

DNA Characterization

Since the seminal work of Crick and Watson in the early 1950's it has been commonly accepted that DNA is the macromolecule that encodes hereditary information for all known living organisms and most viruses. Characterizing DNA, especially its *size*, has played a critical role in biomedical research, diagnosis and forensic science.

The size of DNA is commonly measured by electrophoresis and is detected by the fluorescence of ethidium bromide, a potent carcinogen, which has raised many safety and environmental concerns.

For this Application Note, a DAWN multi-angle laser light scattering (MALLS) detector was connected to an HPLC system with a size exclusion chromatography (SEC) column to determine the molar mass and size of a super-coiled circular duplex DNA. This DNA often shows multiple bands on a gel as a result of different degrees of superhelicity (a phenomenon which frequently causes confusion in size measurements using electrophoresis).

The results from these multi-angle light scattering measurements prove that the DNA has a uniform molar mass and root-mean square radius of 1.77 million Daltons and 82 nm, respectively. The superb agreement between the theoretical value for this DNA molecule, 1.73 million, and the results obtained by the light scattering measurement show the remarkable and profound ability of the DAWN detector to determine absolute molar masses *without* making assumptions about this intriguing molecule's size, shape or composition.

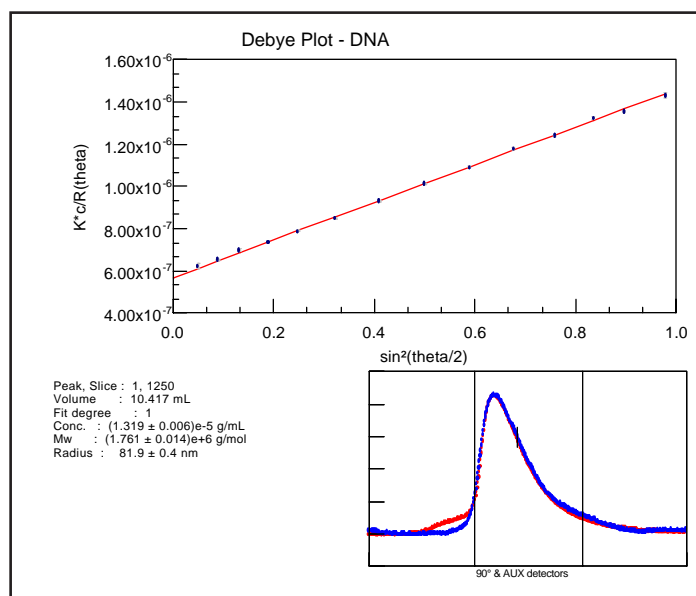


Figure 1. The Debye plot of a "slice" of the DNA chromatogram showing the large radius at a given elution volume.

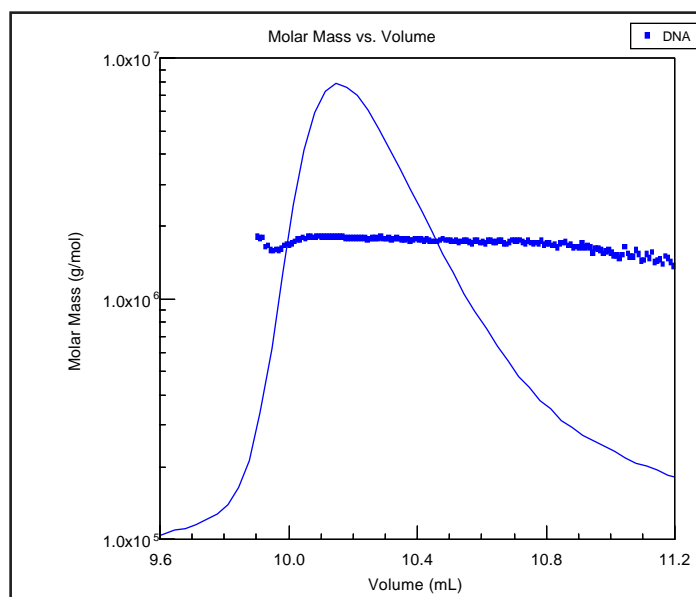


Figure 2. ASTRA's Molar Mass vs. Volume plot shows the constant mass of the DNA molecule over the entire peak width.

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