Instruction Manual for Using the

BI-ZPMwA Zimm Plot Software

Molecular Weight Determination using Batch Mode

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Please Read

This is your instruction manual using your Brookhaven BI-ZPMwA Batch Mode software. Please read it carefully before making measurements. The How To section describes how to install the software and what the manual covers. You may familiarize yourself with some of the features of this software by reloading data files (Files/Database). Then choose one or more of the plots by clicking on Graphs in the main menubar. If you have any questions or suggestions, please contact Brookhaven Instruments.

Software is never really finished: there are always additions and changes. As these become available, they will be added to the back of this manual as appendices. Please look at the appendices if you can not find the answer to your questions in the main part.

Please contact the factory at info@brookhaveninstruments.com if you have questions. Please refer to the instrument model number (BI-MwA), the serial number, and date of manufacture as indicated on the identification sticker affixed to the instrument. Please refer to the BI-ZPMwA software version number found by clicking Help on the main menubar. Please also indicate from whom the instrument was purchased.

Remember the old saying: "When in doubt, read the instruction manual." Sometimes the solution to your problem has already been addressed. You just need to find it. Thanks for purchasing a Brookhaven.

This program requires Windows 9X or higher and at least 8 Mb of RAM.

Important Warning

Never allow any liquid to dry inside the instrument. Always flush the solute or polymer with solvent. If you are not going to work with the instrument for more than a day, **AND** you are working with a solvent in which bacteria may grow, flush the instrument with one or more compatible solvents. Flush with one or more intermediate solvents until you can replace the final liquid with ethanol or some other alcohol in which bacteria will not grow. Then cap the inlet and the outlet ports with the plastic DELRIN plugs that were included when we shipped the instrument to you.

If a blockage occurs due to dried salt or polymer, or if the instrument was left with water and bacterial growth is suspected, refer to Appendix A of this manual.

For a list of compatible solvents, see Appendix B of this manual.

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First Printing: January 2003

Catalogue Number: BI-ZPMwA, Ver. 1.0

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Information produced by using this BIC software and its manual, including the resulting displays, reports, and plots, are believed to be accurate and reliable. However, Brookhaven Instruments Corporation assumes no responsibility for any changes, errors, or omissions.

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Section I: How to Use this Manual and Software

Introduction

The BI-MwA is a seven-angle, static light scattering instrument used to determine properties of polymers in solution. These properties include M_w, the weight-average molecular weight, R_g, the root-mean-square radius of gyration, and A_2, the second virial coefficient. The BI-MwA can be used with GPC/SEC software to determine molecular weight distributions. The BI-MwA can be used with special software to determine the time-dependence of molecular properties during polymerization/depolymerization and aggregation (BI-ACOMP). Finally, the BI-MwA can be used with the BI-ZPMwA discussed in this manual to determine polymer properties in batch-mode.

<u>Before using the instrument</u> please read the BI-MwA Installation manual. Make sure your instrument is operating properly by following instructions in the installation manual.

Using the batch mode requires manual injection of solvent and polymer solutions of known concentrations. Use either a manual syringe or a syringe pump (BI-SPump). Periodic normalization and calibration are also required.

Fiber optics are used in the BI-MwA at each of the fixed scattering angles to collect the scattered light. A highly sensitive CCD detector (2048 pixels) registers the light from each fiber. A signal is generated, and subsequently digitized, at each pixel upon which the light falls. The light from any one angle's fiber falls onto many pixels and the sum over all the values associated with a single angle is called the integrated intensity. [Here the word integrated refers to a spatial integration over the detector.] The integrated intensity is proportional to the total scattered intensity at that angle. A measurement is also made of the laser's intensity before the beam enters the cell. Dividing the angular intensities by this reference intensity takes care of slow drifts in laser power over time.

The BI-ZPMwA application program allows you to control the BI-MwA, to collect and to correct the measured scattered light intensities, and to plot the results. You can choose from a variety of plots including Zimm, Debye, and Berry plots. In all the plots, you can determine M_w and A_2. In the Zimm and Berry plots, you can also determine R_g. A few sets of sample data are included. Results can be printed, archived, and reloaded for future reference. An ASCII file can be created for use in spreadsheets and plotting programs.

This type of light scattering is variously known as static light scattering (SLS), *c*lassical *l*ight *s*cattering (CLS), and *t*otal or *i*ntegrated *l*ight *s*cattering (TLS or ILS). [The word integration here refers to the averaging over time.] It has even been called TILS for *t*otal *i*ntensity *l*ight *s*cattering. If measurements are made at a single angle, it is called

SALS for *s*ingle-*a*ngle *l*ight *s*cattering. MALLS is an acronym for *m*ulti-*a*ngle *l*aser *l*ight *s*cattering. One "L" would have been good enough, because, before the introduction of lasers, Hg-arc light sources were used with good results too. Whatever the name, the measurement is always the same: Average the intensity of light over a time that is long compared to the fluctuations due to thermal motion of the polymer molecules. Typically any time greater than a few milliseconds is sufficient, even for the largest polymers encountered. Most intensity measurements are averaged for less than one second in order to identify outliers from dust/aggregates.

In this manual we prefer the term SLS to distinguish it from DLS, dynamic light scattering, a form of light scattering typically used for particle size determination calculated from diffusion coefficient measurements and polymer relaxation times due to various modes of motion.

Software Installation

To install the BI-ZPMwA software, place the installation CD in the appropriate CD drive (here assumed to be the D: drive). Click on Window's START button and select RUN. Type D:\ZPMWA\Setup. Press the Enter key. Follow any on-screen instructions. When finished, a BIC icon with the name Zimm Plot Software will be added to your BIC program group that was created when you installed the BI-TAS program during installation.

References

Most good physical chemistry textbooks have a few pages on Zimm Plots and light scattering, though in most cases they describe instrumentation that is decades out of date, showing mercury arc lamps instead of vertically polarized lasers, for example. Nevertheless, these accounts serve as an easy introduction to the subject.

If you want more information than is available in undergraduate textbooks, read **Section II: Theory** in this manual.

Specialized information is also available. If you are unfamiliar with the Zimm Plot method then read chapter 14 in reference 1 or chapter 5 in reference 2. Both are presented at the undergraduate level. Reference 3 is an introductory textbook on classical light scattering from polymer solutions. Reference 4 is a review chapter on SLS presented at the intermediate level. Reference 5 is the classic text on SLS written mostly before the widespread introduction of lasers, personal computers, and DLS. It is, however, full of valuable data and insight. Long out of print, look in a university library to find a copy. Reference 6 is an advanced treatise on light scattering on branched polymers. It contains, among other advanced information, tables of particle scattering factors for a variety of molecular shapes. It also summarizes different methods for plotting angular SLS and DLS data. Reference 7 is a textbook on DLS. Despite its title, it is suitable for use in a graduate

level course.

1. Allcock and Lampe, Contemporary Polymer Chemistry, Prentice-Hall publisher, 1981.

2. Hiemenz and Rajagopalan, Principles of Colloid and Surface Chemistry, Third Edition, Revised and Expanded, Marcel Dekker publisher, 1997.

3. Kratochvíl, Classical Light Scattering from Polymer Solutions, Elsevier publisher, 1987.

4. Casassa and Berry, "Light Scattering from Solutions of Macromolecules", chapter 5 in Polymer Molecular Weights, Part I, P.E. Slade editor, Marcel Dekker publisher, 1975.

5. M.B. Huglin, editor, Light Scattering from Polymer Solutions, Academic Press, 1972.

6. W. Burchard, "Static and Dynamic Light Scattering from Branched Polymers and Biopolymers", in Light Scattering from Polymers, Advances in Polymer Science series, Number 48, Springer-Verlag, 1983.

7. K.S. Schmitz, An Introduction to Dynamic Light Scattering by Macromolecules, Academic Press, 1990.

Though an understatement, this list of references is by no means complete.

Section II: THEORY

The Basic Equation

In the limit of low concentration, the data obtained from SLS measurements in the BI-MwA for a dilute, homopolymer solution in a single solvent can be expressed as:

$$H*c/\Delta R(\theta,c) = 1/[M_w P_z(q)] + 2A_2 c \quad . \qquad (1)$$

The optical constant H is given by:

$$H = 4\pi^2 n_o^2 (dn/dc)^2/(N_o \lambda_o^4) \ , \qquad (2)$$

where

n_o	is the refractive index of the solvent. Values from 1.30 to 1.60 are typical.
dn/dc	is the specific refractive index increment of the solution. Values from -0.05 to 0.30 cm^3/g are typical. See Appendix C.
c	is the polymer concentration. Values from 0.01 to 10 mg/cm^3 are typical. The lower concentration range is more common when measuring high molecular weight polymers, above 1M Daltons, for example; the upper concentration range is more common when measuring low molecular weight polymers, below 100K Daltons, for example.
N_o	is Avogadro's Number, 6.022×10^{23} mol^{-1}.
λ_o	is the wavelength in vacuum of the laser. For diode lasers in the red it varies from 660 to 680 nm, depending on the particular laser.
θ	is the scattering angle in degrees.
ΔR	is the excess Rayleigh Factor (also called Rayleigh Ratio) in units of cm^{-1} calculated at different angles and concentrations. Excess means it is the <u>difference</u> between the value for the solution and the value for the solvent. R is proportional to the corrected†, scattered light intensity, the proportionality constant is determined by calibration against a substance (toluene, benzene) with a known Rayleigh Ratio. (†Corrections include normalization, reflection, and refraction.)
M_w	is the weight-average molecular weight (molar mass). Values in the range from a few hundreds to tens of million g/mol (Daltons) are measureable, more commonly 10^3 to 5×10^6 Daltons.
$P_z(q)$	is the z-average particle scattering function or particle structure function. (In more concentrated and strongly interacting systems a solution structure function is also important.) $P_z(q)$ yields R_g.
q	$= (4\pi n_o/\lambda_o)\sin(\theta/2)$ is the magnitude of the scattering wave vector.
A_2	is the second virial coefficient, values in the range 0 to 10^{-4} $cm^3 \cdot mol/g^2$ are typical. For polydisperse systems, A_2 is also an average quantity; however, the average is not a simply defined one like the weight- or z-average.

The factor 4 in the optical constant H is a result of using incident light that is linearly polarized perpendicular to the horizontal scattering plane. This is the most common configuration and is typically referred to as vertical polarization. The BI-MwA uses vertical polarization. For unpolarized incident light, the factor is 2. In older textbooks, you will see the factor as "2".

The particle scattering function can be expanded in terms of q^2, and the first three terms are

$$P_z(q) = 1 - (R^2_{g*}q^2)/3 + O(q^4) \quad , \quad (3)$$

where R_g is shorthand for $\langle R^2_g \rangle^{0.5}_z$, the z-average root-mean-square radius of gyration, a measure of the molecular size. R_g is almost universally called the radius of gyration, even though that is a misnomer. It is a root-mean-square value. In fact, even for a monodisperse polymer system, where the z-averaging equals all other average values, R_g is still an average value. A random coil polymer takes up a large number of configurations and R_g even for a single molecule is thus an average value over time.

The coefficient of q^2 is independent of the particle shape. Coefficients of higher order terms do depend on shape. Therefore, the radius of gyration (more properly, the z-average, RMS radius of gyration) is a primary parameter obtained from light scattering: primary in the sense that no model for the shape is assumed.

The Zimm Plot

Combining Equations 1 and 2, and expanding $1/P_z(q)$ to first order in q^2 with a Taylor series, gives the following:

$$H*c/\Delta R(\theta,c) = [1/M_w]\cdot[1 + (R^2_g \cdot q^2)/3] + 2A_2c \quad . \quad (4)$$

This equation is the basis for the famous Zimm Plot. Scattered light intensities are measured at several angles for each of several solution concentrations and for the pure solvent at each angle. Subtracting the solvent scattering from the solution value yields the excess intensity scattered by the polymer. After reflection, refraction, and volume corrections, and calibration, the excess Rayleigh Factors are calculated from measured intensities.

If the left hand side of Equation 4 is plotted against $\sin^2(\theta/2) + k_{p*}c$, where k_p is an arbitrary plotting constant chosen purely for convenience in viewing the results, a grid-like plot results. Two sets of, typically, parallel lines make up the grid. One set consists of angular measurements at each concentration, and one set consists of concentration measurements at each angle. Extrapolating the angular measurements to zero angle for each concentration yields a straight line in c. The slope of this line yields A_2. Extrapolating the concentration measurements to zero concentration for each angle yields a straight line in $\sin^2(\theta/2)$. The slope divided by the intercept of this line yields Rg.

The intercept of both extrapolated lines—the double extrapolation to zero angle and zero

concentration—yields M_w the weight-average molecular weight of the polymer in solution. M_w is the most important parameter of a polymer distribution. Light scattering offers an absolute* method for its measurement.

The value of dn/dc, several other parameters in Equation 1, and a calibration constant must be known in advance in order to calculate M_w and A_2 from a Zimm Plot. This is also true for the Debye and Berry Plots described below. Interestingly, however, R_g can be calculated without knowing either dn/dc and without a calibration. Both parameters cancel from the calculation of R_g.

The Debye Plot

When the longest dimension of a scatterer is small compared to the wavelength of light in the liquid, then by definition the particle/polymer is called a Rayleigh scatter. In this case, the angular term in Equation 3 is negligible, and it can be approximated as:

$$H*c/\Delta R(\theta,c) = 1/M_w + 2A_2c \quad . \qquad (5)$$

This equation is the basis for the Debye Plot. At one angle, intensities are measured as a function of concentration. The results are plotted according to Equation 4. A_2 is obtained from the slope of this plot; the intercept yields $1/M_w$. Typically the angle used is 90°, but this is not necessary. This angle is convenient because flare light should be negligible and because residual dust or microgel contributions are usually insignificant.

Whenever the molecular weight is small, approximately 100 kDa and lower, consider collecting data for a Debye Plot. The interpretation of possible downward curvature at low angles is avoided. Downward curvature may be due to dust, microgels, colloids, or flare. Downward curvature can be misinterpreted as a large molecular weight with a corresponding large radius of gyration.

The BI-ZPMwA program allows one to make Debye Plots even from data collected at several angles. Click **Graphs** and choose the Debye plot. Then select the angle at which you want to plot the data.

NOTE: Some authors graph plot $[H*c/\Delta R(\theta,c)]^{-1}$ vs. q^2 or $\sin^2(\theta/2)$ and call it a Debye Plot. For high M_w values, the argument goes, the intercept will be easier to determine.

The Berry Plot

* Though commonly referred to as an absolute measurement, since calibration is required, it is not absolute in the sense of a first-principles measurement where there are no calibration or adjustable parameters. However, compared to viscosity measurements, where many more assumptions are required to obtain a molecular weight, light scattering is—if you will excuse the oxymoron—relatively absolute. In addition, compared to calibration using a polymer of known molecular weight, calibration with pure toluene is apt to result in a more accurate M_w since it is impossible to produce monodisperse, synthetic polymer distributions. So-called “standards” are in fact narrow distributions.

The term linear in c in Equation 1 is the first in a polynomial expansion in c. These terms come from the expansion of the osmotic pressure in c. At higher concentrations or for strongly interacting particles (polyelectrolytes not screened by free salt ions) they may be important. They are also important, for different reasons, for branched polymer systems. Whatever the reason, the Zimm Plot shows upward curvature, and it is no longer a simple set of straight lines.

The curved data may be fit to higher order terms in c and $\sin^2(\theta/2)$. Brookhaven software provides for fits up to 5th order in either or both variables. With a few data points, it is always possible to fit the measured data quite well with a high-order polynomial. Nevertheless, the parameters calculated with high-order fits are often not meaningful. Whenever possible select a linear fit, or try to linearize the data. The Berry Plot was developed for this purpose.

The right-hand side of Equation 3 can be written as:

$$[1/M_w]*[1+(R^2_{g*}q^2)/3]*[1+2A_2M_wc+3A_3M_wc^2+ ...] \quad . \qquad (6)$$

For hard spheres, $A_3M_w = (5/8)*(A_2M_w)^2$, while for flexible coils in a theta solvent the term is zero. In general it can be written as $g(A_2M_w)^2$. When the 3rd order term is small but not negligible, an approximation for g might be 1/3, the approximate average between 0 and 5/8 (.625). With g = 1/3,

$$[1 + 2A_2M_wc + 3A_3M_wc^2 + ...] \cong [1 + A_2M_wc]^2 \quad ,$$

and Equation 5 becomes,

$$H*c/\Delta R(\theta,c) \cong [1/M_w]*[1 + (R^2_{g*}q^2)/3]*[1 + A_2M_wc]^2 \quad . \qquad (7)$$

This equation can be made to yield 2 sets of lines by taking the square root. The result, linear in q^2 and c, is

$$H*c/\Delta R(\theta,c) = [1/M_w]^{0.5}*[1+(R^2_{g*}q^2)/6]*[1+A_2M_wc] \quad . \qquad (8)$$

This equation is the basis of the Berry Plot. The square root of $Hc/\Delta R$ is plotted against $\sin^2(\theta/2) + k_{p*}c$.

