Rapid Determination of Molecular Weight (M_w) and Second Virial Coefficient (B_{22}) of a Monoclonal Antibody (mAb) by Debye Analysis using ARGEN™

Fluence Analytics

INTRODUCTION

Protein aggregation generally occurs as the result of either conformational or colloidal instability. Conformational stability is the free energy difference (ΔG) between folded and unfolded states. Although not direct measurements of free energy, melting temperature value (T_m) and aggregation temperature (T_{Agg}) can be used as qualitative assessments of conformational stability. Colloidal stability is determined by the balance of repulsive and attractive intermolecular interactions between protein molecules to conserve the native folded state. Simply stated, the propensity for aggregation is reduced by less intermolecular interaction. Therefore, determination of the second virial coefficient (B_{22}) is a valuable screening tool to predict aggregation propensity. ARGEN™ is the ideal tool to assess quantitative and qualitative properties of all classes of biotherapeutics and determine T_{Agg}, B_{22}, molecular weight (M_w), as well as changes in weight average molecular weight (aggregation) under various thermal, chemical, and mechanical stressors. This technical note outlines the method in which ARGEN™ is utilized to determine the second virial coefficient (B_{22}) and molecular weight (M_w) of a monoclonal antibody.

ARGEN™: SMART & RAPID THERAPEUTIC BIOPOLYMER DEVELOPMENT

ARGEN is a high throughput tool for rapid assessment of the stability and viability of therapeutic proteins, peptides, and biopolymers. The instrument uses a multi-stressor testing platform powered by static light scattering detection and intuitive data processing. These features enable teams to develop biologic formulations up to 16-fold faster.

HOW ARGEN™ WORKS

ARGEN utilizes fixed angle (90°), SMSLS (simultaneous multiple sample light scattering) technology which provides rapid, real-time, continuous data collection for characterizing qualitative and quantitative properties of target molecules. The device is equipped with 16 independently controlled sample cells, permitting the user to establish thermal, chemical, and mechanical (stirring) stress parameters on each sample concurrently. This allows for a highly flexible approach to experimental design.

ARGEN™ INTUITIVE CONTROL SOFTWARE

The ARGEN control software features an intuitive interface for all aspects of experimental design and independent control of each cell for parallel parameter adjustment and real time data processing.

EQUATIONS & ROADMAP FOR DETERMINATION OF M_w & B_{22} USING STATIC LIGHT SCATTERING (90°)

STEP 1: CALCULATING THE EXCESS RALEIGH RATIO AT 90°, *I*(90)

ARGEN detects the intensity of scattered light (90°) from a solution subjected to a vertically polarized laser source. The intensity of the scattered light is directly proportional to the size and concentration of the molecules analyzed as defined by the Zimm equation (**Equation 3**) and determined by solving for the excess Rayleigh ratio (**Equation 1**).

Below is the equation used to calculate the excess Rayleigh light scattering intensity "*I*" at a 90° scattering angle:

$$I(90) = \frac{[90Scat_{sample} - 90Scat_{solvent}]}{90Scat_{reference}} \times I_{Abs,Tol} \times F$$

Equation 1

- ***90Scat*$_{solvent}$** is the scattering intensity of the solvent or buffer only (minus protein). This value is automatically stored for each cell when a "Solvent Baseline" experiment is performed.
- ***90Scat*$_{reference}$** is the scattering intensity of the reference (toluene). This value is automatically stored for each cell when a "Reference Baseline'' experiment is performed.
- ***I*$_{Abs,Tol}$** is the absolute Rayleigh scattering ratio for toluene at the laser wavelength. ARGEN uses a laser with λ = 660nm, therefore $I_{Abs,Tol}$= 1.19E-5 cm^{-1}.
- *F* is determined by the optical correction factor of the instrument. For ARGEN this value is ***F = 0.95.***

STEP 2: M_W & B_{22} DETERMINATION USING THE ZIMM APPROXIMATION

When determining the molecular weight of a synthetic or natural polymer, the Zimm approximation is made at a low concentration when $q^2 \langle S^2 \rangle_z \ll 1$. The following equation can be used for a polydisperse polymer population:

$$\frac{K \times Conc.}{I(\theta)} = \frac{1}{M_W}\left(1 + \frac{q^2 \times \langle S^2 \rangle_z}{3}\right) + 2 \times B_{22} \times Conc.$$

