

Heparin Characterization

Heparin is well-known as an anti-coagulant, anti-thrombotic drug. Chemically, it is a linear polysaccharide that is derived from animal tissues. For some time it has been known that heparin is not a homogeneous substance; rather, it is a heterogeneous mixture of molecules ranging in molar mass from less than 5,000 to more than 30,000 Daltons. Heparin can be chemically or enzymatically depolymerized to obtain low molecular weight (LMW) heparin products, which exhibit an improved pharmacological profile.

The U.S. Food and Drug Administration (FDA) has scrutinized LMW-heparin products closely due to their inherent variability and polydispersity. Previously, these materials had to be characterized by *relative* techniques involving viscometry or standard elution-based chromatographic methods. Fortunately, the advent of multi-angle laser light scattering combined with SEC means that authentic calibration standards derived from heparin are no longer necessary. LMW-heparins may now be characterized *absolutely*—without regard for the molecules' elution time or conformation in solution.

For this application note, Scientific Protein Laboratories (Waunakee, WI), a subsidiary of American Home Products Corporation, analyzed three commercial LMW heparin products. They employed a Waters 510 pump and 401 refractometer, a Shodex OHpak SB-803HQ analytical column (8.0 x 300 mm) with an SB-G guard column, and a miniDAWN, triple angle light scattering instrument. The mobile phase was 0.1 M ammonium acetate, and the specific refractive index increment, dn/dc was measured off-line in a Wyatt/Optilab DSP operating at the same wavelength as the miniDAWN. Details of this study were published in *Analytical Biochemistry* **245**, 231—241 (1997).

The results show unequivocally the power of the miniDAWN to resolve the three LMW-heparin products, each of which was manufactured by a different depolymerization process. Wyatt Technology's ASTRA software indicates that clear differences exist in these products' differential molar mass distributions (Fig. 2).

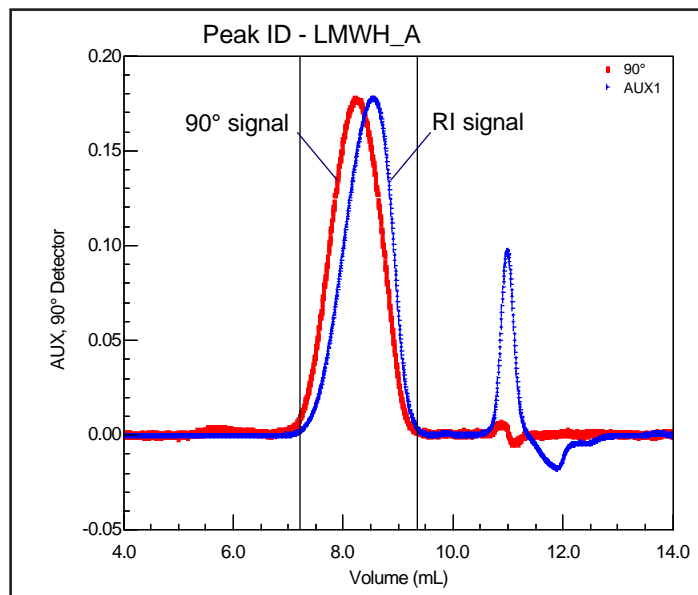


Figure 1. One of the three samples displayed by ASTRA. Note the different response of the miniDAWN and the RI detector at the same elution volumes.

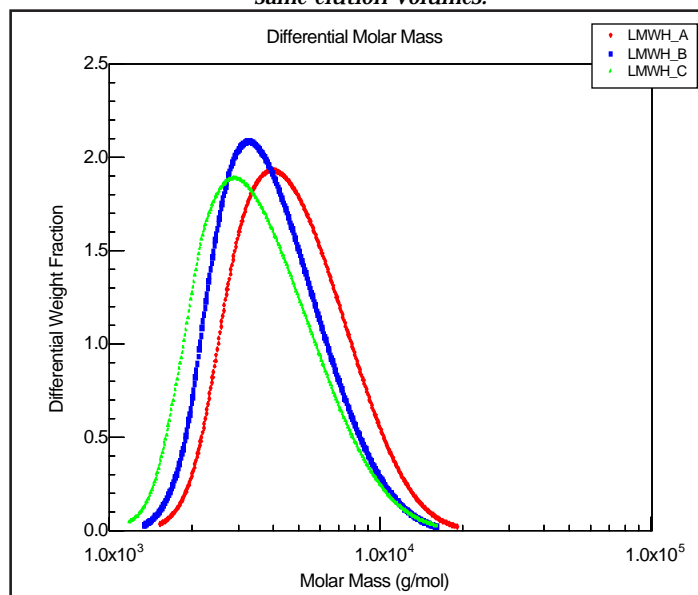
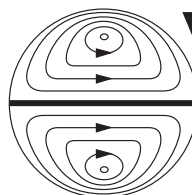


Figure 2. The Differential Molar Mass Distributions tell the entire story: the three samples are different! An indication of different derivitization processes.



**Wyatt
Technology**
CORPORATION

6300 Hollister Avenue ■ Santa Barbara, CA 93117
Phone (805) 681-9009 ■ FAX (805) 681-0123
E-mail: info@wyatt.com ■ URL: <http://www.wyatt.com>

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