In the polymer and light scattering literature, one often finds an alternate designation for the second virial coefficient, Γ. The value of Γ typically varies from about 10 to 1,000 cm^3/g, and it is a measure of molecular volume. Γ is the symbol often used in the discussion of viscosity of polymer solutions. Γ is related to A_2 as follows:

$$A_2 = \Gamma/M_w \qquad (9)$$

Corrections for Depolarization

For optically isotropic molecules, the polarization of the scattered light is parallel to the polarization of the incident light. The most common configuration with a laser source is incident light that is linearly polarized perpendicular to the scattering plane. This is commonly called vertical polarization. For many, large, flexible-chain polymers depolarization effects are negligible, and, as a result, no polarization corrections are necessary. M_w, R_g, and A_2 are correct as calculated from the Equations 3, 4, and 7. [Many solvents are anisotropic scatterers and depolarize the scattered light significantly; however, these effects are negated upon subtracting the solvent scattering from that of the solution.]

For optically anisotropic molecules, the polarization of the scattered light has components both parallel and perpendicular to the incident polarization. For short, flexible-chain polymers and chains in or near the rigid rod limit, depolarization effects are not negligible, and, as a result, polarization corrections are necessary. Under these circumstances M_w, R_g, and A_2, as used in the equations above, should be considered *apparent*, not *true* values.

To make the corrections, two limiting cases of the worm-like chain model must be distinguished: flexible chain and rigid rod. In addition, each of these two cases can be measured in either of two common experimental setups: measurements with linearly polarized incident light perpendicular to the scattering plane and no analyzer R_{uv}; or, measurements with an analyzer set to pass only scattered light with polarization parallel to the incident polarization R_{vv}.

For R_{uv} measurements on flexible chain molecules:

$$M_{w,app} = M_{w*}[1 + (7/5)\delta^2] = M_{w*}[(3 + 3\rho_v)/(3 - 4\rho_v)] \quad , \qquad (10)$$

$$A_{2,app} = A_2/[1 + (7/5)\delta^2] = A_2/[(3 + 3\rho_v)/(3 - 4\rho_v)] \quad , \qquad (11)$$

$$R_{g,app} = R_g/[1 + (7/5)\delta^2]^{0.5} = R_g/[(3 + 3\rho_v)/(3 - 4\rho_v)]^{0.5} \quad . \qquad (12)$$

For R_{uv} measurements on rigid rod molecules:

$$R_{g,app} = R_{g*}\{[1 - (4/5)\delta + (47/35)\delta^2]/[1 + (7/5)\delta^2]\}^{0.5} \quad . \qquad (13)$$

For R_{vv} measurements on flexible chain molecules:

$$M_{w,app} = M_{w*}[1 + (4/5)\delta^2] = M_{w*}[3/(3 - 4\rho_v)] \quad , \qquad (14)$$

$$A_{2,app} = A_2/[1 + (4/5)\delta^2] = A_2/[3/(3 - 4\rho_v)] \quad , \qquad (15)$$

$$R_{g,app} = R_g/[1 + (4/5)\delta^2]^{0.5} = R_g/[3/(3 - 4\rho_v)]^{0.5} \quad . \qquad (16)$$

For R_{vv} measurements on rigid rod molecules:

$$R_{g,app} = R_{g^*}\{[1 - (4/5)\delta + (4/7)\delta^2]/[1 + (4/5)\delta^2]\}^{0.5} \quad . \qquad (17)$$

In these equations δ is the molecular anisotropy and ρ_v is the depolarization ratio. The depolarization ratio is an experimentally measured parameter. It is operationally defined as:

$$\rho_v = R_{hv}(90°)/R_{vv}(90°) \, , \qquad (18)$$

where $R_{hv}(90°)$ is the excess scattered light intensity measured at $\theta = 90°$ with polarization perpendicular to the incident polarization, and $R_{vv}(90°)$ is the excess scattered light intensity measured at $\theta = 90°$ with polarization parallel to the incident polarization. The excess is defined by the difference between the value measured with the solution and the value measured with the solvent. Since many common solvents can depolarize the light significantly, one must use the excess values.

The molecular anisotropy can be written in terms of the experimentally determined depolarization ratio as:

$$\delta^2 = 5\rho_v/(3 - 4\rho_v) \qquad (19)$$

Literature or measured values of ρ_v can be input, and its effect on recalculated values M_w, R_g, and A_2 determined. The BI-MwA does not provide for ρ_v measurements though our BI-200SM does.

For most polymer samples the depolarization corrections are negligible. This is so because the normally random orientation of dipole moments from the anisotropic monomeric units in a polymer cancel as the molecular weight increases. In fact, it is often stated that if the measured depolarization ratio is not negligible, the measurement is probably wrong. The older literature values of depolarization ratios are suspect. Be careful in applying these corrections. If in doubt, do not apply them. This is accomplished by setting ρ_v in the BI-ZPMwA program, to zero, the default value. With ρ_v equal to zero, there should be no effect on the calculation of M_w, R_g, or A_2 even if the flexible chain or rigid rod choices are selected.

For rod-like polymer molecules, for short-chain molecules where end-groups may contribute significantly to depolarization, ρ_v may not be negligible and corrections are necessary.

WARNING

Real samples and real measurements almost never fit exactly the simple theory just presented above. As the angle is decreased, the intensity is often anomalously large due to dust, microgels, other supramolecular entities, and flare light at the cell-window interfaces. These effects cause a downward curvature in the Zimm and Berry Plots. The user is advised to consider whether this downward curvature is real. If real, it will lead to lower intercepts and greater slopes, resulting in anomalously large M_w and R_g values. Such curvature is easily fit to higher order polynomials that are available in the BI-ZPMwA program. Though the fit will certainly improve as the order of the polynomial is increased, the user must use judgment in accepting these results. Are they repeatable results? If not, then dust, microgels, and other supramolecular entities (colloids, perhaps) are most likely the problem. Look at plotted points. If all the values at a particular angle are shifted low or high, consider that the normalization at that angle is suspect and make the necessary changes. If all the values at a particular concentration are shifted, consider that the concentration is not correct. Make the necessary changes. Clean up the sample before proceeding. If the results are repeatable, do they make sense? If not, perhaps flare light is the problem; perhaps the calibration was not done with a clean solvent; perhaps the polymer concentrations are not correct; perhaps the value of dn/dc is wrong.

Section III: Software Tour & Definitions

Figure III-1 shows the main window when entering the program.

Click **Setup** to access a menu for setting Experimental and Sample Parameters (see Section VI), or click the command buttons labeled Experimental Parameters and Sample Parameters at the bottom of the main window. Also available from **Setup** is the Trend/Noise menu used to set values defining acceptable signals.

Click **Graphs** to select the type of plot that will be used to analyze the data. The choices are Zimm, Berry, and Debye, further discussed in Section VII. You can also select an expanded view of CCD Monitor and the Stability Graph, both of which are described below.

Click **Help** to see the software version number and how to contact Brookhaven Instruments Corporation. If you contact us, please include the software version number as it will help us understand any problems you may be having.

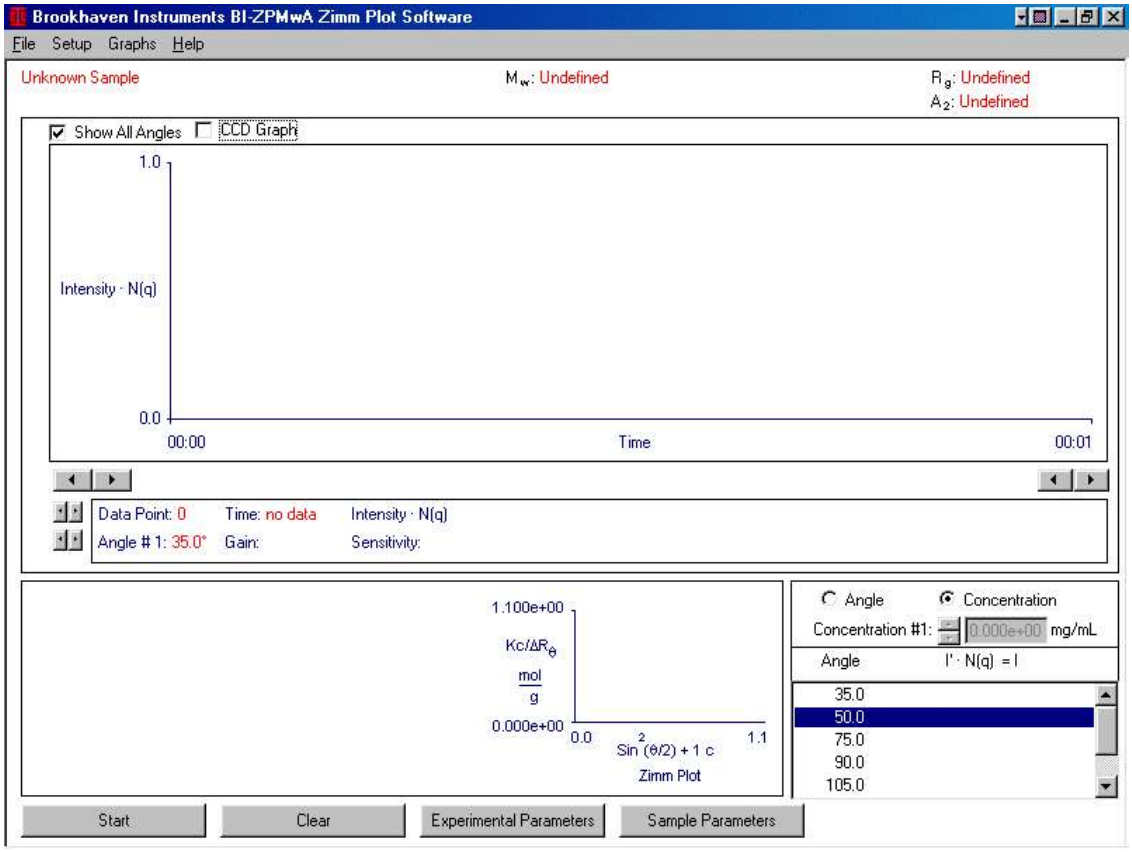


Figure III-1: Main Window

The upper graph shows the normalized intensities vs. time for all angles (check box) or a single angle. (See Section IV for a detailed explanation of normalization.) You can highlight any angle, any time point, and you have complete freedom to expand or contract the time axis.

The box in the lower right shows the angles, the concentrations, and the corrected, normalized intensities, I. If you have previously measured the solvent, then it is subtracted and the result is the excess intensity, I_{ex}. After calibration, it is the excess intensity that yields the excess Rayleigh Ratios used in calculating molecular properties from light scattering data.

Here are the seven angles used in the BI-MwA along with the values of $\sin^2(\theta/2)$ used in plotting:

Angle, θ	$\sin^2(\theta/2)$
35°	0.0904
50°	0.1786
75°	0.3706
90°	0.5000
105	0.6294
130	0.8214
145	0.9096

There are three pairs of supplementary angles—35º + 145°, 50° + 130°, 75° + 105°—useful in applying reflection corrections, and 90°.

Click **Graphs** and select *CCD Monitor*. An example is shown in Figure III-2.

The graph shows the raw signal values, 16-bit representations of the voltages developed at each of the 2048 pixels on the CCD. Since $2^{16} - 1 = 65{,}535$, this is the maximum value on the Y-axis that can be registered. After that, the detector is saturated and the peak is truncated and becomes flat. This is the overflow limit and values above this are shown in gray. When viewing the CCD Monitor, you can increase the Gain and/or the Sensitivity and watch some values turn from red to gray, indicating an overflow condition. When making actual measurements, the Gain and Sensitivity are automatically adjusted for optimum signal-to-noise ratio conditions and you do not set them manually.

Dark voltage values are automatically subtracted in the BI-MwA. There is no need to make separate dark voltage measurements.

There are eight peaks shown on the CCD Monitor and there are eight corresponding rows in the table to the left of the graph. These are listed from 35° to 145° plus the reference beam value. In order to confuse you, we have reversed the order on the graph: The first peak is the reference beam followed by the highest angle, with the lowest angle furthest to the right. To be sporting, however, we have listed under the column marked Peak Index, the pixel number at which the peak occurs. The third column gives the value corresponding to the peak. The last column, marked Intensity, is the area under the curve (divided by the sensitivity). This value represents the raw, total intensity value before applying rejection, smoothing, and stability criteria. These criteria are discussed in Appendix E.

These intensity values —after rejection, smoothing, and stability criteria are applied— are multiplied by normalization constants, N(q), determined with a Rayleigh scatterer. The normalized intensities are then multiplied by a calibration constant and corrected for refraction and reflection (See Appendix D), before being used in the calculation of molecular properties.

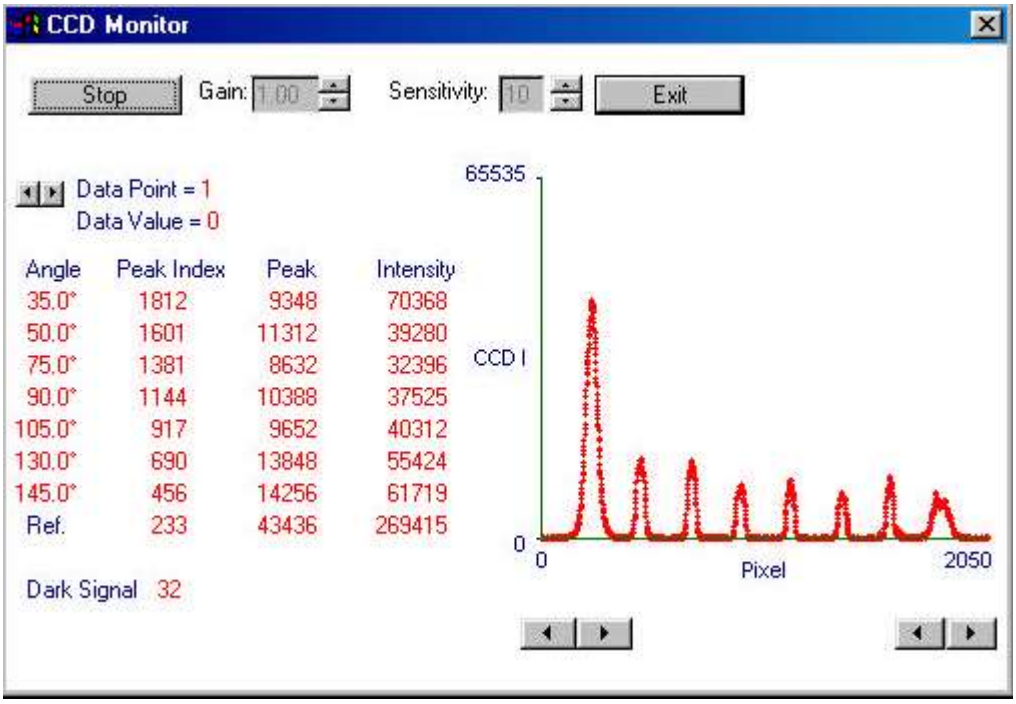


Figure III-2: CCD Monitor Window

Click **Graphs** and select *Stability Graph*. An example is shown in Figure III-3.

Here we plot integrated intensity versus time for one or all the angles if you check *Show All Angles*. You select which of the angles is highlighted with the horizontal cursor by using the right/left arrows at the bottom left of the display. You select which time point to look at by clicking on it or using the right/left arrows next to the label "Data Point".

You can normalize these intensity values by dividing each by the reference intensity. Check *Normalize to Reference Beam* to do this. You can also normalize each intensity by multiplying it by the previous normalization constant. Check *Normalize to N(q)* to do this. In general, both normalizations should be used when viewing this graph. If you want to see the stability of your laser, do not select either normalization. An eighth line appears showing the reference beam intensity versus time.

