Is It Alright To Estimate dñ/dC?

Due to the complexity of measuring the specific refractive index increment (dñ/dC), static light scattering molecular weight measurements are often conducted using an estimated dñ/dC value. When making such an estimate however, it is important to recognize the effect of error in the estimate on the resultant molecular weight and 2nd virial coefficient values calculated from the SLS measurement. The Rayleigh equation utilized for single angle static light scattering measurements can be written in the form shown below, where ñ is the solvent refractive index, R_θ is the Rayleigh ratio of scattering intensities, λ is the wavelength of the incident light, N_A is Avogadro's number, A_2 is the 2nd virial coefficient, and C is the concentration.

$$M = \left[\frac{2\pi \tilde{n}^2}{R_\theta \lambda^4 N_A} \left(\frac{d\tilde{n}}{dC} \right)^2 C - 2A_2C \right]^{-1}$$

After differentiating with respect to dñ/dC and rearrangement, the relative errors in the resultant molecular weight and 2nd virial coefficient values can be expressed as:

$$\frac{\Delta M}{M} = (1 + 2A_2CM)\left(\frac{\Delta\, d\tilde{n}/dC}{d\tilde{n}/dC} \right) \qquad \frac{\Delta A_2}{A_2} = \frac{1 + 2A_2CM}{A_2CM}\left(\frac{\Delta\, d\tilde{n}/dC}{d\tilde{n}/dC} \right)$$

As evident in the above expressions, the relative error in the molecular weight and 2nd virial coefficient values is dependent not only upon the error in the dñ/dC estimate, but also upon the magnitude of the M and A_2 values and the concentration range over which the measurements are collected. Figure 1 shows a graphical representation of the influence of dñ/dC error on the calculated molecular weight and 2nd virial coefficient values for a low molecular weight protein (lysozyme) under near theta solvent conditions ($A_2 \sim 0$). For this specific example, the relative error in MW and A_2 is approximately twice the relative error in the dñ/dC estimate.

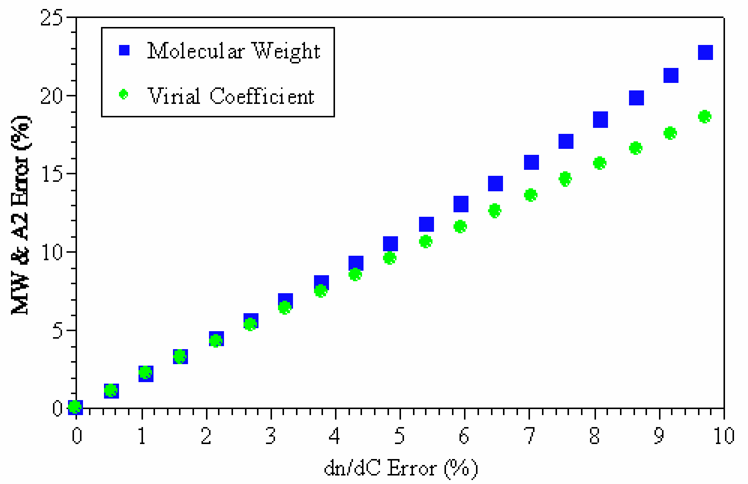

Figure 1: Relative error in molecular weight and 2nd virial coefficient as a function of error in the dñ/dC estimate for lysozyme under near theta solvent conditions.

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The specific refractive index increment is a property associated with a given particle-solvent pair, under a specific set of conditions. It is dependent upon the temperature, the wavelength of the light used in the measurement, the conformation of the particle, and the presence of additives in the dispersant. Ideally, dñ/dC should be measured under the appropriate solution conditions and at the specified wavelength, prior to beginning any static light scattering experiment. In the absence of the ideal, then yes, dñ/dC can be estimated, but one should take the time to insure that the estimate is reasonable. For most static light scattering samples, the dñ/dC value will fall within a range of 0.1 to 0.2 mL/g, with 0.185 being a commonly used value for globular proteins in aqueous buffer and 0.1 being a commonly used value for linear polymers in organic solvents. Since this range amounts to 100% variation, it is instructive to examine the influence of various parameters on the dñ/dC value of the analyte-solvent pair.

Wavelength Effects

The wavelength dependence of dñ/dC is expected to follow the Cauchy relationship, with dñ/dC being proportional to λ^{-2}. Figure 2 shows an example of this λ^{-2} dependence of dñ/dC for a series of globular proteins in water. Note that the dñ/dC values in this and subsequent figures within this document are extracted from Chapter 6 of Light Scattering From Polymer Solutions (Ed. M.B. Huglin; Academic Press: New York, 1972).

