

Infrared (IR) Laser Utility

When using MALS detectors to characterize polymers that have chromophores, corrections are needed to compensate for the absorption and/or fluorescence at the operating wavelength in order to obtain accurate molar mass determinations. Procedures for making such corrections have been discussed elsewhere (1, 2).

Absorption and fluorescence effects may be eliminated if the light scattering measurements are carried out at a different (generally higher) wavelength. In this application note, we demonstrate the use of an Infrared (IR) laser as the light source in a miniDAWN detector for characterizing allophycocyanin (APC), a fluorescent protein.

APC, isolated from *Spirulina platensis*, a blue-green alga or cyanobacterium, is one of the brightest fluorescent probes used in a variety of assays. It has the excitation and emission maximums of 650nm and 660nm, respectively. Because of its broad absorption and fluorescence spectra, light scattering measurements at wavelengths *lower* than 750nm would introduce significant errors *unless* the fluorescence is corrected.

For this experiment, we used a miniDAWN MALS detector equipped with a 50mW IR laser ($\lambda=785\text{nm}$) to measure the molar mass of the APC protein following separation by size exclusion chromatography. An HP 1100 HPLC system with a Shodex KW 804 column were used to carry out the experiments.

As seen from the plots in Figure 1, the molar mass of the APC monomer is approximately 110 kD, which agrees well with the one in the literature. The results also reveal aggregates in the sample, under the conditions of this analysis.

A miniDAWN with a Gallium-Arsenide laser ($\lambda=690\text{nm}$) was also used to illustrate the erroneous effects of fluorescence on the light scattering measurements. Molar masses of *more than 10 million* Daltons would have been obtained if measured at 690 nm—wrong by a factor of at least 100. It is evident, therefore, that MALS detectors employing different laser options can facilitate the characterization of colored polymers. Indeed, in applications similar to this one, the IR laser greatly simplifies the measurements.

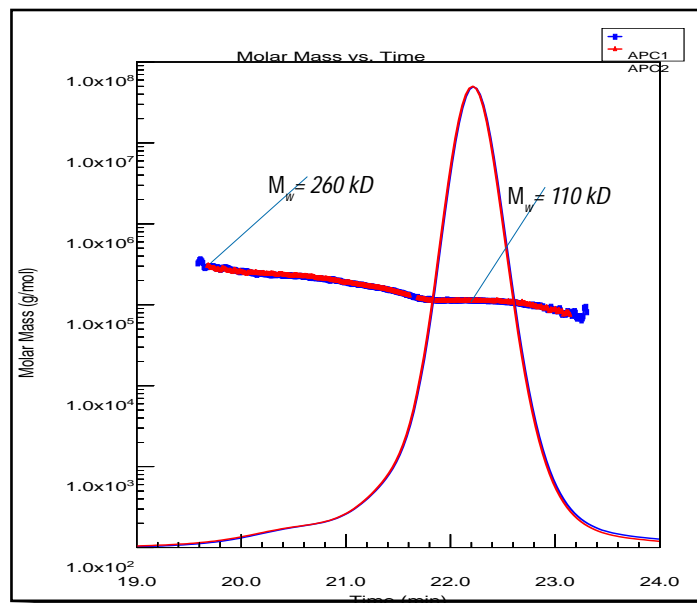


Figure 1. Molar mass versus elution volume of APC protein superimposed with the signal from the UV detector. Note the excellent reproducibility from duplicate injections.

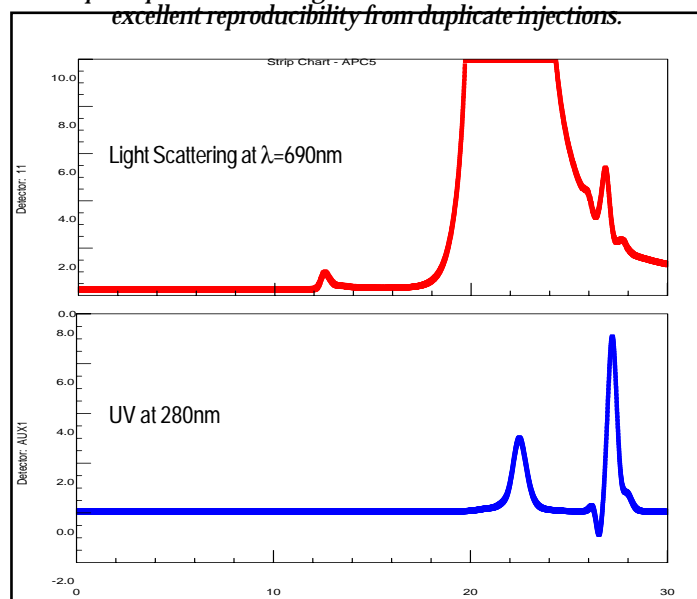
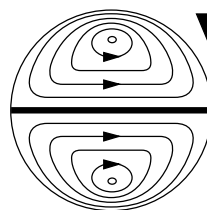


Figure 2. Chromatograms obtained from SEC of the APC protein using a miniDAWN with a solid state laser at 690 nm.

1. "MALS Detection of Chromophoric Polymers", 8th WTC Annual Users' Forum.
2. "Lignins and Fluorescence: Lignosulfonates", Biopolymers, Wyatt Application Note.



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