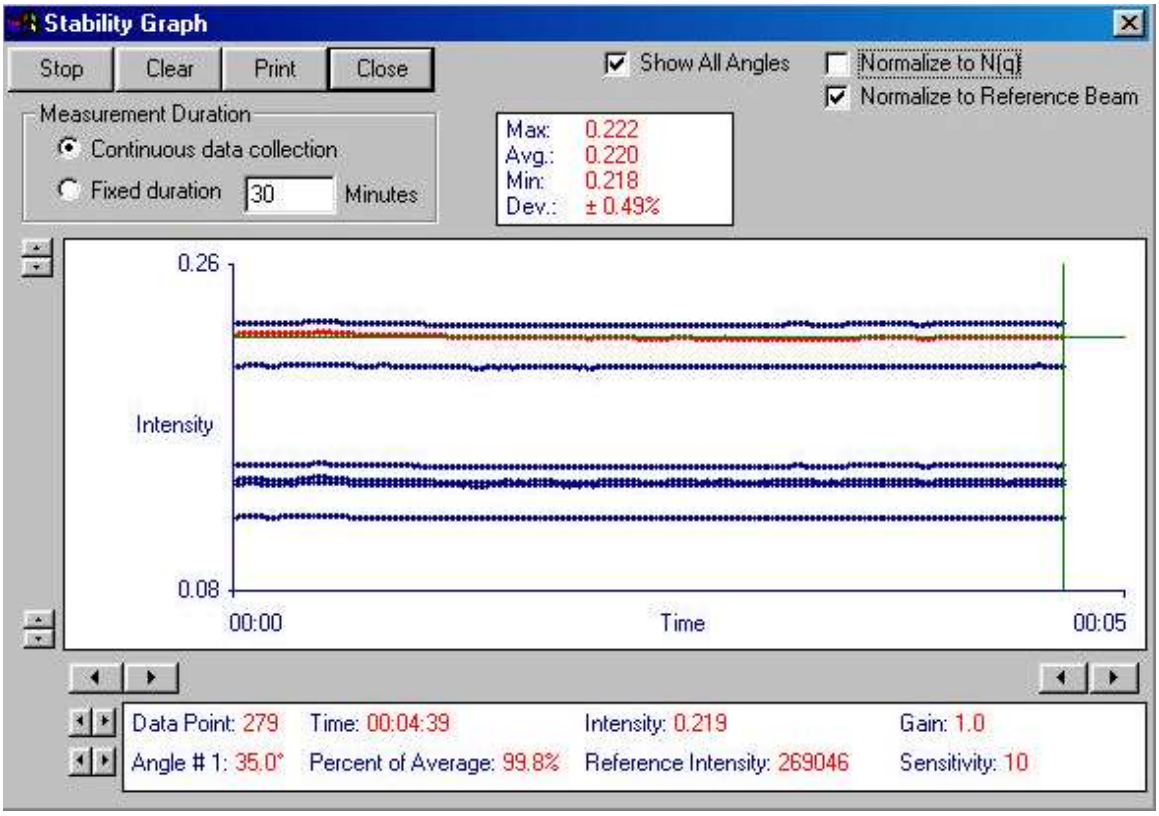
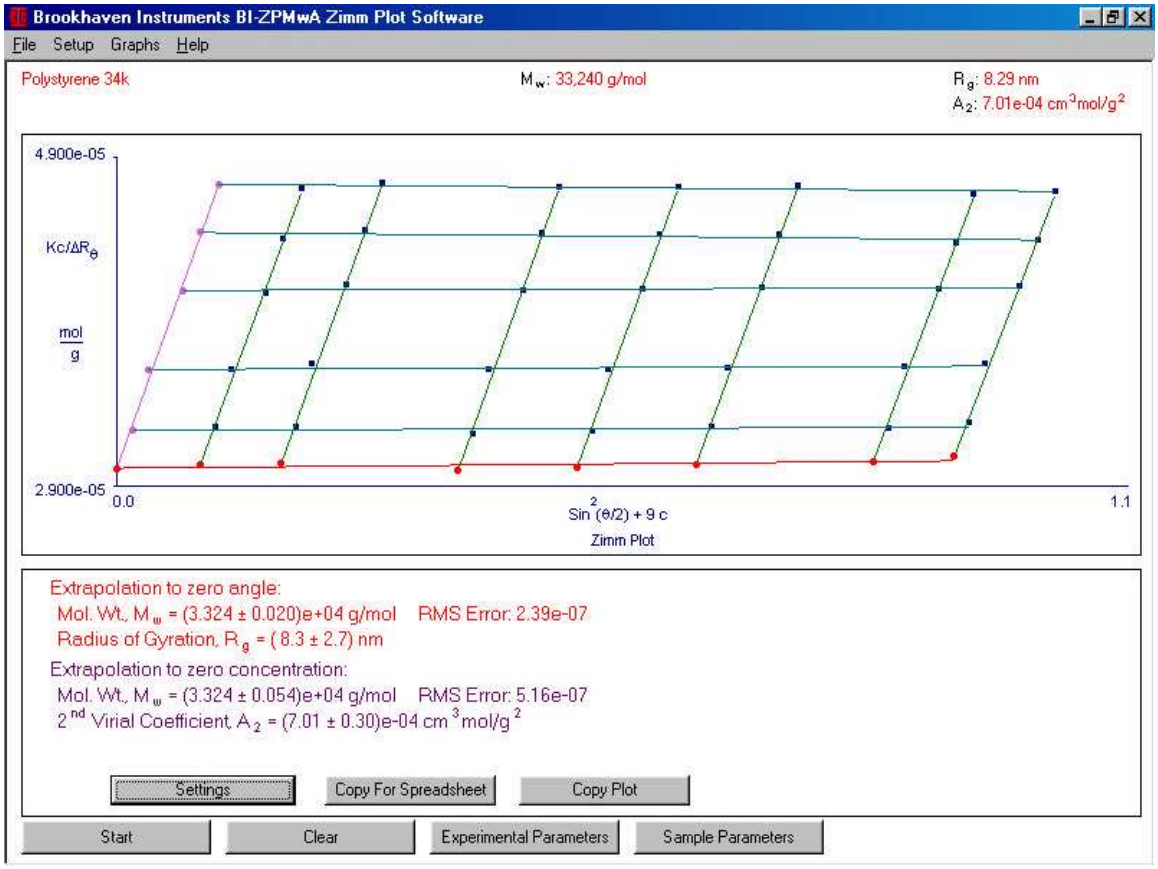


Figure III-3: Stability Graph

As a final introduction to the software, consider Figure III-4 showing the end-result of a measurement on five concentrations and seven angles for a 34 kDa standard of polystyrene in THF.

The intercept from the double extrapolation to zero angle and zero concentration yields M_w = 33,240 g/mol. The slope/intercept of the zero concentration line yields a radius of gyration, R_g = 8.3 nm. The slope of the zero angle line yields a second virial coefficient, $A_2 = 7.01 \times 10^{-4}$ cm^3·mol/g^2. These values are obtained from a least squares fit and the fitting errors are also given. Details on this and other plots are discussed in Section VII: Analyzing Data and Interpretation.



The plot is a classic Zimm Plot. Notice the double grid pattern. Poor data, including a bad normalization, would result in points not lying on a regular grid with a correspondingly poor extrapolation.

Figure III-4: Zimm Plot for polystyrene in THF.

To end this brief tour, click **File** on the main menu toolbar. Click Database. Double click on the folder labeled *Sample Data* and it should open. Click once to select one of the data files. Click on **Open File** to recall the file. Click **Results** to enable get the grid lines of a Zimm Plot. Click **File/Print Preview** to view what will be printed. Click **Close**. To print, click **File/Print Report** to obtain a printout.

For more information on the database and how to manipulate folders and files, see Section VIII.

Section IV: Normalization and Calibration

Normalization Defined

A normalization factor, N(q), is applied to all scattering angles. The factor accounts for the differences between the fibers and fiber positions, minor differences in sensitivity across the CCD, and the differences in scattering volume as a function of angle. This latter correction gives rise to a sin(θ) factor that is absorbed into N(q).

N(q) is defined to take advantage of the fact that, for a Rayleigh scatterer, the scattered intensity is independent of the angle. A Rayleigh scatterer is any particle or molecule whose longest dimension is much smaller than the wavelength of light in the liquid, λ_o/n_o. For a typical BI-MwA, with water as the liquid, this ratio is 675 nm/1.33 = 507 nm. Historically, a value of $\lambda/20$ is used to define the upper limit of a Rayleigh scatterer. In this case, 507 nm/20 $\cong$ 25 nm defines the upper limit for a Rayleigh scatterer.

The definition of N(q) is as follows:

$$\mathbf{N(q) \equiv \frac{I'_{samp}(90^o) - I'_{solv}(90^o)}{I'_{samp}(q) - I'_{solv}(q)}}$$

Here 90° is the angle at which N(q) is defined to be exactly 1. The primes indicate smoothed and averaged, integrated intensities. By taking the difference between the sample and solvent values, flare light is cancelled. [NOTE: The subscript "samp" for sample is used because the sample could be a dissolved polymer (solution) or a suspended colloid (suspension). Either is permissible for normalization.]

The difference between a sample and a solvent is called excess intensity. Thus, N(q) can also be written as:

$$\mathbf{N(q) \equiv \frac{I'_{ex}(90^o)}{I'_{ex}(q)}}$$

Since the numerator is a constant for a given Rayleigh scatterer, the product of N(q) and the excess intensity at any other angle is also a constant. This is the definition of a Rayleigh scatterer and the definition of N(q) follows.

Suitable Samples for Normalization

The longest dimension of a particle or polymer used for normalization should be less than about 25 nm. Thus, a nominal 20 nm diameter polystyrene latex sphere in water will suffice when normalizing in water. We find that 4 drops of a 1% solids, Duke

Scientific[1], nominal 20 nm polystyrene latex in 20 mL of DI water is suitable. The stock concentration is 1% solids.

It may be more convenient, especially in solvents other than water, to use a small polymer molecule for normalization. Its radius of gyration, R_g, normally defines the size of a polymer molecule. This is not its longest dimension. For polymer molecules, it is easier to consider the angular dependence of the scattered light when defining size. To first order, the angular dependence in a Zimm Plot (see Section III: Theory) is given by:

$$\mathbf{1 + R_g^2 \cdot q^2 \,/\, 3}$$

The angular dependence is negligible—the definition of a Rayleigh scatterer—when

$$\mathbf{R_g^2 \cdot q^2 \,/\, 3 \leq 0.02}$$

The 2% rise from the minimum to the maximum angle is a reasonable choice but by no means universal. In the BI-MwA, with $\lambda_o = 670$ nm, $\theta_{max} = 145°$, and $n_{solv} = 1.42$, this means $R_g \leq 10$ nm.

If the scatterer were a sphere, $R_g = 10$ nm corresponds to a radius $R_H = 13$ nm or diameter $D_H = 26$ nm, in agreement with the historical rule of $\lambda/20$.

Polystyrene, a typical homopolymer, with a random coil configuration in toluene or benzene, has a $R_g = 10$ nm at a molecular weight of about 80 kDa. In a theta solvent like toluene, at the theta temperature of 34.5 °C, a molecular weight of 127k Da yields a 10 nm R_g.[2] For stiff chains, R_g is larger at the same molecular weight. As a first approximation, using a polymer sample of 50,000 g/mol or less, with a random coil configuration, should be suitable for normalization.

Since the scattered intensity varies with polymer concentration, molecular weight, and the square of the specific refractive index increment, dn/dc, consider using the values in Table IV-1 as a guide for preparing a polymer concentration for use in normalization.

Table IV-1: Recommended Polymer Concentrations in mg/mL for Normalization

dn/dc	2 kDa	5 kDa	10 kDa	20 kDa	50 kDa
0.05	40	16	8	4	1.6
0.10	10	4	2	1	0.4
0.15	4.4	1.76	0.88	0.44	0.18
0.20	2.5	1	0.50	0.25	0.10

[1] Duke Scientific Nanosphere Size Standards, Catalogue Number 3020A, 20 nm nominal diameter. Contact at www.dukescientific.com.

[2] See pages 38 and 104, Polymer Solutions, Iwao Teraoka, Wiley Interscience, 2002.

Rod-like polymers should be avoided for normalization unless you already know they are Rayleigh scatterers. Globular and cross-linked polymers are suitable even up to molecular weights of 1,000,000 g/mol since they are very compact.

Always normalize with the same liquid that will be used to dissolve the unknown polymer. While normalization should be independent of liquid (after a small correction for differences in fiber acceptance angles (see Appendix D), it is usually convenient to use the same liquid.

Preparing the BI-MwA for Normalization: Achieving Stability

Clean a 20 mL syringe with soap and water, scrubbing all internal parts and the barrel. Rinse thoroughly in water and then with an intermediate solvent that is both miscible in water and with the normalization liquid. See Appendix B, the last section entitled **Compatible Solvents Used for Flushing,** to determine a good intermediate solvent.

Use a fresh 100 nm (0.1 micron) filter that is suitable for use with the normalization liquid. Since filters often have dried material on their surface from the manufacturing process, flush with 20 mL of liquid before attaching to the BI-MwA. With the filter attached, inject 20 mL into the inlet port of the BI-MwA. Use a syringe pump like the BI-SPump. Use approximately a 1 mL/minute flow rate.

Click **Setup/*Stability Graph***. See Figure IV-1. Click **Start**.

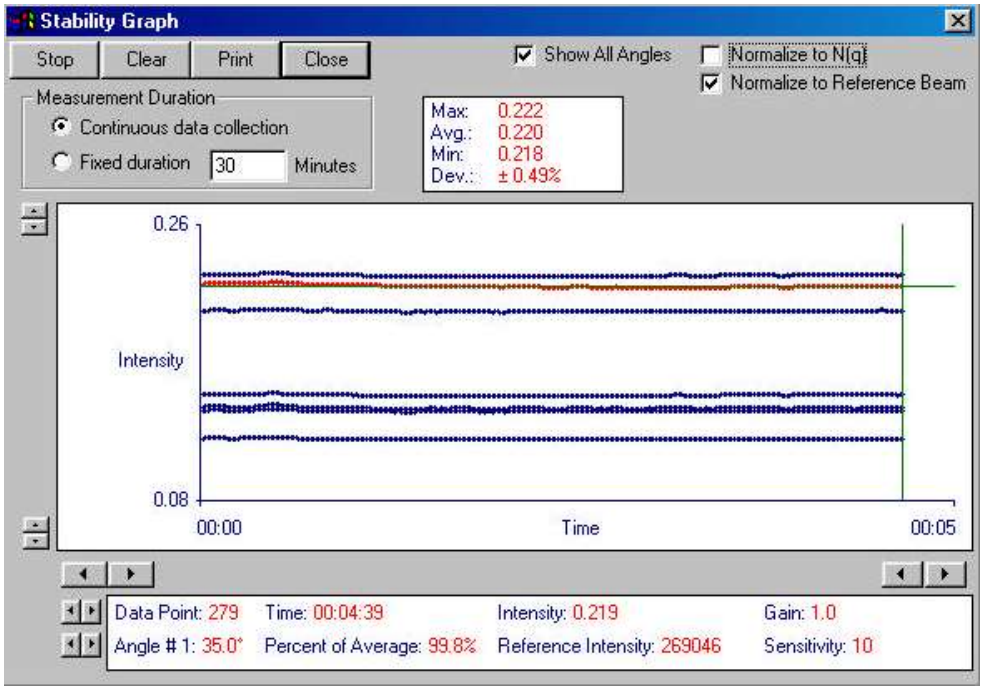


Figure IV-1: Stability Graph

When the box labeled *Normalize to N(q)* is checked, the current normalization is used. The instrument was normalized at the factory; however, if it was not, there is a default set of normalization values (all equal to1.000) that is used when the software is first installed. These default values must not be used when making measurements on a polymer sample, but they are acceptable at this initial stage.

Look at the plots of Intensity$_*$N(q), where the "Intensity" is the smoothed and integrated, averaged values divided by the smoothed and integrated, averaged values of the reference intensity if the box labeled *Normalize to Reference Beam* is also checked. If that box is not checked, you can also see the reference intensity as a function of time. It should be more smooth than any of the other plots. It should be horizontal.

If one or more of the seven plots for the various angles contains obvious peaks, dips, drifts or random jumps, continue flushing with more filtered liquid until they are all flat with very small random fluctuations. Use the Up/Down and Left/Right arrows on the X- and Y-axes to expand/contract the scales for easier viewing. Use the Left/Right arrows to isolate any particular angle or time point. You can also click on the currently highlighted angle plot to place a vertical and horizontal cursor at a particular point. The horizontal cursor is useful in judging stability over time. Right clicking the mouse button will place the cursor at the right edge of the plot. The cursor will move as each new point is added.

Preventing Air Bubbles

When attaching the tip of the filter housing to the fitting on the tubing of the Inlet port, tap the fitting to force air bubbles to rise to the top of the fitting. Then, holding the fitting, squeeze one of two drops of liquid into the end of the fitting. Insert the filter/syringe into the fitting. This should minimize entrained air bubbles.

If after injecting 40 mL of liquid the stability is not acceptable, consider the following procedures. First, use a smaller-diameter syringe without a filter. Manually force liquid through in two or three jerky motions. By applying greater pressure, you can sometimes dislodge adsorbed impurities and air bubbles. Second, inject a different liquid with a lower surface tension and a different solubility factor for polymers. For example, if you are working with water, use acetone. Inject 20 mL slowly without a filter or use a filter suitable for use with acetone (Nylon or PTFE/Teflon). Then start over again with the normalization liquid until the stability graphs are flat.

Avoiding Precipitation

If the last material in the BI-MwA was a salt or a polymer solution, do not initially use a liquid in which the salt or polymer is not soluble. If you do, the salt or polymer may precipitate, coming out of solution, and stick to surfaces or clog small orifices. Therefore, always flush with a compatible solvent before switching to a new liquid.

Normalization

Click on the **Experimental Parameters** command button. See Figure IV-2.