Equation 2

- ***Conc.*** is the concentration of the sample (mg/ml or mg/cm^3).
- $q^2 \langle S^2 \rangle_z$ is the term for the z-average mean square radius of gyration.
- B_{22} is the 2nd Virial Coefficient for the sample dissolved in solvent.
- ***K*** is the optical component for vertically polarized light and is determined from the following equation:

$$K = \frac{(2\pi)^2 \times n^2 \times \left(\frac{dn}{dc}\right)^2_{sample}}{(\lambda \mathrm{cm})^4 \times N_{Avogadro}}$$

Equation 3

- ***n*** is the index of refraction of the pure solvent
- $\left(\frac{dn}{dc}\right)_{sample}$ is the differential index of refraction increments of a solvent with respect to the concentration of sample dissolved in that solvent.

ARGEN automatically determines the value of ***K*** upon entry of ***n*** and $\left(\frac{dn}{dc}\right)_{sample}$ on the experiments page.

Within the limitation of measuring scattering at 90°, the Zimm Equation reduces to:

$$\left.\frac{K \times Conc.}{I(90)}\right|_{q=90} = \frac{1}{M_W} + [(2 \times B_{22}) \times Conc.]$$

Equation 4

Or simply:

$$y = b + mx$$

Equation 5

With these values, a Debye plot can be generated using **Equation 6**:

$$\frac{K \times Conc.}{I(90)} vs. Conc.$$

Equation 6

The Y-intercept is equal to $\frac{1}{M_W}$ of the molecule in solution, and the slope of this plot is equal to 2x the virial coefficient (B_{22}) as displayed in **Figure 3.**

EXPERIMENTAL METHODS

SAMPLE PREPARATION: SOLVENT & STANDARD (TOLUENE) SCATTERING BASELINE DETERMINATION

A 2 ml stock solution of [mAb] = 0.05 mg/ml was prepared and subsequently syringe-filtered using a 0.22 µm cellulose acetate filter. All data was collected at 10 Hz. After establishing solvent baseline and standard scattering intensities, filtered buffer solution was used to dilute the stock mAb sample to [mAb] = 0.05 mg/ml. Since there is a significant increase in scattering intensity between the solvent and mAb solution, the neutral density (ND) filter was adjusted to mitigate scattering signal saturation. Next, the sample was serially diluted, and scattering intensities were collected for each dilution in series. When the scattering signal was <20% of the maximum, the ND filter was adjusted to increase signal intensity as depicted in **Figure 1** (steps **C5** and **C6**). Normalized scattering intensities for each dilution are shown in **Figure 2**.

