APPLICATION NOTE

Molecular weight measurement of Polyethylene Oxide & Dextran

Introduction

Molecular weight determination of polymers provides useful information such as stability, structure and complexation. Several techniques exist for measuring molecular weight but either yield relative values or have low resolution. However, one method of characterizing molecular weight is light scattering from macromolecules in solution. The relationship between molecular weight and scattering intensity is based on the Brownian motion of the particles in solution. This scattered light is referred to as Rayleigh scattering which is when the particles' scattered light is much smaller than the wavelength of the incident light.

DYNAMIC LIGHT SCATTERING

Microtrac Dynamic Light Scattering (DLS) instruments of the Nanotrac series are capable for measuring the molecular weight along with particle size of macromolecules. One big advantage is the simultaneous measurement of particle size and molecular weight over a concentration range.

The Nanotrac DLS product line consists of the Nanotrac Flex, Wave II and Wave II Q which measures particle size, zeta potential, concentration, and molecular weight. The difference in design gives the choice between a wide array of measurement cells used to satisfy the need of any application. It also allows the measurement of samples over a wide concentration range, monomodal or multimodal samples independent of prior knowledge of particle size.

Calculation of molecular weight

Nanotrac's series of DLS instruments has the ability to measure and calculate the molecular weight (MW) of particles suspended in solution. The molecular weight calculation utilizes the Debye plot which is comprised of the particles' total light scattered (Rayleigh scattering) at known concentrations. The Debye equation (eq.1) relates the MW to the Rayleigh ratio and concentration, (C_{WV}, g/mL) used to generate the linear plot.

In order to determine molecular weight from the Debye Plot, the value of the differential index of refraction, dn/dc, is required for the molecular solution being measured. Microtrac's software provides a means of measuring dn/dc by utilizing the magnitude of the Fresnel reflectance from the sapphire window of the Nanotrac probe. In operation the light collected from a known water/sapphire reflectance is compared with the light collected from a molecular suspension with a known C_{WV}.

$$\frac{KC}{R} = \left(A_2 C + \frac{1}{MW}\right) \quad \text{(Eq. 1)}$$

Where the Rayleigh ratio, $R = \frac{I_{scat}}{I_0}$,

A= 2nd viral coefficient

K is the optical constant= $\left(\frac{4\pi^2}{\lambda^4 N_A}\right)(n_0^2)\left(\frac{d_n}{d_c}\right)^2$

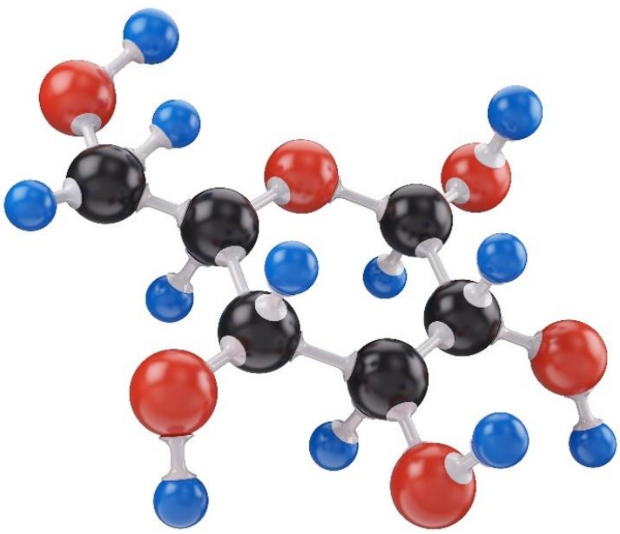
N_A = Avogadro's number = 6.03 x10^{23},

λo= instrument laser wavelength = 780nm,

n_o = index of suspending medium = 1.333 for water,

$\frac{d_n}{d_c}$ = differential index of refraction.

A plot of $\frac{KC}{R}$ versus C yields the intercept of $\frac{KC}{R}$ at C =0 is equal to $\frac{1}{MW}$. The slope of the plot is A, the 2nd Virial Coefficient, a measure of the molecular interactions at high concentration. The value and sign of A is important especially in polymer solutions. If A positive, then particle-solvent interactions are favorable, whereas a negative value suggests aggregation. Microtrac's MW analysis separates the molecular suspension scattering intensity from interfering larger particle scattering intensity by utilizing the Brownian motion signature measured with the Nanotrac dynamic light scattering techniques. The ability to separate the interfering scatter avoids complex filtering of molecular suspensions.



Experimental

Polyethylene oxide (PEO) and dextran polymers were prepared by serial dilution at concentrations of 0.01, 5.00x10^{-3}, 2.5 x10^{-3}, 1.25 x10^{-3} g/mL and mixed for 30 minutes using a magnetic stir plate. The value of dn/dc was automatically calculated in the Microtrac FLEX software using the input concentration value, C_{wv}, of the suspension. As a result, the optical constant K was also calculated. Weight concentrations in ascending order were then collected for size measurement and used to create the Debye plot. Data from the size measurement generated data points to be used for the calculation of the molecular weight.