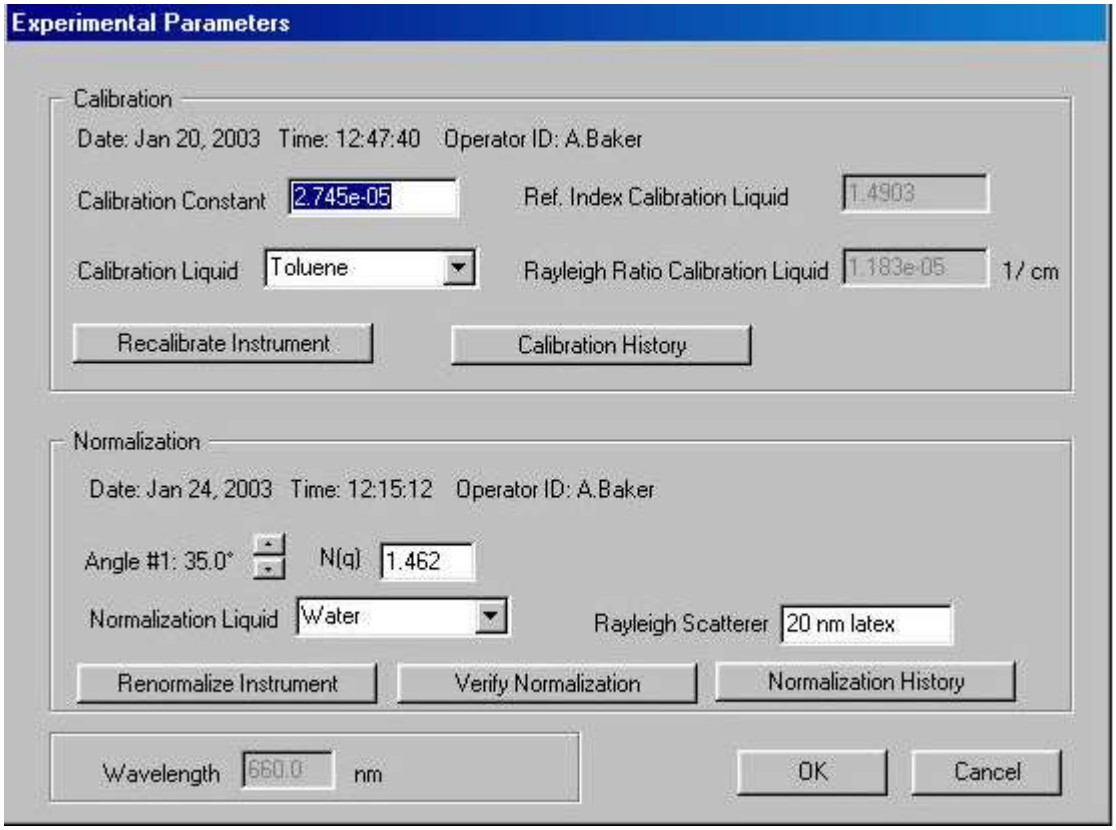


Figure IV-2: Experimental Parameters window.

In the bottom half of this window, notice the "Normalization" information. The date, time, and operator are listed for the last normalization. If you were the first to install new software, any normalization information comes from the factory or from the default

values [N(q) = 1.000 for all angles]. By using the Up/Down arrows, you can view the individual N(q) values. The value for 90° is by definition always the same: 1.000.

Select from the pull-down list a normalization liquid. If your liquid is not listed, select *Unspecified* and then type in the chemical name. In the box labeled "Rayleigh Scatterer", type in a short description as a reminder.

Now click on **Normalize Instrument**. A window will appear instructing you to inject your liquid first without the scatterer. If you are using a syringe pump, and have just finished the preceding instructions for flushing and stability check, wait until the syringe bottoms out or, if you are confident of having a clean, dust/bubble-free liquid, turn off the pump. Then click OK and wait. A window will appear showing a small stability graph so you can judge if the system is still stable. In addition, after N seconds, where N equals the number of stability points (20 by default, see Appendix E), the seven angular values for the solvent (suspension liquid) are obtained. If there are occasional bubbles, you may have to wait longer and then you will notice the red/green lights associated with the Noise and Trend criteria as explained in Appendix E.

When finished, a window will appear instructing you to inject the sample with the Rayleigh scatterer. Again, when inserting the filter into the fitting, take care to dislodge bubbles and top it off with a few drops of liquid. Then inject 10 mL slowly, using the syringe pump. When finished, click OK. Do not click OK until after at least a few millilitres of solution/suspension are injected; otherwise, you will record values that are distorted by the mixing of two solutions/suspensions of different concentration in the cell. Often the scattering is higher and not indicative of a Rayleigh scatterer. So, be patient.

When finished, a new set of N(q) values is automatically recorded. It becomes the current set and is used to calculate molecular properties.

Click **Normalization History**. Figure IV-3 shows an example of what you might see.

This ASCII text file is found in C:\Windows\bi_mwa_NormalizationLog.txt. When you click **Normalization History,** we use Windows' Notepad editor to read it.

The first line gives labels to the history that follows. The seven columns of numbers are the N(q) values. Values should agree to within 4% for 35° and 145° and 2% at all other angles.

Notice that the values obtained on December 20 at 10:40 in the morning do not meet the criteria at 35°, 105°, and 130°. Since you do not know which set is correct, you only have one choice: Repeat until at least two consecutive measurements agree within 4%/2% at the exterior/interior angles. This is basis of what happens when you click the **Verify Normalization** command button. You are led through another normalization until two consecutive ones satisfy the criteria.

The values on December 20 at 11:09 in the morning do not meet the criteria at 130°. However, the 4th set of values on December 20 at 11:36 in the morning meets the criteria 2% when compared to the 5th set of values on December 20 and all further measurements.

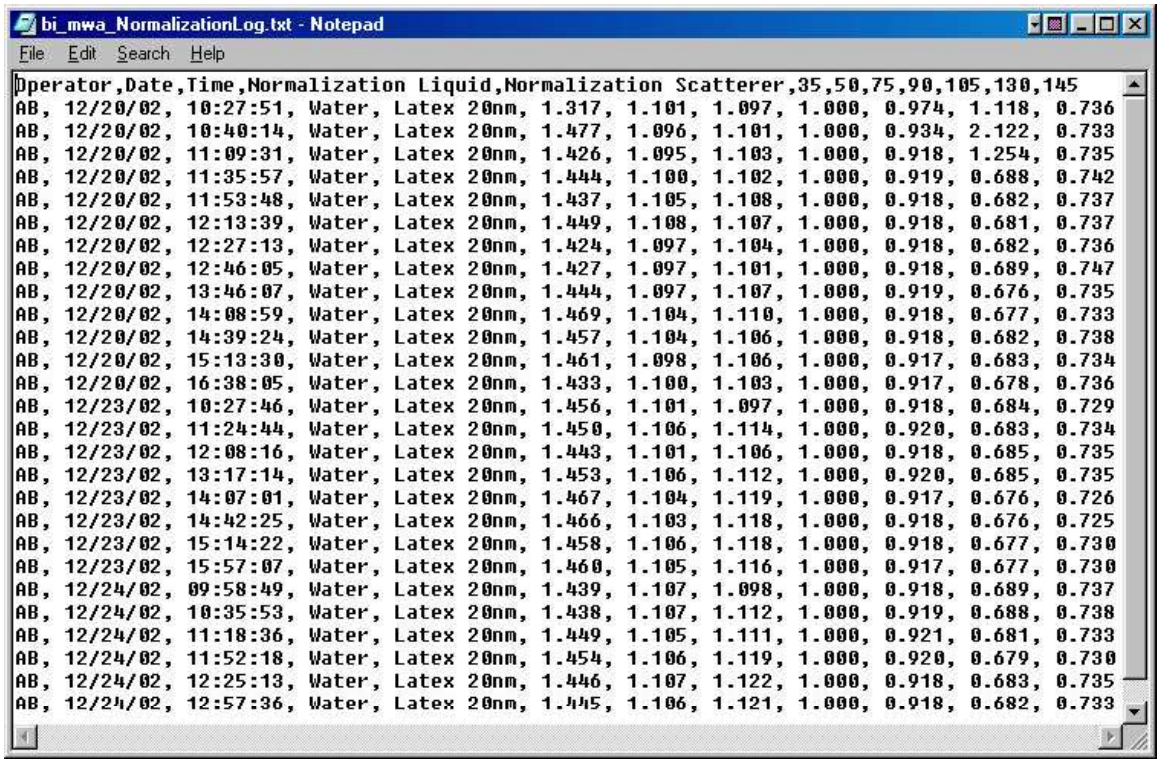


Figure IV-3: Normalization History

If sin(θ) were the only variable controlling N(q), the values would be as follows:

θ	sin(θ)
35	0.574
50	0.766
75	0.966
90	1.000
105	0.966
130	0.766
145	0.574

Clearly, sin(θ) is not the only contributor to N(q). There is no way to predict which values will be larger and which values will be smaller than 1.000. Therefore, only a set of values that are repeatable should be used. Experience does suggest that values over

approximately 3 to 4 are indicative of bubbles, dried salt or polymer. Continued flushing is required.

Once normalized, an instrument should remain normalized for many months unless impurities, salts, or polymer are allowed to dry inside the instrument. We do recommend, however, renormalization every couple of weeks or before a new series of measurements.

Calibration

A calibration constant is obtained by measuring the intensity of light scattered from a standard at 90°. It is assumed that $R_c = k_c * I_c$, where k_c is the calibration constant and R_c is the known Rayleigh Ratio. R_c is known for toluene and benzene at various laser wavelengths. These values are encoded in the BI-ZPMwA software. Rayleigh Ratios scale inversely with λ^4. Such a calibration is only good if the flare light at the calibration angle, typically 90º, is insignificant.

Figure IV-4 shows the Experimental Parameters window again. In the top half of this window, notice the "Calibration" information. The date, time, and operator are listed for the calibration. If you were the first to install new software, the word UNCALIBRATED appears instead of the date, time, and operator identification. In this case, you cannot make any measurements until you calibrate the instrument.

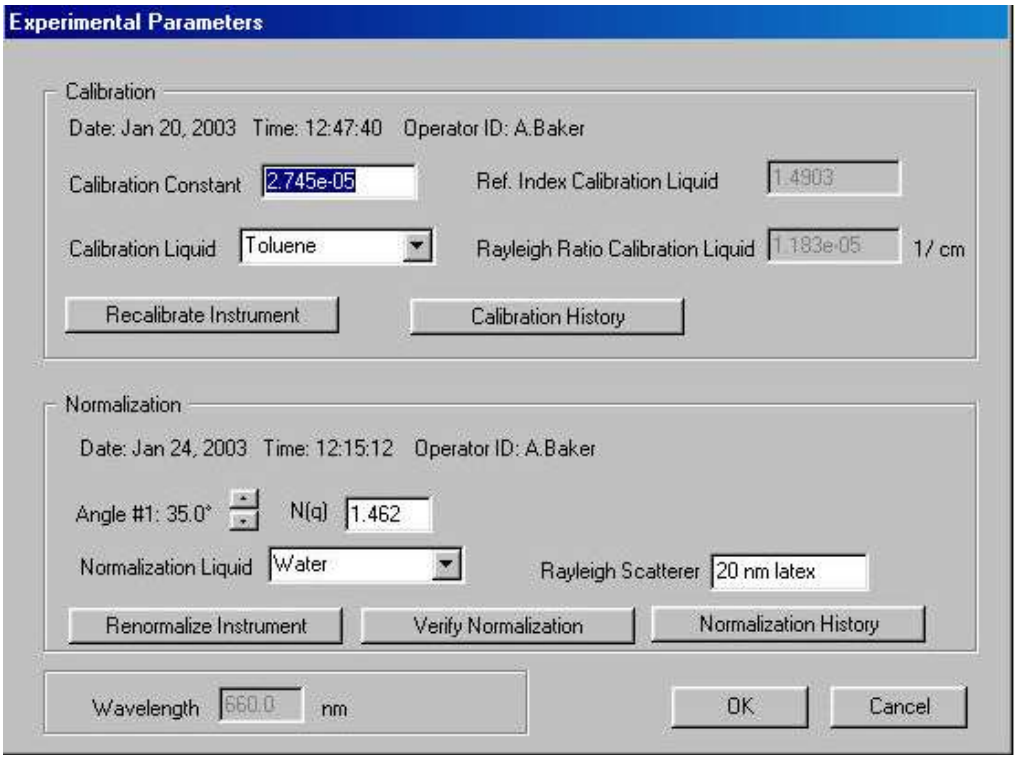


Figure IV-4: Experimental Parameters window.

Proper calibration ensures accuracy when calculating values for M_w and A_2. Select a calibration liquid, typically toluene, from the drop-down list box labeled "Calibration Liquid". Given the wavelength of the instrument shown at the bottom of the page—a value automatically read by the software from the BI-MwA—the Rayleigh Ratio and refractive index are calculated and displayed as shown above in Figure IV-4. The previous calibration constant, here 1.474E-005, is also displayed.

Before calibrating, it is necessary to replace any liquid in the system with the calibration liquid. If the liquid in the instrument is a solution or suspension, first flush with the pure liquid to prevent precipitation. Use 20 mL, a filter, and the syringe pump. Monitor the progress with the *Stability Graph*. If an intermediate liquid is required—as it would be when changing from water to toluene, for example—flush with 20 mL of the intermediate liquid. [When going from water-to-toluene or toluene-to-water, we recommend ethanol as an intermediate liquid.] Finally, using an appropriate filter and the syringe pump, flush the system with the calibration liquid for as long as it takes to obtain stability.

Click on **Recalibrate Instrument**. Follow the instructions. At the end, a new calibration constant will appear. Click on **Calibration History** to review past calibration constants. Calibration constants should agree within 2% if repeated over a short period of time. If they do not, it indicates that not enough care was taken in flushing the system. Repeat the procedures until you get this type of repeatability.

Figure IV-5 shows a calibration history.

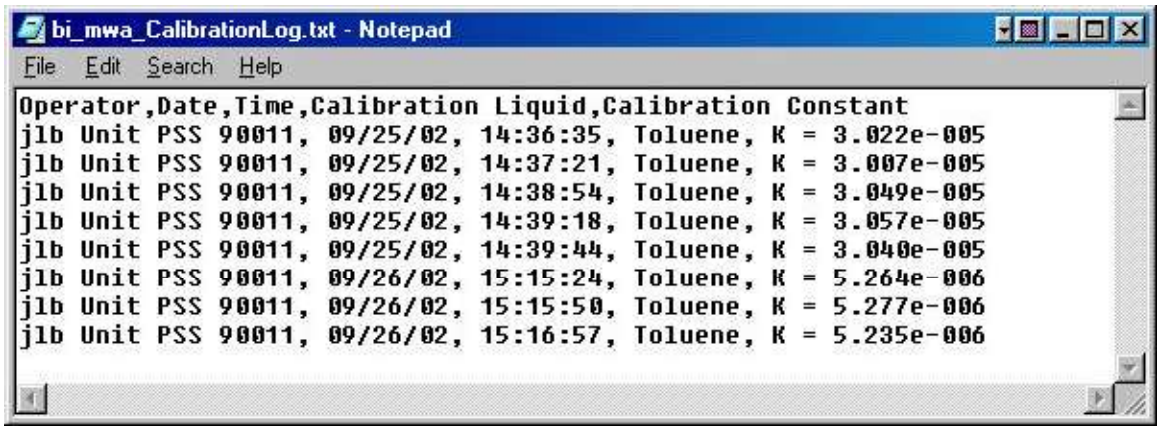


Figure IV-5: Calibration History

The average of all the Sept. 25, 2002 calibration constants is (3.0350 ± 0.0204)e-005 (± 0.67%); whereas, the average of all the Sept. 26, 2002 calibration constants is

(5.2587 ± 0.0215)e-006 (± 0.41%). Repeatability should be 2% or better, as it is here, for different optical configurations. The configuration on Sept. 26 included a neutral density filter that was added in anticipation of batch-mode and TDSLS measurements that are generally made at higher concentrations; whereas, the Sept. 25 calibration was done without the neutral density filter. Such a configuration is best for GPC/SEC measurements.

Since the absolute value of M_w will depend on the calibration constant, make sure to use HPLC grade toluene or benzene. Once opened, a new bottle will begin to absorb moisture from the air. Therefore, do not use old bottles of toluene. Purchase small, new bottles of the purest grade available. Alternatively, use triple distillation to purify a reagent grade bottle of toluene. You will need about 100 mL, which includes enough for flushing and repeated measurements.

Once calibrated, an instrument should remain calibrated for many months unless impurities, salts, or polymer are allowed to dry inside the instrument. We do recommend, however, recalibration every couple of weeks or before a new series of measurements.

Section V: Sample Preparation

Sample Preparation: Generalizations

This is the single most difficult part of making a good light scattering measurement. Most errors in a light scattering measurement can be traced to poorly prepared samples. This applies to the normalization and calibration procedures also. Be patient. Clean everything twice. Use pure materials. Avoid manual injections. Instead, use a syringe pump. Take care to remove visible bubbles from flasks of liquid and the syringes. Use appropriate, rinsed filters.

References 3, 4 and 5 in **Section I: How to Use This Manual and Software** each contain chapters devoted to sample preparation.

It is much easier to prepare samples in nonpolar liquids than it is in water. Water dissolves more air, increasing the possibility that bubbles will form. Water is polar and keeps more charged impurities in suspension.

