# Protein Solutions™

# Protein PEGylation

The rise of biotechnology has spawned different means of delivering drugs to the human body. Among the many problems encountered is the rapid rejection or clearance of a protein before it has accomplished its task.

A variety of methods exist for modifying proteins so that they are more effective in the bloodstream. Among them, glycosylation and PEGylation are perhaps the most popular. In the former, sugars are attached to the protein, in the latter, polyethylene glycol strands are attached as "whiskers" to increase the half-life of the protein and make it less likely to be hydrolized or cleared from the bloodstr eam.

In this application note, a monoclonal antibody was measured before and after PEGylation. As illustrated in Fig. 1, the antibody has a constant molar mass of 150K Daltons across the entire peak.

Figure 2 shows what happens when the same antibody has been conjugated with PEG molecules of 5K Daltons each. Using a self-consistent, iterative method, the number of PEGs per protein was calculated, and can be seen on the graph itself.

The chromatography set-up consisted of a miniDAWN multi-angle light scattering detector, Optilab DSP interferometric refractometer, HP 1050 HPLC system, and a Pharmacia Superose 12 30/10HR column.

The results are, perhaps, most remarkable for their specificity. Never before has it been so easy to determine the number of PEG attachments to a protein. Now, using the miniDAWN it has become a straightforward experimental procedure.

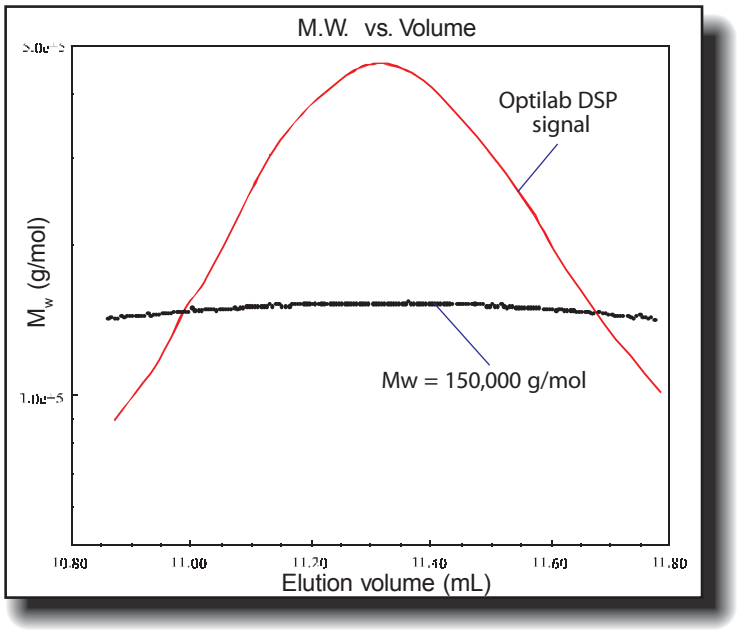


Figure 1. 150K Dalton protein before PEGylation. Notice the constant molar mass across the entire peak.

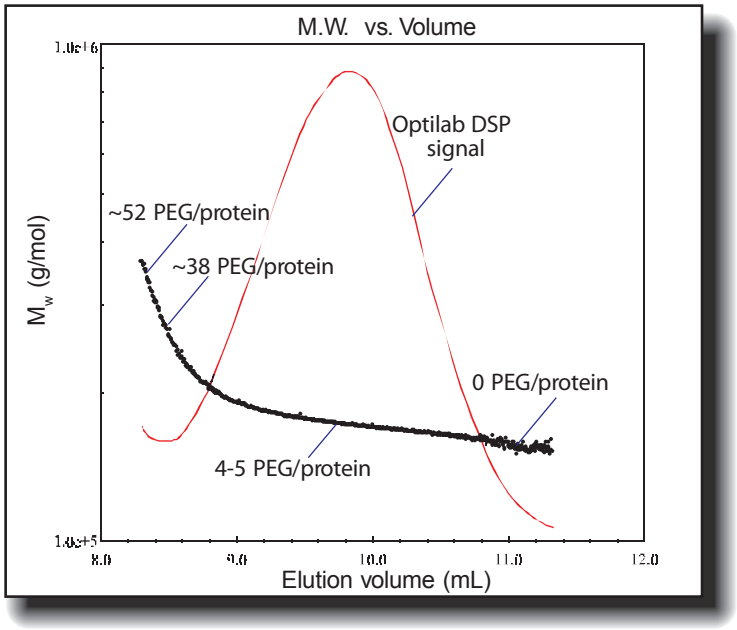


Figure 2. The same protein that has been PEGylated. Notice the dramatic change in molar mass across the peak, depending on the number of PEG attachments.

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