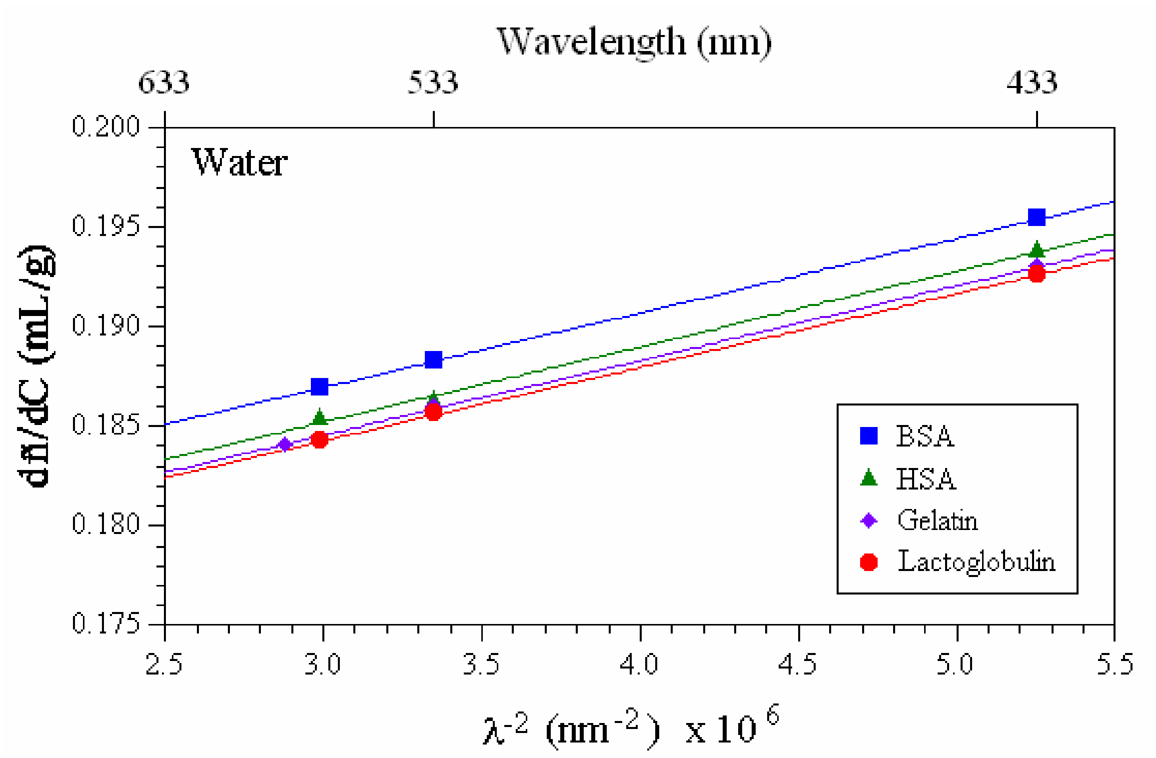

Figure 2: Influence of wavelength on dñ/dC for a series of globular proteins in water.

Of particular interest regarding the above figure, is the observation that the slope $[\Delta(\text{dñ/dC})/\Delta\lambda^{-2}]$ is independent of protein type. As discussed in the following section, this observation should not be too surprising, in that the refractive index (ñ) is strongly dependent upon the partial specific volume (inverse density), which tends to be relatively constant for globular proteins.

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The data in Figure 2 also helps to justify the 0.185 rule of thumb value for dñ/dC for globular proteins. The standard laser wavelengths for light scattering instruments are 633 and 532 nm. For this wavelength range, the range of dñ/dC values for the globular proteins shown in Figure 2 is 0.182 to 0.188, or better stated as 0.185 ± 1.5%.

Conformation Effects

As noted earlier, the independence of $\Delta(dñ/dC)/\Delta\lambda^{-2}$ on protein type is likely a consequence of the globular nature of proteins. The refractive index of a solution is a function of the solution density. For globular proteins, the specific volume ($1/\rho$) is relatively independent of protein type (v_{sp} = 0.73 cm^3/g). Hence the similarity between the dñ/dC and $\Delta(dñ/dC)/\Delta\lambda^{-2}$ values for the globular proteins examined here. As shown in Figure 3 however, significant changes in both dñ/dC and $\Delta(dñ/dC)/\Delta\lambda^{-2}$ are observed when the macromolecules are coiled, rather than globular. With regard to error, if one were to use the 0.185 rule of thumb value for proteins for a static light scattering measurement of Dextran, the ~25% error in dñ/dC could propagate into very large errors in the calculated molecular weight and 2nd virial coefficient values.