Water that sits around for more than a day or so is usually not suitable as bacteria begin to grow. Use freshly distilled or filtered water. In winter, when the temperature of water may be lower than usual, allowing more air to dissolve in the water, consider degassing it or letting it stand at room temperature and then sonicating it.

When using other solvents, do not use old, half-filled bottles, as these solvents are very likely to be contaminated. Use either triply distilled solvents or the very best HPLC or reagent grade solvents. The money spent on purchasing the best solvents and correct filters will be worth it because of the time saved in making good measurements.

Gravimetric methods are more exact, even for the determination of the solvent volume. (Multiply by the solvent density determined to three significant figures and include a small temperature correction if available.) Yet, it is more convenient to determine the volume of solvent using a calibrated pipette. Automated pipettes are sometimes very accurate, but first verify they are correctly calibrated by weighing a liquid of known density. Never rely on the markings of a typical syringe for accurate volume determinations unless you have calibrated the syringe by weighing liquids of known density.

A minimum of a four-fold change in polymer concentration from the least to the most concentrated sample measured is recommended. A 10-fold change is preferred. Use either a 1:2:5:10 or 1:2:4:6:8:10 layout in concentration. A minimum of three concentrations is required; we recommend five.

Verify that Filtration does not effect Concentration

If it is possible to dry a solution without decomposing the polymer itself or incorporating some of the solvent into the newly dried polymer, then prepare a solution by weighing dried polymer and adding a known volume of liquid. Then filter. Then dry the solution and see how accurately the two polymer weights agree. It is best to cover the sample that is drying to prevent

airborne materials from adding to the dried weight. This entails placing a low-lying canopy over the sample to be dried. You should expect concentrations to be accurate and repeatable to 1% or better. If not, a significant amount of polymer is on the filter.

If evaporation to constant weight is not suitable, consider the following choices: UV absorption (if appropriate) at a wavelength where the extinction coefficient is known or measured; measurement of the differential refractive index, Δn, where dn/dc is already known (BI-DNDC).

Two Methods for Preparing A Set of Concentrations

You can prepare a single stock solution, quantitatively divide it into separate vessels, and add known amounts of filtered solvent to each. Alternatively, you can prepare individual sample concentrations.

The advantage of the first method is that only solvent is filtered to make dilutions. If significant change occurs upon initial filtration of the stock solution, only this filtered solution concentration needs to be determined as described above. However, errors compound with serial dilution, and an error made at any step propagates into all succeeding steps.

You must use a precision pipette or a calibrated, automated pipette. Volume delivery is always more prone to error than the less convenient but more accurate method of weighing samples. In addition, some polymers do not rapidly disperse when new solvent is added. You may have to wait for spatial homogeneity before injecting the freshly diluted sample into the BI-MwA.

Preparing individual sample concentrations is more time consuming, but it is the preferred method for experienced users.

Stock Solution Concentration

The scattered intensity depends on many factors including polymer concentration, molecular weight, and specific refractive index increment, dn/dc.

As a first guess make a stock solution of 10 mg/mL for a polymer with M_w = 100,000 g/mol and dn/dc = 0.1 mL/mg. Since I ~ c*M*(dn/dc)^2, you can use these numbers to estimate a stock concentration given a guess for M and knowing dn/dc for the unknown polymer.

Preparing a Stock Solution

This procedure assumes you have plenty of dry polymer.

Wash with soap, water and a bottle brush any syringes, vials or dilution vessels including any screw caps. Wear clean, initially dry gloves to prevent finger oils from contaminating the glassware. If you are using a glass pipette, you must clean it too. Automatic pipettes with disposal polyethylene tips are better in this regard, but make sure you have calibrated the pipette

and understand that a loose fitting pipette tip introduces a false volume reading.

Let glassware dry thoroughly.

Choose a filter and filter housing suitable for use with the solvents to be used. For water and similar, polar, non-aggressive solvents, standard cellulose filters are sufficient. For more aggressive solvents, or ones that will not wet cellulose, consider Nylon and Teflon filters and polycarbonate housings. Disposable filters are convenient, though the traditional stainless steel filter housings and replaceable filters are also choices to consider.

Use a nominal 0.1 micron pore size filter. Rinse the filter at least once with a syringe full of liquid to remove residue from the filter manufacturing process.

Prepare a 20 mL dilution vial with cap by rinsing cell, cap, and upper portions of the cell several times with filtered solvent. Cover it while it dries thoroughly. Weigh it. Weigh in roughly 100 mg of polymer. Add approximately 10 mL of filtered solvent. Weigh the vial. By subtraction, determine the solvent's weight. Use the density of solvent at the room's temperature to calculate its volume. Calculate the stock solution concentration. In this example, it will be close to 10 mg/mL. Alternatively, use a **calibrated**, volumetric pipette to determine the volume of solvent.

Even though the solvent was filtered, it often happens that dust is introduced into the solution from the surface of the dry polymer. Therefore, you need to filter the stock solution. If you have not done so already, verify that filtration does not significantly change the polymer solution concentration. If polymer is left on the filter, then you should use the dilution method discussed previously, and you must determine the filtered, stock solution concentration using one of the two methods previously discussed.

During the preparation of the stock solution and in the subsequent handling of polymer solutions, remember that volatile solvents will result in increasing concentration with time. Also, minimize contact between samples and lab air. It is laden with dust, and all your previous efforts are wasted if well-prepared samples are not capped.

The highest measured concentration, using any of the measurement schemes described previously, should be about half the stock solution concentration.

We recommend measuring five concentrations, though a minimum of two will give you a straight line. [Note: If you use statistical weighting, a minimum of three data points for a straight line are required to calculate χ^2.]

Before You Take the Plunge --A Trial Measurement, Please

Do not start with the most difficult sample you can find. In particular do not try to measure that 30 million molecular weight DNA first; do not try to measure sucrose in water (300 g/mol) first; and do not try to measure the exotic polymer you just synthesized first. There is, after all, only 3 micrograms of it in existence and you cannot get the Nobel prize if there is no

more left for another lab to verify your work.

Now that you have read this manual and played with the software using stored data, there is an overwhelming tendency to make your very first SLS measurements on either your own samples or the most difficult one you can find. Resist the temptation until you have experience.

Instead, try something simpler to get use to the program, and, if you have never made SLS measurements before, gain some experience with sample preparation. We suggest measuring polystyrene in toluene, perhaps in the range of 100,000 g/mol. It dissolves almost immediately; no gels are formed; scattering is good; the dn/dc is approximately 0.105 mL/mg; and inexpensive reference standards are available that are good to about ± 5 %. Check with a supplier of GPC/SEC standards for calibration of columns. They offer polystyrene “standards”.

Do not be fooled when purchasing GPC/SEC standards. Some are not narrow distributions defined by $M_w/M_n \leq 1.05$. Some are characterized by M_p, the “peak” molecular weight obtained in a GPC/SEC fractionation. Advice: Be sure you know what the weight-average molecular weight, M_w, is supposed to be and its expected uncertainty.

Section VI: Making A Measurement

Normalize and calibrate the instrument as discussed in Section IV. The current normalization and calibration values can be reviewed by clicking on **Experimental Parameters**. Prepare samples as discussed in Section V. Then click on the **Sample Parameters** command button. See Figure VI-1

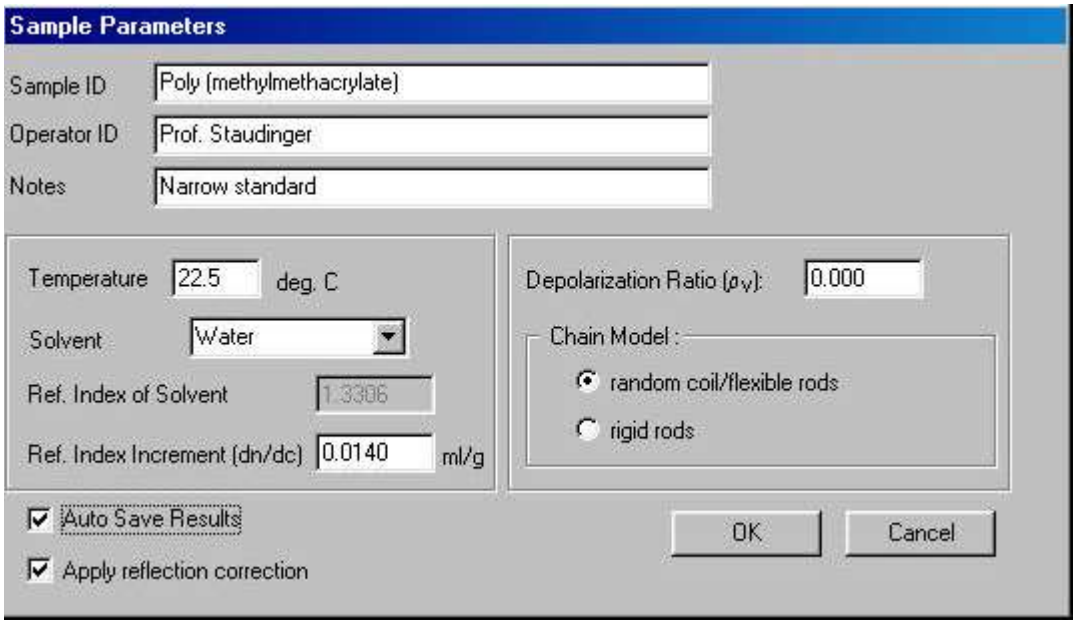


Figure VI-1: Sample Parameters window.

Fill in an appropriate "Sample ID". This name is used in the database and a little thought in assigning an identification will make it easier as time passes to identify measurements. Fill in the "Operator ID" and add any "Notes".

Select the "Solvent" from a pull-down list. If your solvent is one of several common ones found in the list, its refractive index will automatically be calculated and displayed (grayed out: visible but cannot be changed). If your solvent is not in the list, select "Unspecified" and then type in the solvent's refractive index.

As discussed in Section II: Theory, the value of dn/dc is required. See Appendix C for more information.

For most random coil polymers, leave the "Depolarization Ratio, ρ_v" at its default value of 0.000. If you know the value of ρ_v and the appropriate chain model, make those choices in this window. NOTE: If ρ_v is zero, it does not matter what chain model you select. The answers for M_w, R_g, and A_2 are then not dependent on the model chosen.

Click on "Auto Save Results" and exit this window by clicking on OK.

NOTE: You can always recalculate results by editing later the solvent's refractive index, dn/dc, ρ_v, and the selected chain model.

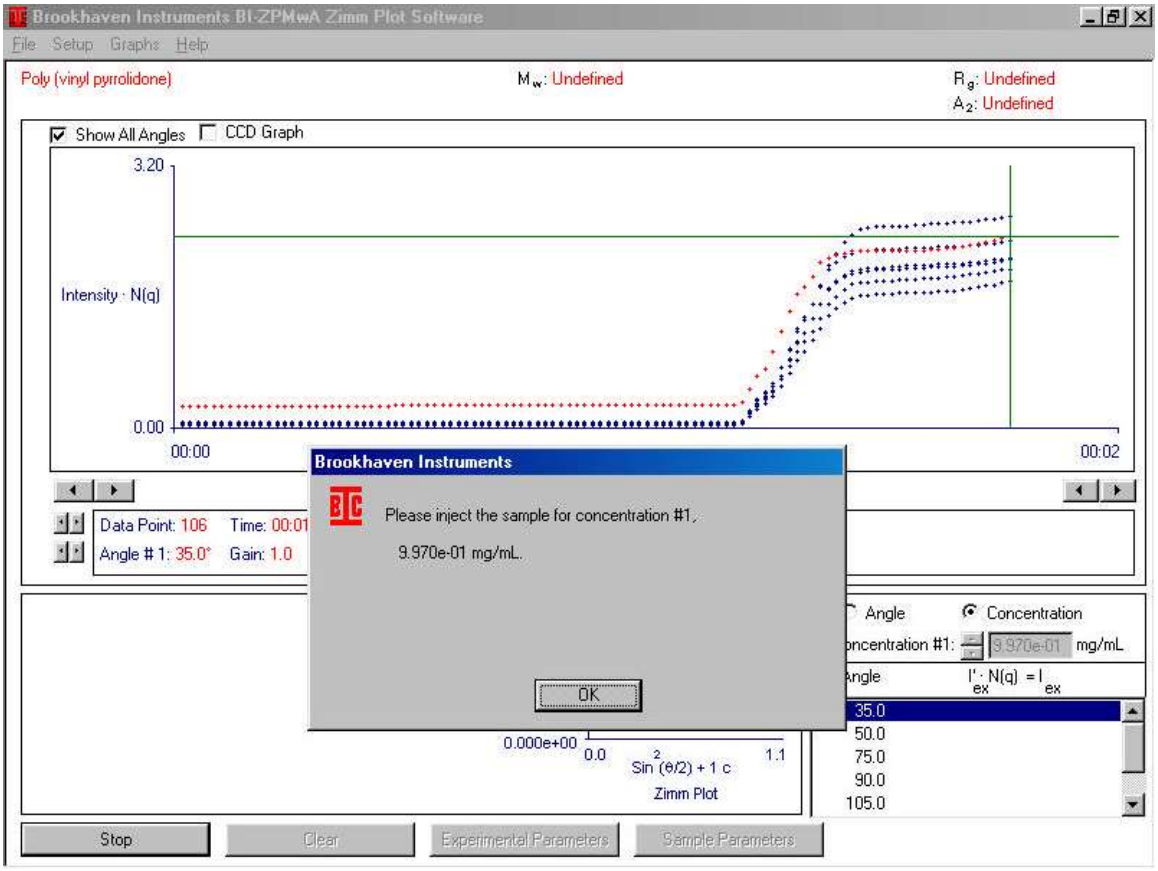


Figure VI-2: Main Page.

Click on the **Start** command button in the lower left of the screen. If the instrument has not been normalized and calibrated, you will be instructed to do so. You cannot make any measurements without performing these separate measurements first.

Figure VI-2 shows the window you will see instructing you to inject your solvent. Press OK only after the miniature stability graph shows that the signal vs. time has reached a new level. This should require a minimum of 3 mL depending on how clean the system was prior to this injection. After pressing OK, you will wait a time in seconds

equal to N, the number of stability points (default 20). Red/green traffic lights will appear indicating whether or not the signal is stable.

Once stable, you will be asked to type in your first polymer concentration and then inject the sample. Again, do not press OK immediately after injecting the sample. Wait for stability. Continue to follow the instructions on the windows requesting you to type in the polymer concentration, inject the sample, and press OK to enable data acquisition.

You can click **DONE** to end a measurement. If you do so before collecting at least five concentrations, you will receive a warning to add more concentrations. A minimum of three concentrations is required.

As you add points, a Zimm Plot (or the plot selected) will begin to form with preliminary results for M_w, R_g, and A_2. Until you have acquired all the points and made decisions about the analysis described in Section VII, do not become overly concerned with these preliminary results. Of course, they should be the right order of magnitude, unless your concentrations are wrong, or your normalization or calibration is wrong.

NOTE: You can always change the type of plot after you have made the measurement since the intensities from all seven angles are collected at each concentration independent of the plot type you have selected for viewing and analysis.

Section VII: Analyzing Data & Interpretation

Figure VII-1 shows the plot before a fit is displayed. Even though the fitted lines are not displayed, a fit has nevertheless been made and the results are shown above the graph in red for M_w, R_g, and A_2. The fit was made using all the angles and all the collected concentrations since to this point the user has made no choices. In addition, the fit order for both the lines of constant concentration and constant angle are, by default, one (linear fits). The plotting constant, here 23, was chosen by default given the layout of the points.