Results

DLS was used to obtain the particle size distribution, dn/dc and molecular weight of polymers dextran and PEO. **Figure 1** shows the Debye plot which is calculated from the experimental data of dextran. Since the measurement of MW using the Debye plot technique depends on accurate measurement of the scattered light from a molecular suspension of known concentration, the R2 of 0.98 shows good agreement between $\frac{KC}{R}$ and the concentrations. Though not shown, PEO correlation coefficient was 0.9.

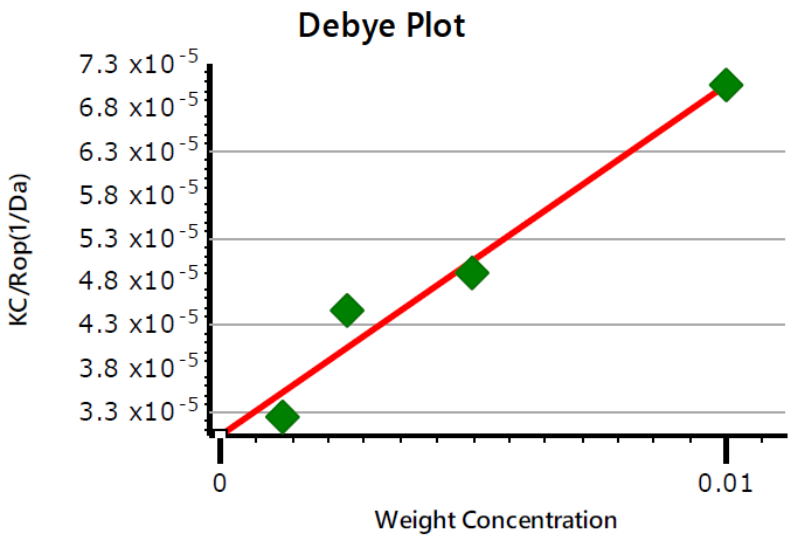


Figure 1. Dextran Debye plot measured with the Nanotrac Flex with a correlation coefficient of 0.98.

Table 1 shows the calculated data from both polymers. The MW and second viral coefficient for each polymer were calculated using linear regression of the Debye plot (eq1). The MW is found by taking is the inverse of the intercept at a concentration zero. The observed molecular weight of dextran and PEO was 33,000 Da and 80,100 Da, respectively. The theoretical values are in good agreement with both percent errors remaining under 20%. In addition, the second viral coefficient values demonstrated the particles had good affinity with the solvent (d_i H_2O) with no aggregation ($A > 0$).

Table 1: Calculated results for dextran and PEO from Microtrac's FLEX software molecular weight utility that are derived from the Debye plot seen in **Figure 1.**

Polymer	Intercept, $\frac{1}{Da}$	$\frac{dn}{dc}$	$A, \frac{mL\,Da}{g}$	Molecular weight, Da	Mean Diameter, nm
Dextran	3.00x10^{-5}	0.14	4.02x10^{-3}	3.33x10^{4}	10.5
PEO	1.25x10^{-5}	0.13	3.12x10^{-3}	8.01x10^{4}	20.1

Figure 2 shows the composite set of particle size distribution of dextran at each of the concentrations used to calculate the MW. This composite data shows the mean diameter to be consistent at all concentration ranges, with good stability over the course of all measurements within a very short time frame. This measurement not only enables the precise determination of the molecular weight, but it also provides information about the dispersion stability along with the second viral coefficient reported in **Table 1**. This simultaneous measurement of particle size with MW data shows the powerful calculation capability of the Microtrac Nanotrac flex and the FLEX software coupled with the high sensitivity detection.

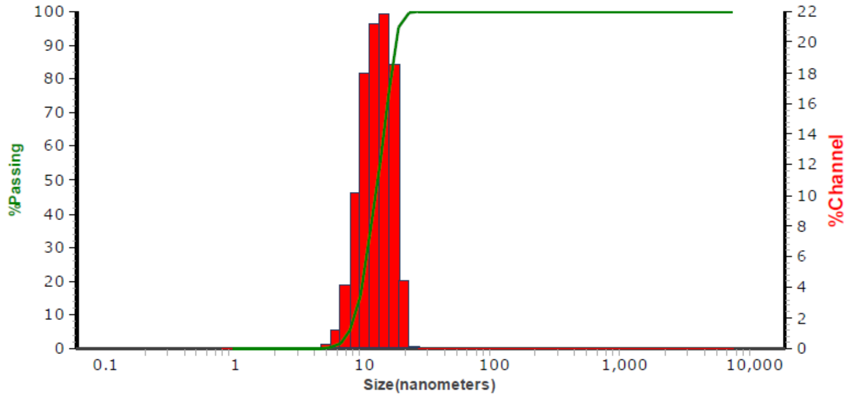


Figure 2. Composite particle size distribution of dextran at different concentration profiles calculated in Microtrac's FLEX software

Summary

We have shown Microtrac's DLS Nanotrac line can simultaneously get size, aggregation and weight while working with polymers, proteins, and biological molecules. The purpose of this application note is to provide further information on Microtrac's DLS analyzers ability to establish molecular weight calculations via the Debye plot technique when used with particle size distributions. Molecular weight determination via light scattering is a quick and useful tool for additional characterization of polymers and proteins' properties.