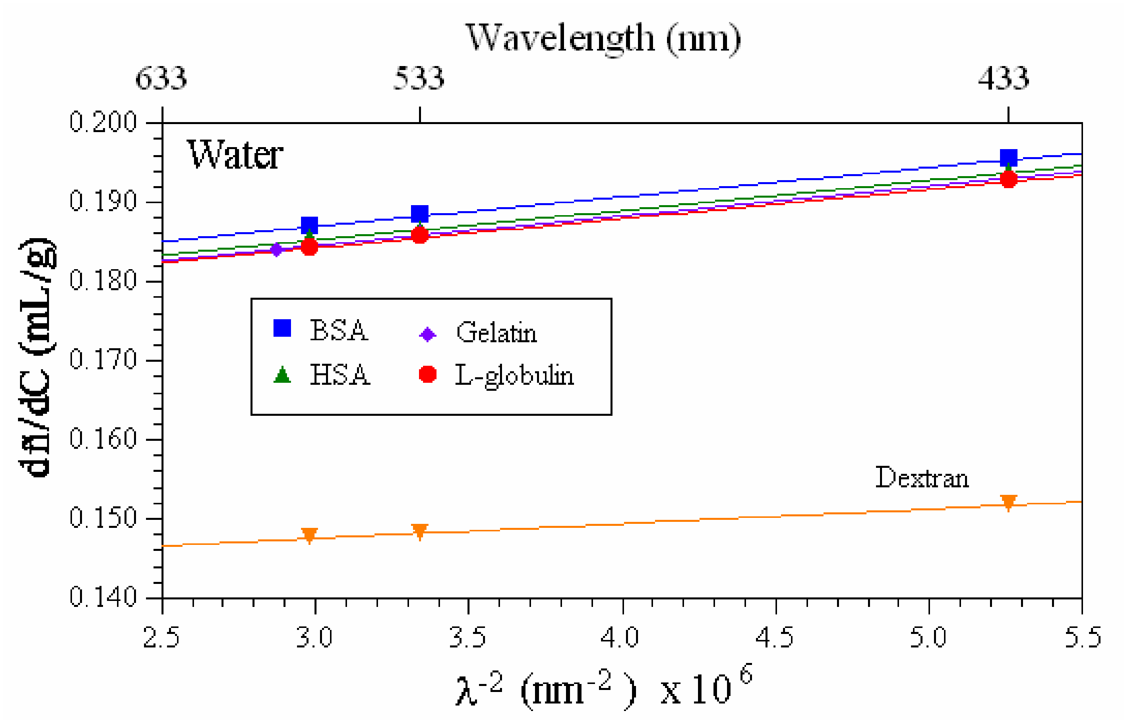

Figure 3: Comparison of dñ/dC values for Dextran, a random coil polysaccharide, to those of a series of globular proteins.

Additive Effects

Additives can influence dñ/dC in two ways. First, the incorporation of additives can change the refractive index of the dispersant or medium surrounding the particle of interest; and second, the presence of additives can lead to a change in the conformation of the particle being measured. Examples of the former effect can be seen in Figure 4, which shows the influence of salt on the dñ/dC values for BSA and lactoglobulin, two globular proteins reported to have stable conformations under the conditions cited. As seen in this figure, the addition of salt leads to a decrease in dñ/dC, but has little to no influence on the wavelength dependence [$\Delta(dñ/dC)/\Delta\lambda^{-2}$].