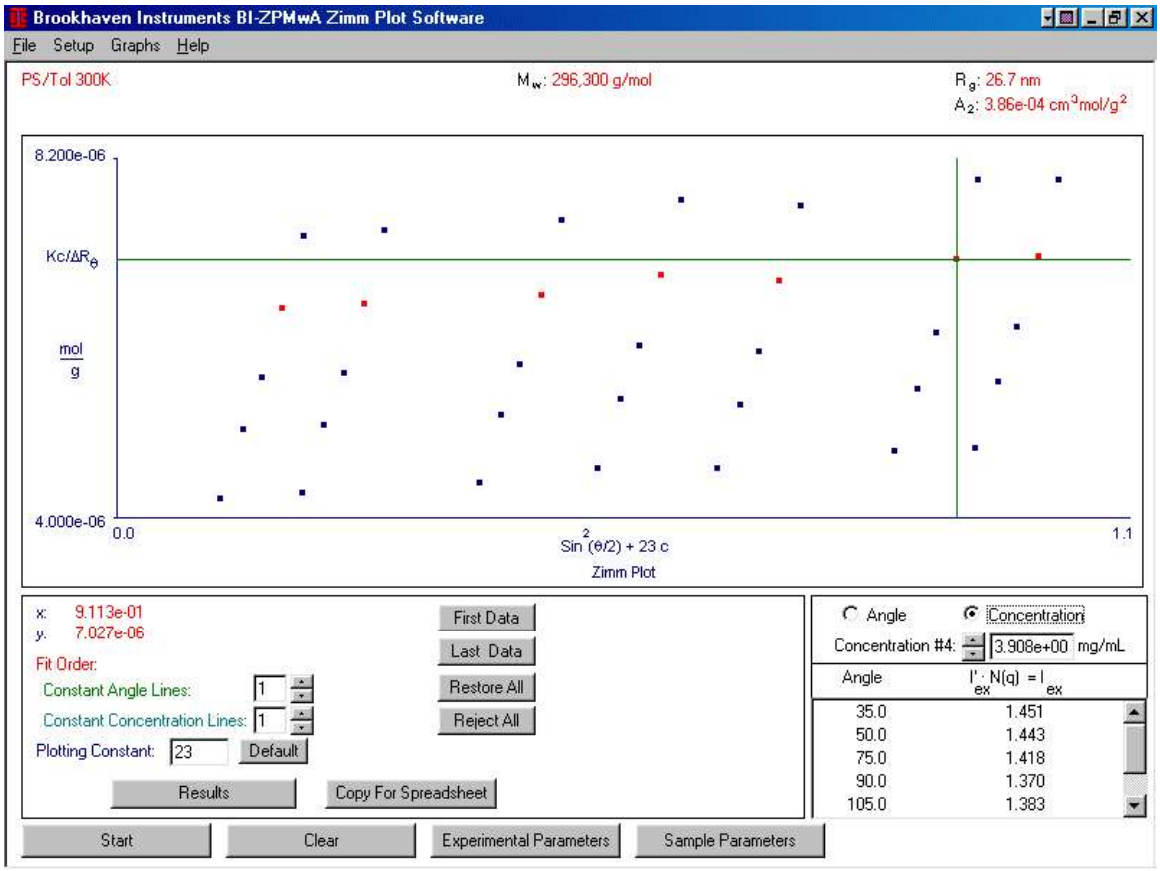


Figure VII-1: Preliminary Zimm Plot window.

If the points are not well-separated, change the plotting constant. Even negative values are allowed. If any single point is significantly off the pattern established by the rest, click on it. The angle and concentration for that point will be shown in reverse video in the table in the lower right of the screen. Double click on the reverse video line and it will be temporarily deleted.

Click on *Concentration* as shown in Figure VII-1. You can then scroll through the concentrations using the Up/Down arrow keys in this table. As you scroll through concentration, the tabular values displayed are the excess intensity, I_{ex}, at each angle for the selected concentration.

You can change the concentration if you wish by editing it; however, do not do this unless you have good reason to believe the originally entered concentration is incorrect. If a concentration were too high, the points would be shifted to the right and high. If a concentration were too low, the points would be shifted to the left and down.

Click on the radio button to the left of *Angles*. You can then scroll through the angles using the Up/Down arrow keys in this table. As you scroll through the angles, the tabular values displayed are the excess intensity, I_{ex}, at each concentration for the selected angle.

If, at constant angle, all the concentration points were shifted up, it suggests that the normalization constant for that angle is too small. If, at constant angle, all the concentration points were shifted down, it suggests that the normalization constant for that angle is too large.

While you can edit the N(q) values in the **Experimental Parameters** window, to do so may introduce a bias. Better to renormalize and apply those values to your saved set of data.

Click on the command button labeled **Results** when finished. Figure VII-2 shows the results for the data points remaining. This is a Zimm Plot using linear fits to both constant concentration and constant angle lines.

At each angle, the five concentrations are extrapolated to zero concentration. This produces seven points on the bottom edge of the grid pattern. These seven points are fitted to a straight line as a function of angle and extrapolated to zero angle. The slope of this straight line, along with the intercept, yields the radius of gyration. The inverse of the intercept of this line yields the weight-average molecular weight. The results of this fit are displayed in the upper half of the lower window and are color matched to the points on the screen.

At each concentration, the seven angles are extrapolated to zero angle. This produces five points on the left edge of the grid pattern. These five points are fitted to a straight line as a function of concentration and extrapolated to zero concentration. The slope of this straight line yields the second virial coefficient. The inverse of the intercept of this line yields the weight-average molecular weight. The results of this fit are displayed in the bottom half of the lower window and are color matched to the points on the screen.

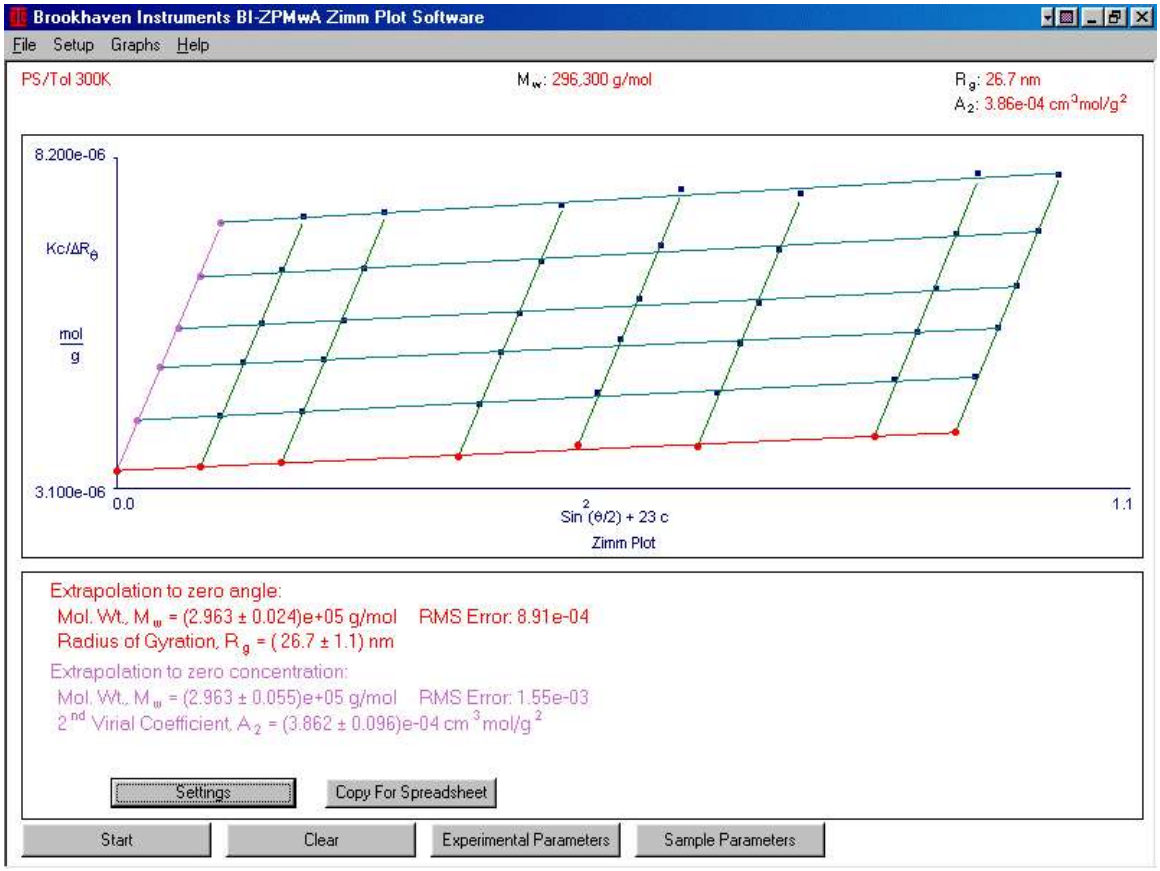


Figure VII-2: Zimm Plot results.

By virtue of the double extrapolation, with all points considered equally uncertain, the two intercepts match, though the random fitting errors are determined by the uncertainty of the respective points obtained from extrapolation. This is the beauty of the Zimm Plot: The double extrapolation yields a common intercept equal to $1/M_w$.

The X and Y values can be copied to a spreadsheet and plotted by the user to suit their needs. Click **Copy for Spreadsheet**. If you choose to accept the plot as it is, click on **Copy Plot**.

Click on the command button labeled **Settings** to return to the previous view. Do this if you want to delete or restore points. Do this if you want to fit to a different order. Do this if you want to fit to a Berry or Debye plot or if you want to scale the plot differently. As explained below, you can even try statistical weighting instead of equal weighting.

Let us examine these choices in more detail.

In this example, all the points appear reasonably correct, with no single point so far off the grid pattern to deserve deletion. As this is a manual, we show a reasonably good result. You will most likely want to delete a few points or even entire angles or concentrations. The command buttons shown in Figure VII-1 allow you to do this. They are labeled **First Data**, **Last Data**, and **Reject All**. If you decide to restore individual points, locate them in the table and double click. If you decide to restore all the rejected points, click on **Restore All**. Which set of points are edited depends on whether the *Angle* or *Concentration* radio button is selected.

It is worthwhile playing a little to satisfy yourself that the final answer is robust and does not depend much on the removal of a few points. Of course, if you only have a few concentrations or if you remove several angles, then your answer is not robust and will be determined by your bias.

It is best to stay with linear fits whenever possible as with few points, it is hard to determine if second or third order fits are worthwhile. For an n^{th} order, least-squares, polynomial fit, you need n+2 data points theoretically. [n+1 points determine the exact fit, not a least-squares fit.] For example, if you retain all seven angles, you can fit up to a 5^{th} order polynomial. If you have five concentrations, you can fit up to a 3^{rd} order polynomial.

Warning

Unless you have strong, consistent, visual justification for 2^{nd} order and higher polynomial fits, stay with the linear fits. Over fitting, to reduce the root-mean-square (RMS) error, may result in the wrong extrapolation for M_w and the wrong slopes for R_g and A_2.

Click on **Graphs** from the preliminary Zimm Plot window. You will see choices shown in Figure VII-3. Check *Autoscale Y-axis* and then click on **OK**. Click **Results** and the plot should fill most of the available space.

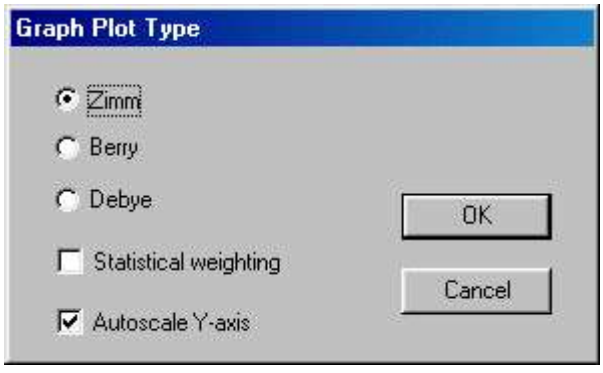


Figure VII-3: Graph Plot Type choices.

Select Berry or Debye plots in this window. Recall that Berry plots are normally preferred when the term $3A_3M_wc^2$ is not small compared to the term $2A_2M_wc$. This typically occurs with polyelectrolytes unless the solutions contain at least a few millimolar of salt ions. We suggest 10 mM $NaNO_3$. A significant 3rd order term can occur even in nonpolar solvents at higher polymer concentrations, depending on the value of M_w.

The Debye Plot is appropriate for a Rayleigh scatterer (R_g less than about 10 to 12 nm). In this case, there is no angular dependence and you can obtain M_w and A_2 only. Figure VII-4 shows an example.

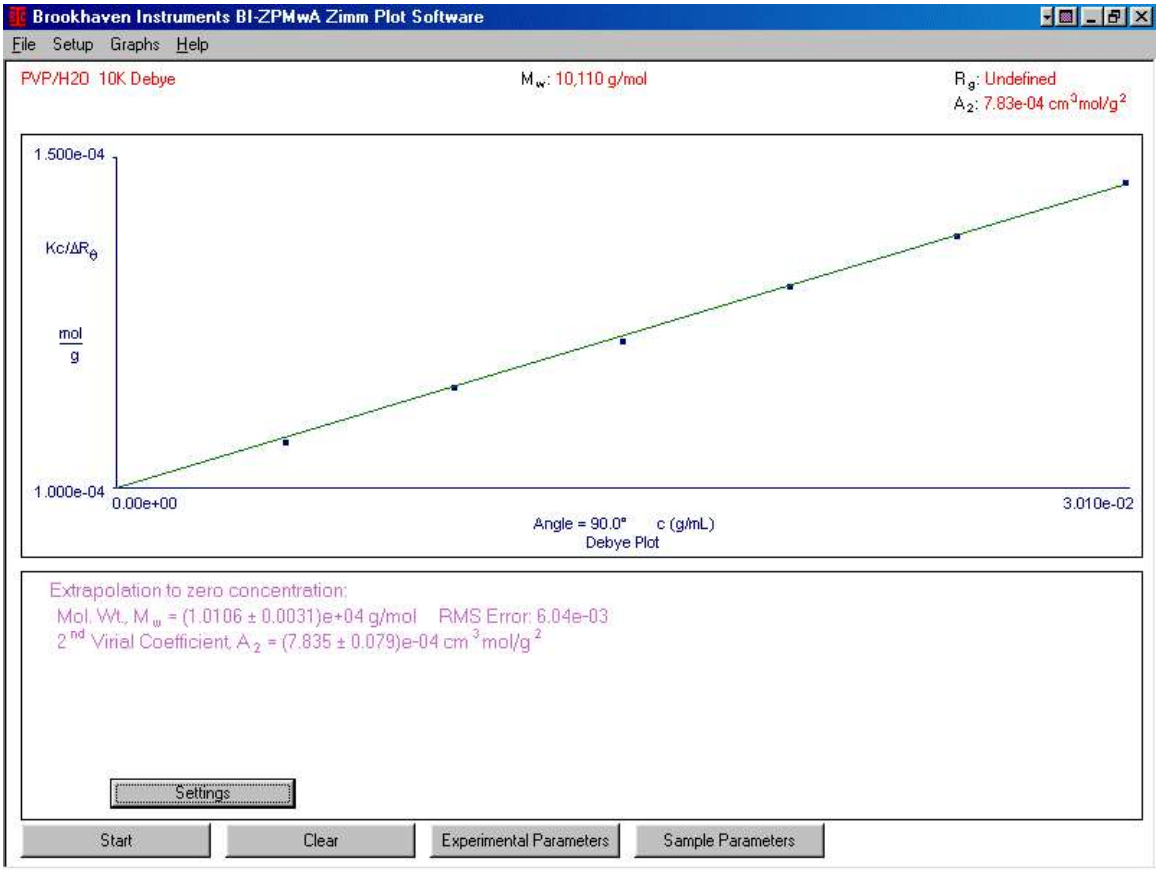


Figure VII-4: Debye Plot

This 10,100 Da polymer is a random coil and its R_g is too small to measure with static light scattering. Thus, as expected, it is a good candidate for a Debye Plot where the A_2 value of 7.84e-04 cm^3mol/g^2 can be determined.

When you select a Debye Plot, you can choose which of the seven angles will be used. We recommend the 90^{o} data since it should be least effected by flare light and reasonably immune to residual dust (forward angle scatterers).

If you have good data, you can try checking the *Statistical Weighting* box in the Graph Plot Type window. Since each data point is determined by averaging a number of points, there is a variance associated with each point. In least squares fitting procedures, the inverse of the variance is the weight. If there are no systematic errors, then with statistical weighting the RMS error is transformed into a reduced χ^2 (pronounced Chi-squared) value. If this unitless number is close to 1, the data and selected equation for fitting are in close agreement. If the value is much larger than 1, then either the data are highly scattered or there is a systematic error or the equation for fitting is the wrong one.

Consult books on statistical fitting procedures for more information on the use of χ^2. Values much less than 1 are also unlikely and indicate something other than a random error is causing the problem.

When you do use statistical weighting, the intercepts for the double extrapolation procedure with the Zimm and Berry Plots do not lead to equal values for M_w unless the fit is very good and then χ^2 will be close to 1.

Finally, it should be noted that if the grid pattern looks good and the fit is good but the resulting value for M_w is wrong, the problem lies with either the value used for dn/dc or with the calibration.

Section VIII: Database

Results are saved in the currently opened **Folder** as a **File** using the **Sample ID, Date,** and **Time** as the file identifiers. Click on **File** in the menubar of the main window to access Folders and Files. Files are automatically saved when the **Auto Save Results** box, under the **Parameters** command button, is checked. If not checked, you can still save files using **Save** or **Save As** in the **File** menu. See Figure VIII-1.

To create folders, select **Database** from the **File** menu. Click on the **Create Folder** command button. Enter a folder name; it can be more than 8 characters; it may include spaces and punctuation. Double click on the folder icon to open it. The file folder icon *opens* when the folder is active. Single clicking on a folder selects the entire folder but does not open it. When selected, but not opened, the entire folder and its contents may be deleted, printed, or archived by clicking on **Delete Selected Folder**, **Print Selected Folder**, or **Archive Selected Folder**.