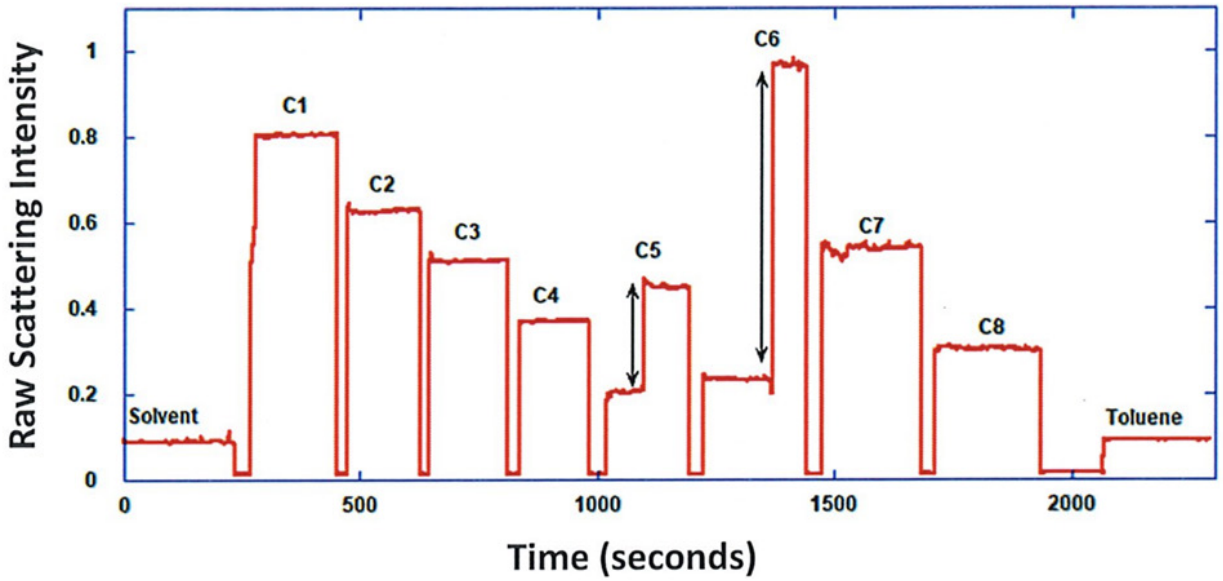

Figure 1: Raw scattering intensities for solvent, mAb dilution series and toluene

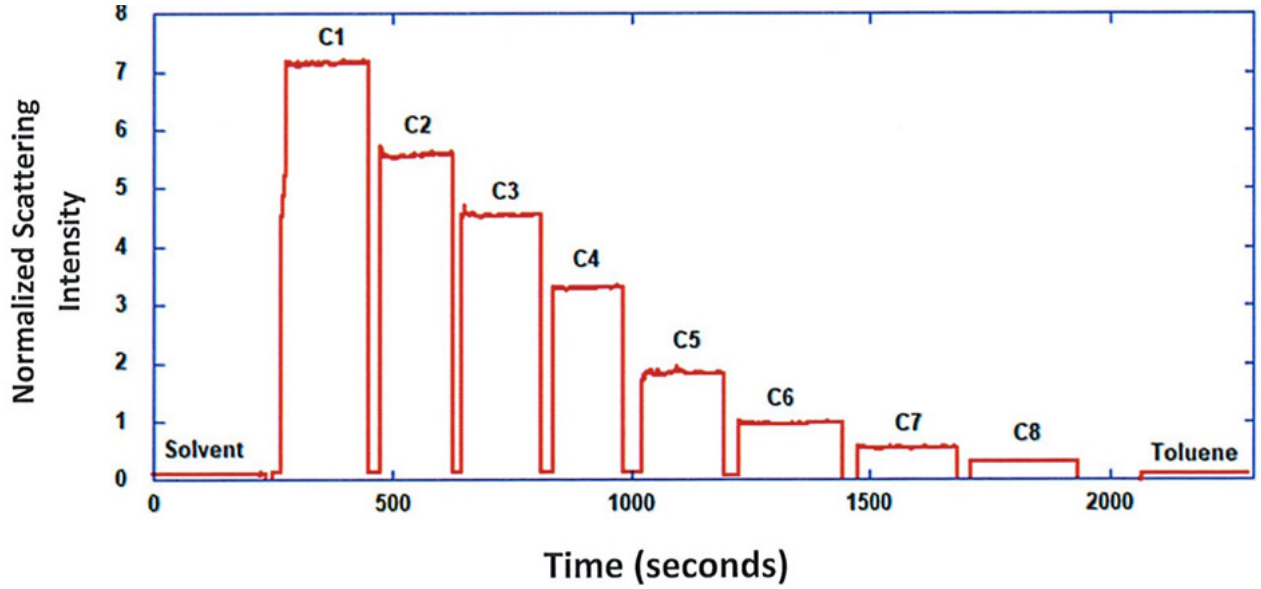

Figure 2: Normalized scattering intensities for solvent, mAb dilution series and toluene

EXPERIMENTAL RESULTS

PARAMETERS FOR DEBYE PLOT

Raw scattering intensities were scaled and normalized to zero neutral density. Scaled scattering intensities (Scaled SLS) for each dilution, solvent, toluene as well as Rayleigh scattering intensities (I(90)(cm^{-1}))) and values of $K^*Conc.$ / I(90) (mol/g) are shown in **Table 1**.