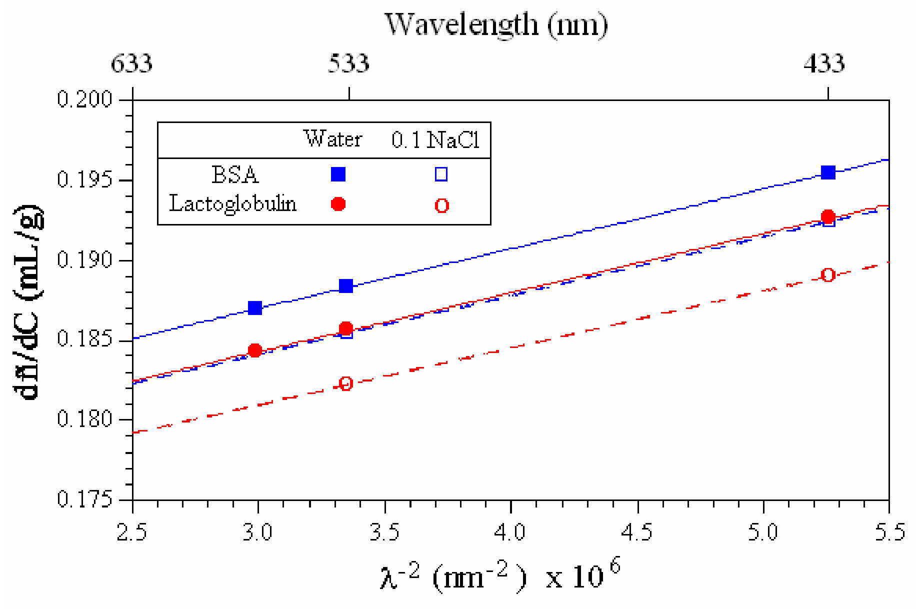

Figure 4: Influence of salt on dñ/dC values for two globular proteins, BSA and lactoglobulin.

The latter effects, i.e. conformational effects, are difficult to deconvolute from dispersant refractive index effects. However, the influence of salt dependent molecular conformation can be inferred from the dñ/dC data shown in Figure 5, which shows the effects of NaCl concentration on the dñ/dC values for a globular protein and a linear polyelectrolyte at 25 C and 633 nm wavelength. As salt is added to the PAA solution, charges are shielded and the polymer collapses into a more condensed conformation, with the dñ/dC value approaching that of a globular protein at higher salt concentrations.

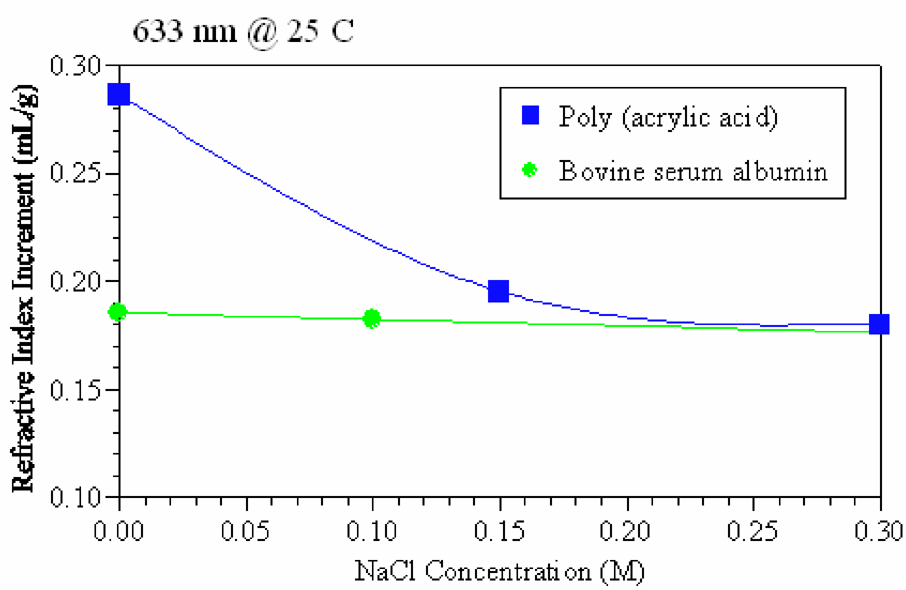

Figure 5: Influence of salt/conformation on dñ/dC values for poly(acrylic acid) & BSA.

Temperature Effects
As noted throughout this document, both the refractive index (ñ) and specific refractive index increment (dñ/dC) are closely correlated with the density of the solvent and particle being measured. As such, one would also expect to see a distinct temperature dependence in dñ/dC values. This dependence is exemplified in Figure 6, which shows the influence of temperature on the dñ/dC values for the series of globular proteins examined earlier. The similarity in the Δ(dñ/dC)/ΔT trends is a direct consequence of the similarity in the partial specific volumes (1/ρ) of the proteins.

