WYATT TECHNOLOGY

APPLICATION NOTE

AN9009: Determination of *dn/dc* with an Optilab®

Introduction

The term *dn/dc*, also called the "specific refractive index increment", represents the change in the refractive index (*dn*) of a solution with respect to a change in its solute concentration (*dc*). The measurement of *dn/dc* is an essential parameter required to analyze multi-angle light scattering for the absolute characterization of molar mass. The *dn/dc* value is unique for each combination of solute, solvent, wavelength and temperature, so it is vital to measure it at the *same* wavelength as the light scattering apparatus and experimental conditions.

Measuring dn/dc with Optilab

Online differential refractive index (dRI) measurements generally serve to measure the concentration of eluting macromolecules. dRI is required for analytes such as polymers which have no intrinsic UV response, and is even commonly used for proteins since the response–quantified as *dn/dc*–is nearly identical for all proteins and there is no need to identify the eluting protein peak or know it's UV absorption coefficient.

The Optilab differential refractometer is capable of measuring dRI online or offline, and in addition to measuring unknown concentrations, it can be used to determine *dn/dc*. Since the Optilab is used in combination with a DAWN®, miniDAWN® or microDAWN® MALS instrument for molar mass determination, the fact that the Optilab operates at the same wavelength as the MALS instruments (658 nm) means that the measured *dn/*dc corresponds to the MALS wavelength to ensures maximum molar mass accuracy.

Measuring *dn/dc* offline is preferred because determining the value online makes numerous assumptions about the total mass which has eluted. In the measurement, several aliquots of known concentrations are injected into the Optilab refractometer. Pure solvent is usually injected before each sample for baseline signals, and after the final sample as a return to the baseline.

As shown in Figure 1, generally 6 aliquots are injected into the refractometer, and may be viewed in the ASTRA® software. Each peak corresponds to an aliquot injected at a certain time, and the "plateaus" of each peak indicate the corresponding differential refractive index for each aliquot concentration.

Using the differential refractive index data measured in combination with the known concentrations entered by the user for each peak, the ASTRA software automatically produces a linear fit. Figure 2 shows the results of this procedure where the slope of the linear fit is assigned as the *dn/dc* value for the injected solute/solvent pair and statistical uncertainty is calculated by the software.

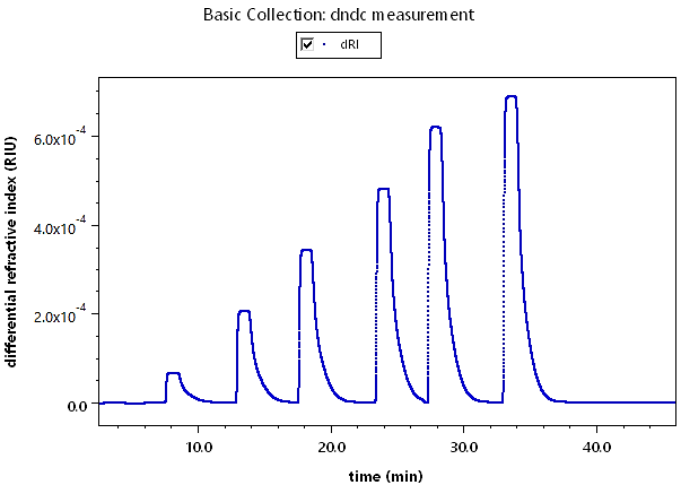

Figure 1. For *dn/dc* analysis, refractive index should be measured at six different concentrations.

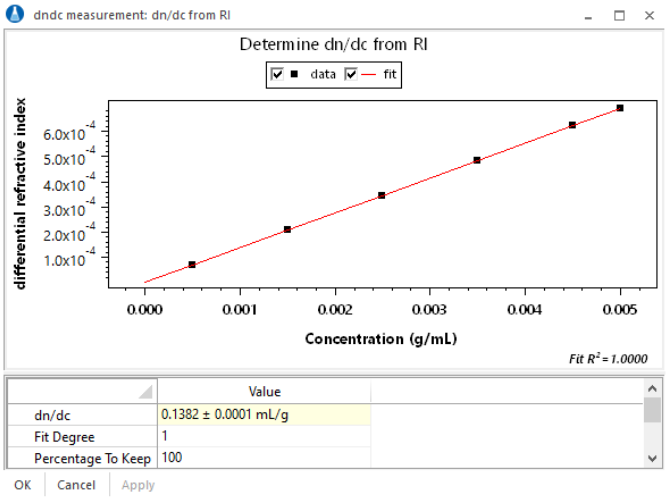

Figure 2. ASTRA software will determine the *dn/dc* value by fitting the data from Figure 1 to a line with zero intercept.

Summary

The result of this procedure is a fast, accurate, highly-reproducible value for *dn/dc* which you can use with WTC's light scattering instruments and software to determine absolute molar masses—without making any assumptions. The Optilab provides the ideal platform for making *dn/dc* determinations routinely and precisely. And, when it is not being used for these offline measurements, it is generally used online as an RI detector of unparalleled range and sensitivity.

Click below to request more information on the Optilab and its unqiue capabilities as a universal concentration detector for online and offline measurements:

Optilab Info Request