WYATT TECHNOLOGY

APPLICATION NOTE

AN1612: Protein PEGylation processes characterized by SEC-MALS

Introduction

Polyethylene glycol (PEG) strands are conjugated to therapeutic proteins to increase the half-life of the protein in the bloodstream and make it less likely to be hydrolyzed. Since PEGylated proteins have a very different conformation and hydrodynamic size-molar mass relationship than globular proteins or linear polymers, they cannot be analyzed by standard analytical SEC using column calibration. In this application note we demonstrate the information that can be obtained on the results of a PEGylation process by combining size-exclusion chromatography with multi-angle light scattering (SEC-MALS) to analyze conjugation.

Materials and Methods

A 16 kDa protein was reacted with 5 kDa PEG and analyzed using SEC coupled to three detectors: the HPLC's UV detector, a miniDAWN™ MALS detector and an Optilab® differential refractive index (dRI) detector (SEC-MALS-UV-dRI). The ASTRA® chromatography software acquired data from all three detectors and analyzed the signals using the *Protein Conjugate Analysis* method to determine the total protein molar mass per conjugated molecule, total PEG molar mass per conjugated molecule, and overall molar mass of the conjugate at each eluting fraction.

Results

Among the most significant findings from this SEC-MALS analysis were:

1. Protein-PEG-Protein bonding Since the specific protein is known to contain only 8 possible sites for PEG attachment, the maximum molar mass for the conjugation of PEG to a single protein should be 56 kDa; 16 kDa for the protein plus (8 x 5 kDa) for the PEG. The ASTRA software, however, determined molar masses as high as 100 kDa for the complex, which indicates the presence of protein-PEG-protein complexes. These unexpected complexes could affect dramatically the pharmacological properties of the sample.

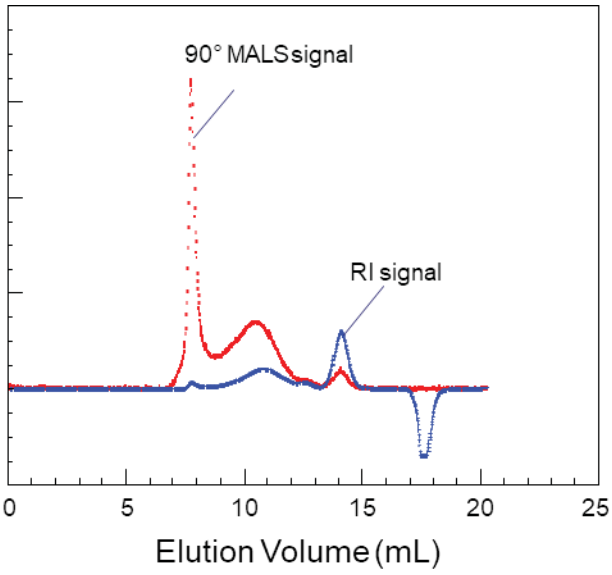


Figure 1. The Peak ID view in ASTRA shows the aggregate at 8.0 mL and the correspondingly large light scattering signal.

2. Degree of PEGylation Since the miniDAWN's molar masses are absolute, the degree of PEGylation can be determined for the PEG-Protein product without making assumptions about conformation.

3. Aggregation detection A small concentration of very-high molar mass (~500 kDa) but very dense (R_g < 10 nm) material elutes at about 8 mL. These aggregates clearly scatter light more than any other sample fractions. Due to the FDA's concerns surrounding aggregates and their potential for creating an immune response in the patient, these aggregates would have to be eliminated prior to clinical trials. SEC-MALS-UV-dRI analysis can help during the requisite process modification by monitoring the decrease in protein aggregation.

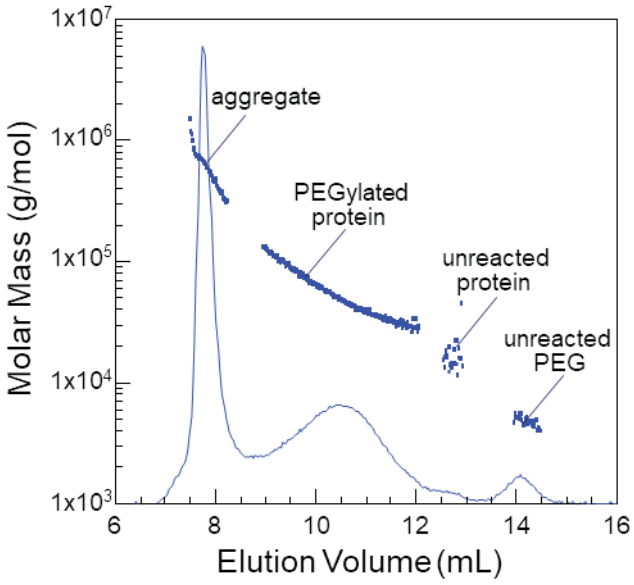


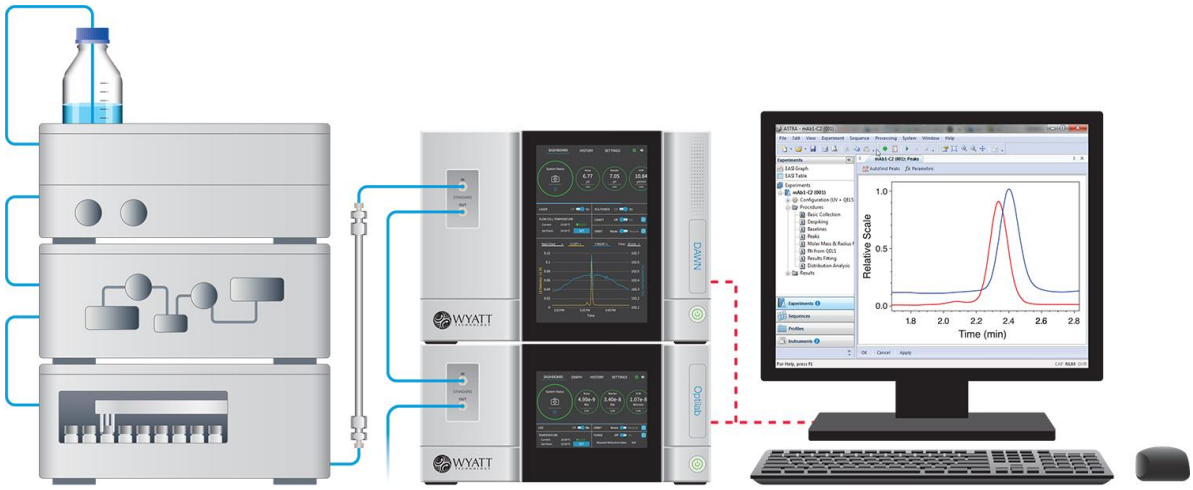
Figure 2. The molar masses, plotted over the 90° light scattering signal, indicate the presence of a large range of molar masses due to different degrees of protein modification.

4. Extent of reaction Based upon the concentration of the unreacted PEG, the extent of the PEGylation reaction can be determined. This information can be used to modify the reaction process in order to improve product formulation effectiveness.

Summary

While SEC alone can provide only semi-quantitative information in the development of PEGylation and other types of conjugation processes, SEC-MALS-UV-RI is an essential experimental technique for such products. Quick, easy and reliable, it provides detailed information on the success of the reaction and the heterogeneity of the reaction products.

More rapid analysis requiring less material can be performed with UHP-SEC-MALS-UV-RI employing a microDAWN™, microOptilab™ and any UHPLC-SEC system.



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