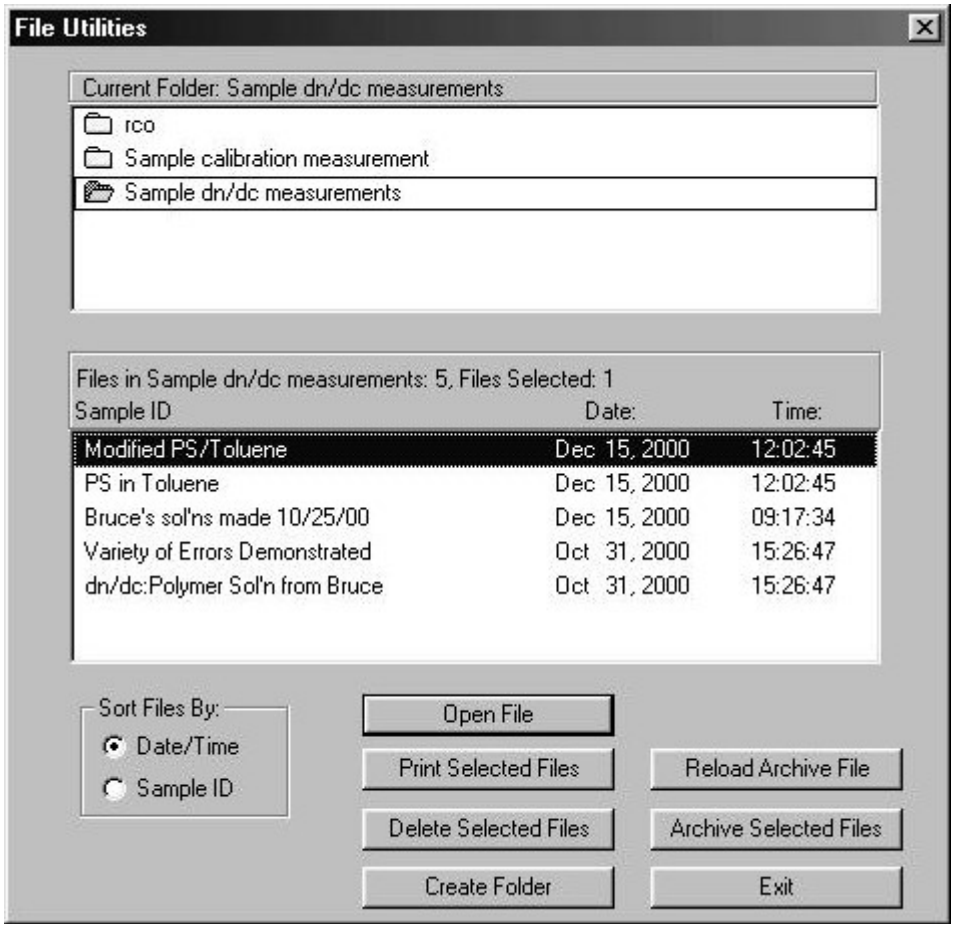


Figure VIII-1: Database window, Folders & Files

Select a single file in an open folder by clicking on it. Select a string of consecutive files by clicking on the first, then, while holding down the *Shift* key, click on the last. Select a string of nonconsecutive files by clicking on the first, then, while holding down the *Control* key, click on any number of files, consecutive or not, one at a time. When selected, a single file or multiple files may be deleted, printed, or archived by clicking on **Delete Selected File(s)**, **Print Selected File(s)**, or **Archive Selected File(s)**.

Once selected, single or multiple files can be moved or copied to another folder by cutting and pasting (CTRL C and CTRL V) or by dragging and dropping.

Files are **archived** in a binary format in the following path:

c:\bicw32\bmzpmwa\data\archive.bak

using the extension .bak by default. You may change the drive\directory, but you will then have to remember it. If you do not change the filename, the default filename, archive.bak, is used. You can archive an entire folder by selecting it, but not opening it. You can archive single files or groups of files. When archived to an existing .bak file, the selected folder or file(s) are added to it.

Click **Reload Archive File** when you want to reload an archived file. Click on the drive and directory where it was stored. The default path, mentioned above, is c:\bicw\dndcw\data. However, if you archived the folder\file(s) in another path, you must select that drive\directory and the correct filename. Files are reloaded and merged into the currently opened folder.

Double clicking on a file opens it. Alternatively, select the file by single clicking on it and then clicking on **Open File**.

Finally, you can export a selected file with an ASCII format. See Appendix F for details.

Appendix A: Clearing Blockages & Removing Bacterial Growth

If liquid (with or without solute or polymer) dries inside the instrument, the signal stability will suffer. The signal will drift continuously. You need to slowly flush the system with a solvent that will dissolve whatever has dried onto the walls of the tubing and cell. This may take many hours depending on the solubility of the dried material in the liquid you use to dissolve it. Use a syringe pump such as the BI-SPump. Alternatively, flush both inlet and outlet with 2-10 mL of solvent many, many times throughout the day.

If solvent will not dislodge the dried particles or polymer, use 0.1 M nitric acid. Wear gloves and safety goggles. You may have to repeat this several times to get even a low flow rate going. Since the blockage is often near the beginning of the internal tubing, it often helps to flush the instrument in the opposite direction.

If the instrument is left with water, bacteria may grow and adsorb onto the walls of the system. When flushed initially, the signal may drift for a long time just as if salt or polymer had dried in the system. This will also require slow flushing with alcohol and then water to remove it.

You can determine when the instrument is stable by monitoring the signal as a function of time using the stability graph.

Appendix B: Solvent Compatibility

Cells

The BI-MwA is supplied with one of two different types of cells: PET, poly(ethylene terephthalate); or PTFE, poly(tetrafluoroethylene). Both are black. DuPont calls its brand of PTFE Teflon. PTFE is more solvent resistant than PET, and it is stable to over 200 °C; however, Teflon is also much harder to machine with accuracy than PET. The black, PTFE used by Brookhaven is filled with carbon black. The filling makes the resulting plastic harder and easier to machine.

The manufacturing I.D. label, after the model name, indicates which type of cell you have: BI-MwA/PET or BI-MwA/PTFE. These I.D. labels are located near the electrical connections.

The only materials in contact with the solvent are PET (or PTFE), stainless steel 316 tubing*, PTFE gaskets, and glass from the entrance/exit windows and the fibers.

WARNING: Do not use the PET cell above a temperature of 80 °C.

Partial List of Compatible Solvents at Room Temperature for the PET cell.

Benzene	Glycerine
Butanol	Heptane
Butyl Acetate	Hexane
Carbon Disulphide	Methyl Alcohol (Methanol)
Carbon Tetrachloride	Methyl Ethyl Ketone (MEK)
Chlorobenzene	Octane
Cyclohexane	Pentanol (Amyl Alcohol)
Dimethylformamide (DMF)	Tetrahydrofuran (THF)
Dioxane	Toluene
Ether	Trichlorotrifluoroethane
Ethyl Acetate	Water
Ethyl Alcohol (Ethanol)	Xylene
Ethylene Glycol	
Formamide	

* If stainless steel is inappropriate for your materials, PEEK tubing is an option. Polyetheretherketone (PEEK) tubing is often used in LC/HPLC for biomaterials. Please contact the factory.

The PET cell is partially resistant to these solvents:

Acetone**
Isopropyl Alcohol**
Trichloroethylene (TCE)

We don't recommend using these solvents with the PET cell:

Chloroform
Dichloroethane
Dichloromethane

WARNING: These solvents will dissolve the PET cell. Do not use them.

Hexafluoroisipropyl Alcohol (HFIP)
Trifluoroethanol (TFE)

Above are partial lists because there are millions of solvents. Contact the factory at info@brookhaveninstruments.com if your solvent does not appear anywhere on these lists.

While PTFE is compatible with many more solvents than PET,

We don't recommend using these solvents with the PTFE cell:

Chlorobenzene
Methyl Ethyl Ketone (MEK)
Trichloroethylene (TCE)

WARNING: These solvents will dissolve the PTFE cell. Do not use them.

Diethylamine
Hydrofluoric Acid (HF, pure)
Methylacrylate

**Acetone and Isopropyl Alcohol are okay to use at room temperature for flushing. Isopropyl alcohol is okay for preventing bacterial growth. Refrain from using these solvents at elevated temperatures or periods longer than a few hours.

Tubing

You can use stainless steel tubing to connect the BI-MwA to any other device. Use 1/16" O.D. (1.59 mm) and 0.010" I.D. (0.25 mm) tubing. For continuous use with a variety of solvents or for higher temperature, stainless steel is preferred.

However, for use with water or in batch-mode operation with less aggressive solvents, consider using PEEK tubing. PEEK, poly(etheretherketone), can withstand temperatures up to 100 °C when used continuously under pressure. It is an excellent choice for most chromatography applications except when used with these solvents:

Do NOT use these solvents with PEEK tubing:

DMSO (dimethyl sulfoxide)
THF (tetrahydrofuran)
CH_2Cl_2 (methylene chloride)
Concentrated HNO_3 (nitric acid)
Concentrated H_2SO_4 (sulfuric acid)

When using PEEK tubing, use 1/16" O.D. (1.59 mm) and 0.010" I.D. (0.25 mm).

Important Warning

Never allow any liquid to dry inside the instrument. Always flush the solute or polymer with solvent. If you are not going to work with the instrument for more than a day, **AND** you are working with a solvent in which bacteria may grow, flush the instrument with one or more compatible solvents (see end of this section). Flush with one or more intermediate solvents until you can replace the final liquid with ethanol or some other alcohol in which bacteria will not grow. Then cap the inlet and the outlet ports with the plastic DELRIN plugs that were included when we shipped the instrument to you.

If a blockage occurs due to dried salt or polymer, refer to Appendix A of this manual.

Compatible Solvents Used For Flushing

Methanol and ethanol are intermediate solvents that are miscible with water and with less polar solvents like THF, ethyl acetate, toluene, and benzene.

When changing from water to a nonpolar solvent, first flush the salt or polymer solution with water until the signal is stable (if you have software running). This should take about 20 mL. Then slowly flush (about 1 mL/minute) with an intermediate solvent like ethanol. Check for signal stability again. This should take about 20 mL.

When changing from a nonpolar solvent to water, first flush the polymer solution with the solvent until the signal is stable. This should take about 20 mL. Then slowly flush (about 1 mL/minute) with an intermediate solvent like ethanol. Check for signal stability again. This should take about 20 mL, depending on the miscibility of the two liquids.

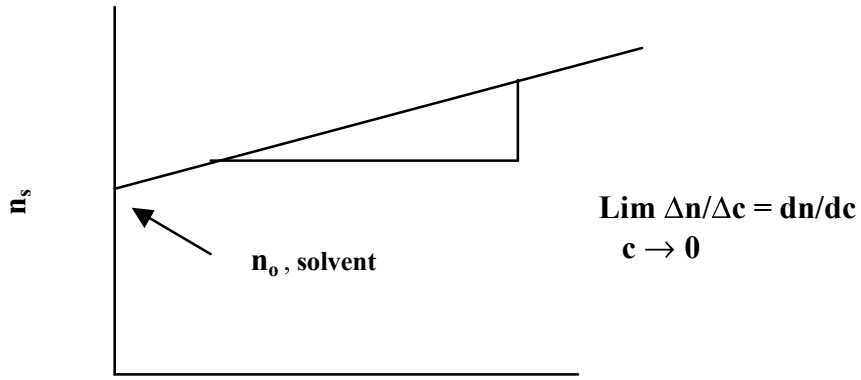
We recommend using a syringe pump like the BI-SPump for slowly flushing the system. The BI-SPump is a fixed-speed, syringe pump. With a 20 mL syringe, the speed is about 1 mL/minute.

By flushing the salt or polymer solution first with the solvent, you prevent salt or polymer from precipitating, and then clogging the small-diameter tubing, in case they are not soluble in the intermediate solvent. For example, if you flush a salt solution with an intermediate solvent, the salt may precipitate. Therefore, in this case, flush first with water and then isopropanol, the intermediate solvent.

Appendix C: Differential Refractive Index Increment, dn/dc

Definition of dn/dc

The differential refractive index increment, sometimes referred to as the specific refractive index increment, or SRII, is the slope of n_s vs. concentration at zero concentration. Here n_s is the refractive index of a polymer solution. The SRII is a limiting slope at zero concentration often referred to as just dn/dc. However, in most polymer/solvent samples n_s vs. concentration is a straight line and the limiting slope is the same as the slope at finite concentrations, even up to 10's of mg/mL.

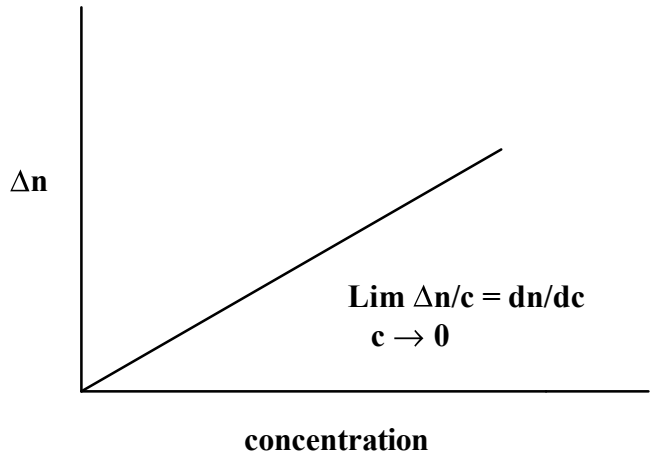


These concepts are illustrated above and serve to define conceptually dn/dc. In practice, the variation in n_s with temperature is larger than its variation with concentration, and the above plot is nearly impossible to obtain with precision unless extreme measures are taken for temperature stability. To avoid this problem, one should use a differential refractometer such as the BI-DNDC.

Differential Refractometer

In a differential refractometer, the solution and solvent are maintained at the same temperature in the same cell separated by a transparent partition. It is not important to know the exact temperature to within a few degrees; it is only important that the temperature difference between the two sides of the cell is small, ≤ 0.01 °C. This is not a particularly stringent condition. It can be accomplished in a well-insulated, small cell by waiting for thermal equilibrium or by controlling the cell temperature to within ± 0.01 °C. The BI-DNDC differential refractometer is capable of maintaining the solvent and solution sides of the cell within ± 0.01 °C of each other from about 5 °C above ambient to about 80 °C.

The signal in a differential refractometer is proportional to the displacement of a beam of light as it traverses the solution and solvents sides of the cell. To improve resolution, the beam is reflected back through the cell to double the displacement. The displacement is proportional to the difference in refractive index of the solution and solvent-sides of the cell. $\Delta n \equiv n_s - n_o$. The basic plot is illustrated below.



The value of dn/dc normally varies from about 0.05 to 0.25 mL/g. Occasionally negative values occur when the refractive index of the solvent is greater than that of the polymer molecule. Since dn/dc is squared in the equations used to determine M_w, a negative dn/dc is physically acceptable.

Establishing Error Limits

When using the batch mode of the BI-MwA with the BI-ZPMwA software, the relative error in calculating M_w is twice the relative error in dn/dc. For example, if you want to know M_w to 5% using static light scattering, a reasonable error limit on M_w, then the error contributed by dn/dc must be less than 2.5%.

Literature Values of dn/dc

Literature values for a very large number of common polymers in a variety of solvents are tabulated in:

1. Huglin, M.B., "Specific Refractive Index Increments", Chapter 6 in Light Scattering from Polymer Solutions, M.B. Huglin editor, Academic Press, London & New York, 1972.

2. Huglin, M.B., "Specific Refractive Index Increments of Polymers in Dilute Solution", pp. IV-267 to IV-308 in Polymer Handbook, 2nd ed., J. Brandrup

and E.H. Immergut, editors, Wiley-Interscience, NY, 1975. Essentially a repeat of values tabulated in reference 1.

3. Timasheff, S.N., p. 372-382 in the Handbook of Biochemistry and Molecular Biology, 3rd edition, Vol. II, edited by G.R. Fasman, 1976. Values for proteins in water.

4. Refractive Increment Data-Book for Polymer and Biomolecular Scientists, compiled by A. Theisen, C. Johann, M.P. Deacon, and S.E. Harding, Nottingham University Press, 2000. Many entries are more recent than the classic compilations above.

