—to distinguish regular peak broadening from the small polydispersity of the sample. Yet this was easily determined by our SEC-MALS measurement.

As a standard protein, bovine serum albumin (BSA) is often used for column calibration. Figure 2 illustrates the molar mass *versus* elution time plot of hemoglobin overlaid with that of BSA. The molar masses of the two proteins are very close, however, the shapes and corresponding elution times of the molecules are entirely different.

In contrast, hemoglobin has a more compact structure than BSA, and consequently BSA elutes *earlier* than hemoglobin. If BSA were used as a calibration standard—a common practice in many laboratories—the molar mass of hemoglobin would have been underestimated dramatically. Yet by simply attaching a miniDAWN or DAWN to the chromatography, all of the assumptions and erroneous conclusions can be avoided.

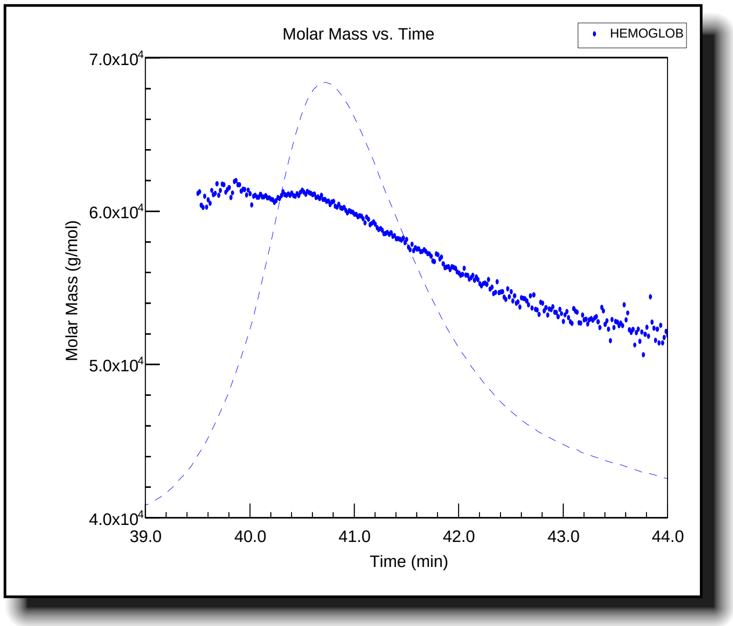

Figure 1. Molar mass versus *elution time of Hemoglobin obtained from SEC using two columns and combined with MALS detection.*

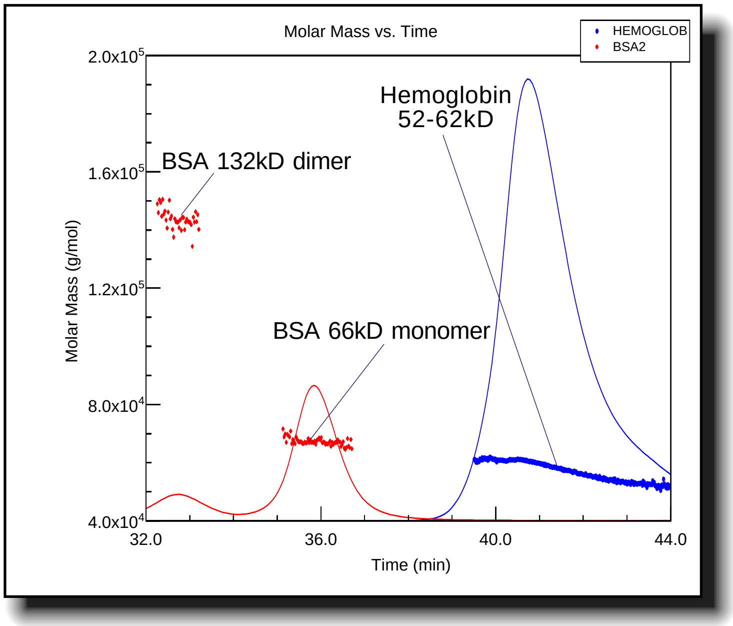

Figure 2. BSA and hemoglobin (proteins with similar molar masses) elute at different volumes, due to the more compact structure of hemoglobin.

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