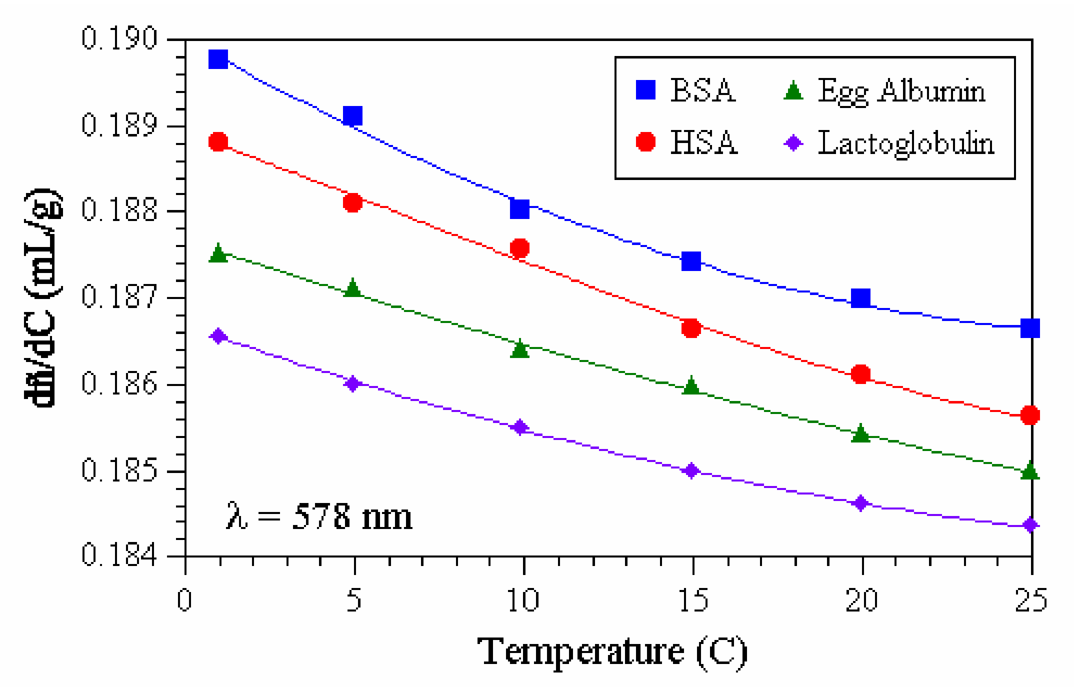

Figure 6: Influence of temperature on the dñ/dC values for a series of globular proteins in water at a wavelength of 578 nm.

Molecular Weight Effects
If the analyte being measured is a linear polymer, molecular weight is another factor that can influence the dñ/dC value. Figure 7 shows the molecular weight dependence of the dñ/dC value for polystyrene in toluene at 25 C and 633 nm. For MW > 1.7 kDa, the refractive index increment values are consistent with the rule of thumb of 0.1 for linear polymers in organic solvents. But for MW < 1.7 kDa, the dñ/dC values drop by ~20% to 0.8 mL/g.

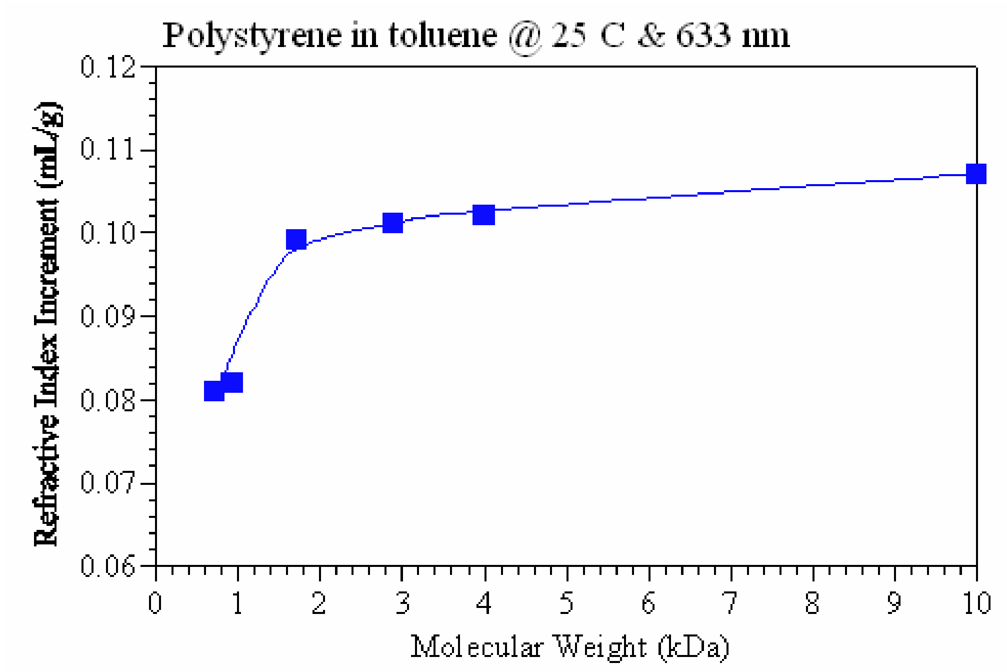

Figure 7: Molecular weight dependence of the dñ/dC value for polystyrene in toluene at 25 C and 633 nm.

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