[mAb] (mg/ml)	Scaled SLS	I (90) (cm^{-1})	K*Conc / I (90) (mol/g)
Solvent	0.091	NA	NA
C1 = 0.0500	7.275	1.05E-03	9.91E-06
C2 = 0.0333	5.589	8.07E-04	8.63E-06
C3 = 0.0250	4.525	6.51E-04	8.02E-06
C4 = 0.0167	3.283	4.69E-04	7.43E-06
C5 = 0.0083	1.819	2.54E-04	6.86E-06
C6 = 0.0042	0.970	1.29E-04	6.74E-06
C7 = 0.0021	0.535	6.52E-05	6.67E-06
C8 = 0.0010	0.310	3.22E-05	6.76E-06
Toluene	0.092	NA	NA

*Table 1: Sample concentrations (g/ml), scaled scattering intensities Scaled SLS), Rayleigh scattering intensities (I(90) (cm^{-1}))), and K*Conc./I(90) (mol/g)*

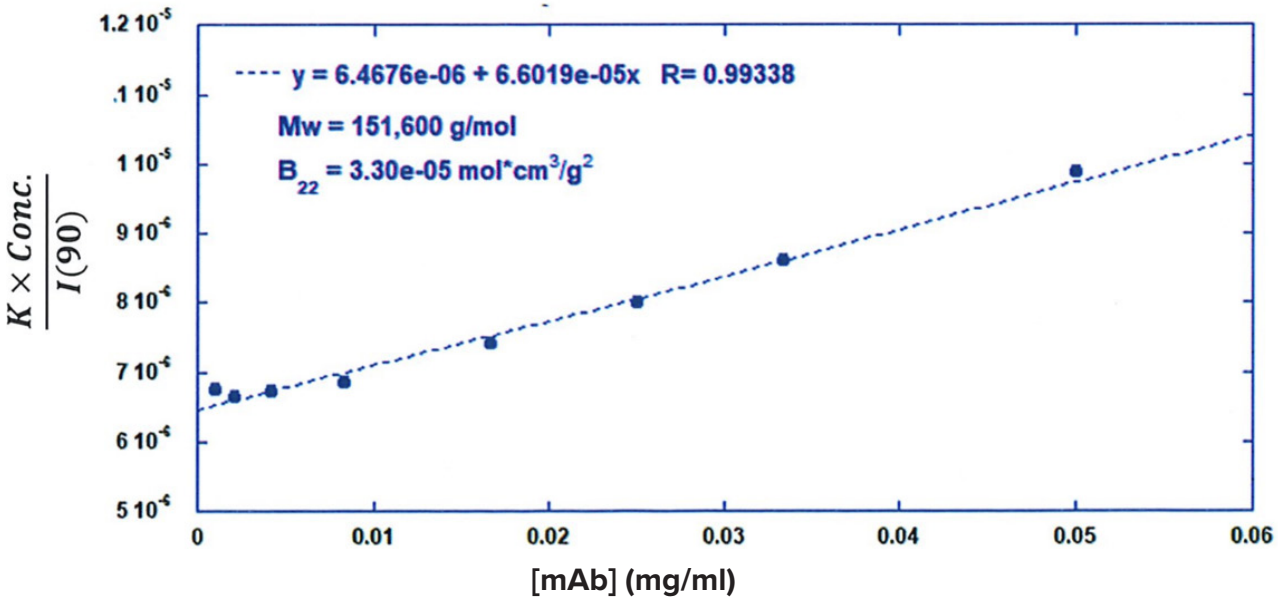

Figure 3: Debye plot for molecular weight and second virial coefficient determination for a mAb

DEBYE PLOT ANALYSIS

To determine K (optical component), the index of refraction for the buffer solution was assumed equal to that of pure water, therefore, n = 1.33. Additionally, the published differential index of refraction increment for monoclonal antibodies is dn/dc = 0.185 cm^3/g with K= 2.089E-7 mol*cm^{2}g^{-2}.

The Y-intercept of the linear fit (regression) indicated that the molecular weight of monoclonal antibody = 151,600 g/mol, and the second virial coefficient is 3.3E-05 mol*cm^3/g^2 (**Figure 3**). These values correlate with reference values found in literature.

CONCLUSION

These experiments demonstrate the utility of ARGEN to determine molecular weight (M_w) and the second virial coefficient (B_{22}) of a monoclonal antibody. The quantitative and qualitative measurements permitted classification and an understanding of the propensity for aggregation in a variety of solution conditions. Furthermore, the high throughput capacity of ARGEN allows users to analyze up to 16 samples or conditions simultaneously, vastly reducing time and resources required for development.