

# Estimating Molecular Weight

Nicomp

## OVERVIEW

It is possible to use the Nicomp to estimate the average molecular weight, MW, of particles or macromolecules suspended in solution. The word "estimate" is emphasized because of the fact that the measurement of molecular weight using the technique of dynamic light scattering (DLS) is not as accurate as the determination of particle size, for which all DLS-based instruments are primarily designed.

## INTRODUCTION

The fundamental quantity measured in a DLS-based instrument is the particle diffusivity, or diffusion coefficient, D. There is a simple empirical formula that can be used to relate the MW of a suspended particle (or macromolecule) to D:

$$D = \alpha \text{ MW}^{-\beta} \dots\dots\dots(\text{Equation 1})$$

The pre-factor constant,  $\alpha$ , is related to the specific composition of both the diffusing particles and the surrounding solvent. The pre-factor depends on the temperature, T, and solvent viscosity,  $\eta$ , as well as the mass density of the particles. Constant  $\beta$  in the exponent is related to the shape/configuration of the particles and macromolecules (which may also be a function of particle and solvent composition, as in the case of polymers). Exponent  $\beta$  depends on the relationship between the hydrodynamic radius and the MW for simple spheres,  $\beta$  equals  $1/3$ .

From Equation 1, a simple, general expression for the MW, as a function of D is shown in Equation 2:

$$\text{MW} = (\alpha/D)^{1/\beta} \dots\dots\dots(\text{Equation 2})$$

In the Nicomp (Figure 1), MW is computed from Equation 2 using the mean D (intensity-weighted) obtained from the simple Gaussian Analysis. Values for  $\alpha$  and  $\beta$  are entered in the Control Menu shown in Figure 2.



Figure 1. Nicomp DLS system

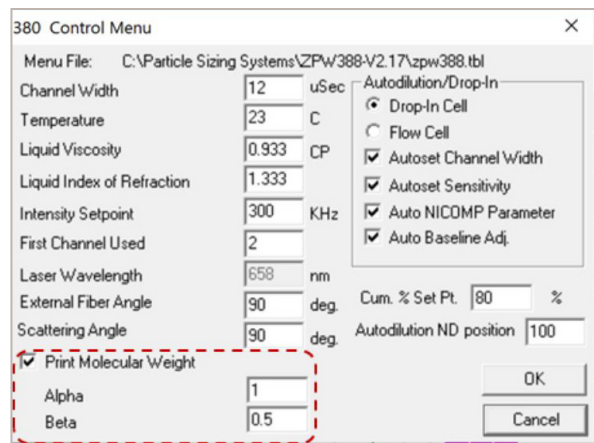


Figure 2. Nicomp control menu

In order to obtain reliable estimates of the mean MW of the particles, reliable estimates of constants  $\alpha$  and  $\beta$  must be available. Values for a few representative polymer/solvent systems are listed in Table 1.

Polymer	Solvent	T	$\alpha$	$\beta$
Polyacrylamide	Water	20°C	$8.46 \times 10^{-4}$	0.69
Polyacrylonitrile	DMF	25°C	$3.2 \times 10^{-4}$	0.63
Polyacrylonitrile	DMF	35°C	$2.19 \times 10^{-4}$	0.58
Polyvinyl acetate	MEK	20°C	$7.8 \times 10^{-4}$	0.63
Polystyrene	MEK	25°C	$3.1 \times 10^{-4}$	0.53
Polyisoprene chloroform	MEK	20°C	$3.5 \times 10^{-4}$	0.42
Poly methyl methacrylate	Ethyl acetate	20°C	$1.61 \times 10^{-4}$	0.48
Polyvinyl alcohol	Water	20°C	$5.5 \times 10^{-10}$	0.68

Table 1.  $\alpha$  and  $\beta$  for the particle/solvent systems

## SAMPLE RESULT

Immunoglobulin G (IgG) lyophilized powder (Athens-Research) was prepared at 1% weight percent by dissolving in PBS buffer and then diluting to 10 mg/mL. The particle size and molecular weight estimate analysis was performed using the conditions shown in Figure 3.

The sample was analyzed seven times; Control Menu settings and the final result showing both particle size and MW results are shown in Figure 4.

The expected MW value for IgG is 145 kDa or 1.45e5 in Daltons, the units shown in Figure 4. The value is slightly higher than the reference value, probably due to aggregation and/or non-expert sample preparation.

## CONCLUSIONS

The Nicomp DLS system is primarily a particle size and zeta potential analyzer. Better and more preferred techniques are available to determine the MW of polymers and proteins, but an estimated value can be calculated using the approach described in this document.

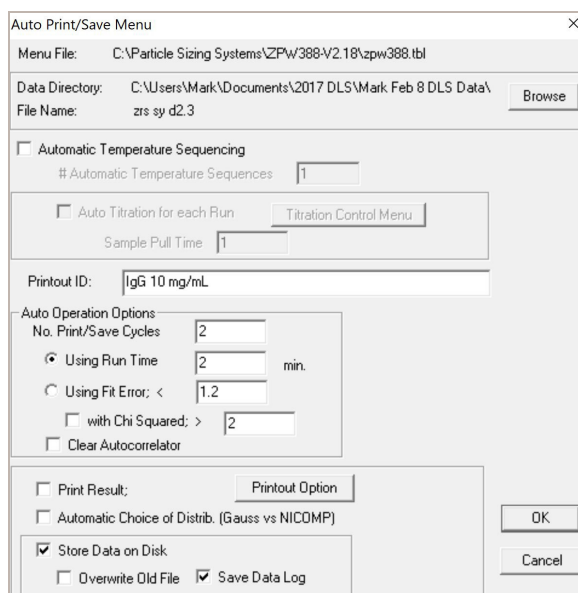


Figure 3. Auto print/save menu

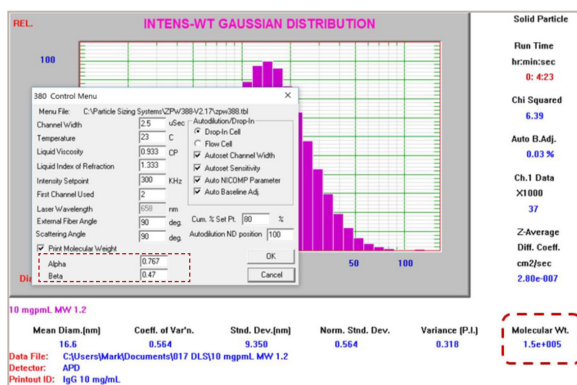


Figure 4. Size and MW results for IgG

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