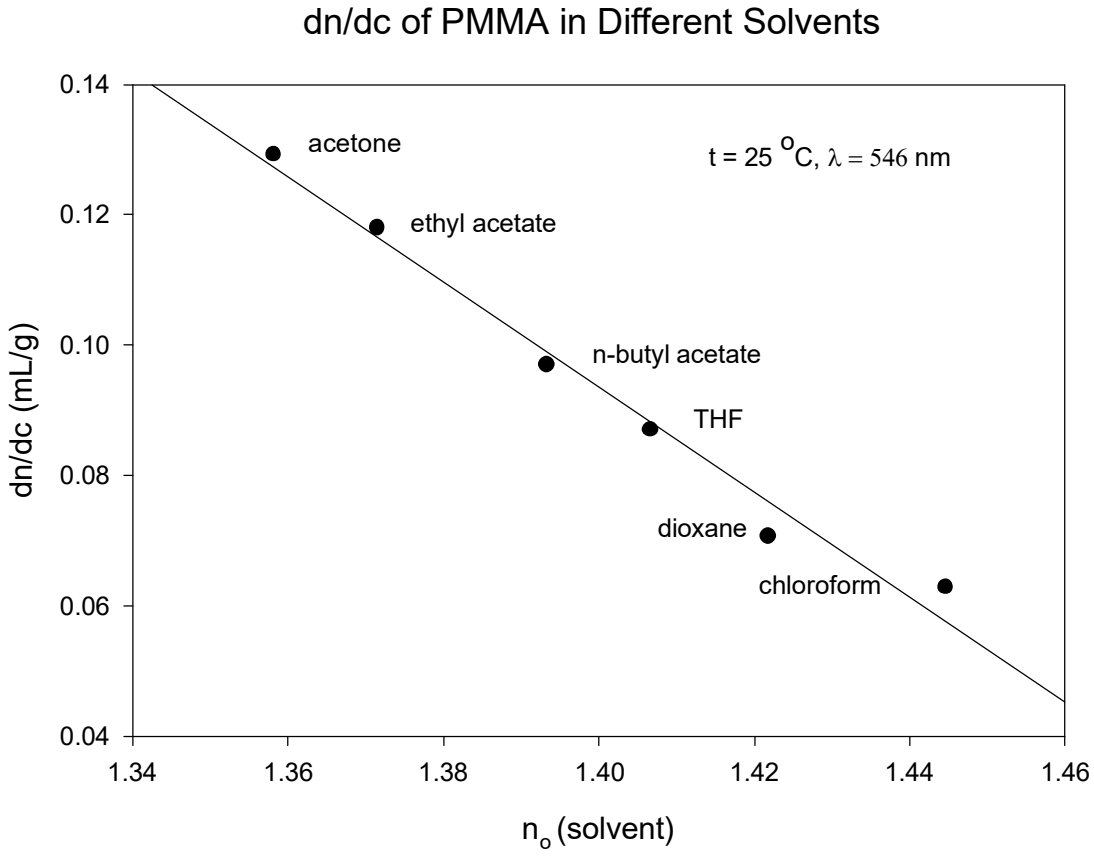
The classic values (before the widespread use of lasers, say before 1970) are given primarily at 436 nm and 546 nm, the mercury lines. Hg-arc sources were the light sources used in differential refractometers and light scattering instruments before lasers and LEDs became widely available. As shown below (**Wave Length Dependence of dn/dc)**, fitting to $A + B/\lambda^2$ using values known at two wavelengths is reasonable for calculating at laser wavelengths of 488 nm and 514.5 nm (Argon-ion laser lines) or 532 nm (newer, frequency-doubled, solid state lasers). However, using the fit to predict dn/dc at 632.8 nm (HeNe laser line) or 660 nm ("red", diode lasers) is less acceptable, but may be necessary.

Without a direct measurement, literature values may be all that you have to work with. It is important, however, to understand that dn/dc varies with the solvent's refractive index, impurities in the solvent and polymer, molecular weight of polymer, temperature, and wavelength. Each of these variables is discussed below.

Effect of Solvent's Refractive Index

If a particular polymer can be dissolved in several different solvents, it is best to choose the one with the greatest difference in refractive index. In this case, dn/dc will be a maximum (scattering intensity varies as square of dn/dc), and errors in determining dn/dc will become less important. While the shape of the molecule (affecting R_g) and its interaction with the solvent (affecting A_2) may change from solvent-to-solvent, M_w will not.

The variation of dn/dc with solvent refractive index is roughly linear. Here is an example.



The data was from Huglin's book, reference 1 above. Note the approximate factor of two in the spread of dn/dc. This leads to a factor of four in the scattered intensity. So using acetone instead of chloroform will yield greater excess intensity, something easier to measure with greater confidence. Note that the near linearity for this particular polymer in several solvents suggests that the partial molar volume at infinite dilute is the same in all the solvents.

Effect of Impurities in Solvent and Polymer

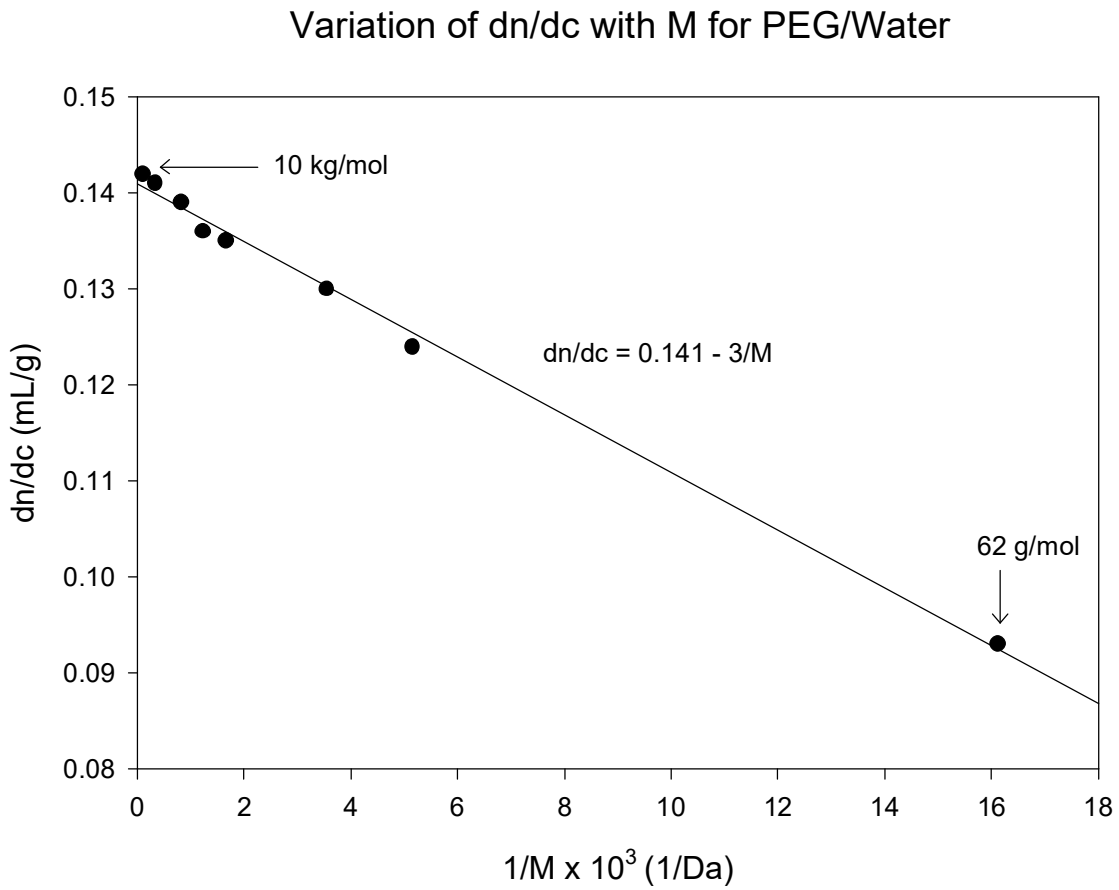
Literature values from several authors for apparently the same polymer/solvent system often differ by several percent, even at the same temperature and wavelength. While this can be due to sample preparation, calibration, and measurement errors, it is sometimes due to impurities in the polymer or solvent. Solvent impurities will cancel when making the dn/dc measurements since they appear in both the pure solvent and the solvent used to prepare the solutions, but then your light scattering measurements have to be made with exactly the same polymer/solvent impurities as that used by the author of the literature article. As this is highly unlikely, it is better to measure dn/dc using your polymer/solvent.

Another manifestation of this same problem may occur if the solutions and solvent have not been kept under the same conditions for both the dn/dc and light scattering measurements. Ideally, the exact same solutions should be used for both measurements, and the solvent used to prepare the solutions should be kept under the same conditions (sealed, no additional moisture or other impurities allowed in contact). Otherwise, the subtraction involved in determining the excess scattered intensity and that involved in determining Δn may be different.

Molecular Weight Dependence of dn/dc

Generally, dn/dc increases with molecular weight and reaches a plateau when the end groups are sufficiently far apart. Below approximately 1,000 g/mol, dn/dc varies considerably. It increases by a few percent up to ~10,000 to ~ 20,000 g/mol, depending on the solvent, polymer shape and end-group contributions to the refractive index. Above this range it is, typically, constant.

An early example from the literature involved polyethylene glycol in water. Here are the results:

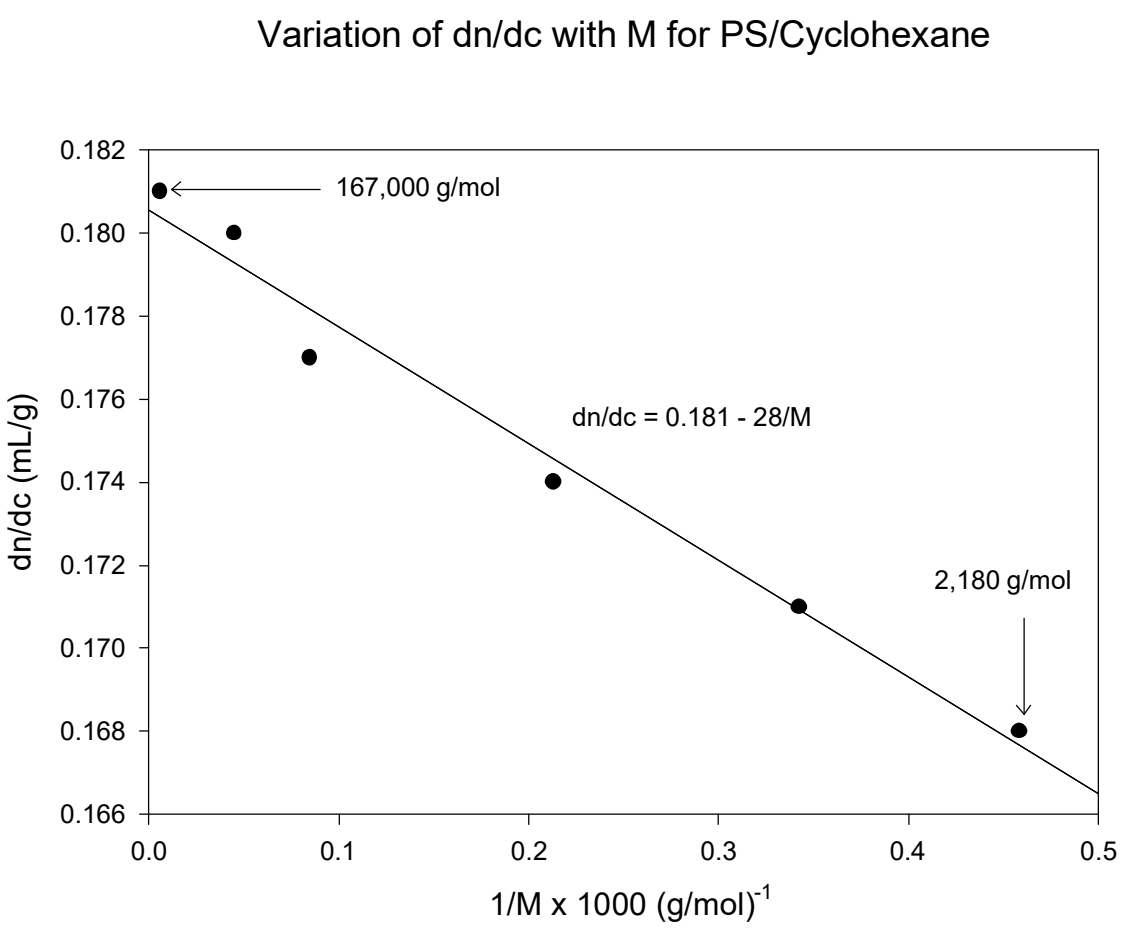


Generally, data like this fits reasonably well to $dn/dc = \alpha - \beta/M$, so when M is large enough, dn/dc does not change. Given the fit above, the change in dn/dc is less than 2% when M > 1,064 g/mol. This is an unusual case and may be because both solvent and polymer have hydroxyl groups.

Polystyrene in cyclohexane is a more common case as shown in the next graph. Here the rate of change with M is 9 times as large and the change in dn/dc is less than 2% when M > 7,750 g/mol. Both sets of data are tabulated in Chapter 6 of Huglin's 1972 book and in the original literature, the references for which are found in Huglin's book.

It is particularly important to realize that each set of data plotted here was measured by the same authors (Kratohvil, J.P. 1968 for PS/cyclohexane and Rempp 1957 for PEG/H_2O). Therefore, it is safe to assume that the samples and corresponding solvents in each run were prepared under similar conditions for the different molecular weights.

If one simply compared a list of dn/dc values for the same solvent/polymer from different authors, all at the same wavelength and temperature, one would be surprised to find variations of several percent. While it is tempting to think that this variation can be explained by differences in molecular weight, more often the reason is that the impurity levels in the polymers and solvents are different from author-to-author. Therefore, if you want to deduce the molecular weight dependence, one must rely on data from the same author.



Temperature Dependence of dn/dc

The refractive index of liquids varies with temperature. For common organic solvents, the range is from -3 x 10^{-4} K^{-1} to -5 x 10^{-4} K^{-1}. Water's value, an exception, is -1.1 x 10^{-4} K^{-1}. Experimental temperature coefficients for dn/dc are usually linear from room temperature up to 100 °C and higher. The coefficients can be zero, negative, or positive, but the variation in the absolute value is typically in the range of 1 to 5 x 10^{-4} $mL \cdot g^{-1} \cdot K^{-1}$. Thus, a few degrees difference in the absolute temperature between the measurement of dn/dc and the light scattering experiment is not significant.

Wavelength Dependence of dn/dc

Experimental observations show that dn/dc decreases with increasing wavelength, with variations of 1 to 3% over the range of 436 nm to 546 nm. These two wavelengths, from a Hg-arc source, represent the two most common ones used in SLS measurements prior to the use of lasers. Since they span the 488 nm and 514.5 nm lines of an Argon-ion laser, and the 532 nm line of a frequency-doubled, solid state laser, one could interpolate

(not linear interpolation, see equation below) values of dn/dc from those measured at the classical values. More uncertainty would accrue to values extrapolated to 632.8 nm, the wavelength of a HeNe laser, or to values extrapolated to ~670 nm, a common wavelength for a "red" diode laser. (Again, the extrapolation is not linear. See below.)

Except for absorption peaks in the spectrum of either the solvent or the polymer, the variation in wavelength is given by:

$$\mathbf{dn/dc} = \mathbf{A} + \mathbf{B}/\lambda^2$$

This equation is also applicable to the variation in refractive index of solvent and polymer (again, assuming no absorption in the wavelength range of interest) and is known as the Cauchy equation. If dn/dc is known at two wavelengths, this equation can be used to calculate it at a third. However, it is much better if three or more measured values fit well to a straight line.

Some investigators ignore the wavelength dependence, and this is usually a mistake. Although there are some polymer/solvent pairs that exhibit very little dispersion, there are others that exhibit quite a bit. For example, poly(vinylacetate)/water has an 8.8% variation in dn/dc from 436 nm to 632.8 nm (see Page 40 of reference 4 listed above). In batch mode, such an error would result in nearly an 18% error in M_w; whereas, PVC/dioxane shows no change in dn/dc from 436 nm to 586 nm (see page 40 of reference 4 listed above). In addition, many researchers working with proteins and protein-like structures, assume a constant dn/dc of approximately 0.18, ignoring wavelength and temperature corrections.

Since the errors in dn/dc arising from differences in samples, even samples supposedly of the same chemical composition and with the same solvent, can be larger than the variations with temperature and wavelength, in order to find suitable A & B coefficients for the equation above, one must choose examples from the same author. The hope is that at least the polymer/solvents were the same. Here is a selection obtained from references 1 and 4 above.

Polymer/solvent	**A**	**B**
Polystyrene/toluene	+ 0.1015	+ 0.00200
Polystyrene/DMF	+ 0.1450	+ 0.00500
Polystyrene/MEK	+ 0.1963	+ 0.00675
Poly(methylmethacrylate)/Dioxane	+ 0.0486	+ 0.00031
Sucrose/water	+ 0.1392	+ 0.00115
Myosin/water	+ 0.1847	+ 0.00121
BSA/0.1M NaCl	+ 0.1791	+ 0.00378
Poly(acrylimide)/acetic acid	+ 0.1857	+ 0.00253
Poly(dimethylsiloxane)/toluene	- 0.0767	- 0.00504

The average value of B for all eight of the positive values is + 0.0028; for the non-aqueous samples the average is a bit higher at + 0.0035; and for the aqueous-based samples the average value of B is a bit lower at + 0.0022. The table is obviously not exhaustive, but the values do cover a range of polymer/solvents. B is calculated with λ in microns.

Here are examples of using the table to estimate small, first-order corrections.

Suppose you measure dn/dc = 0.1500 mL/g for a random-coil polymer in an organic solvent using the 620 nm source in a BI-DNDC. You want to estimate dn/dc at 660 nm, the wavelength of the laser in your BI-MwA. Assuming B = + 0.0035, calculate A = $0.1500 - 0.0035/0.620^2$. The result is A = 0.1409. Now calculate dn/dc at 660 nm. The result is dn/dc = $0.1409 + 0.0035/0.660^2 = 0.1489$ mL/g. The difference in dn/dc at 620 nm and 660 nm is 0.74%. The error in a calculated value of M_w using 0.1500 mL/g instead of 0.1489 mL/g is 1.5%, nearly insignificant compared to other errors. This case demonstrates why using the dn/dc values measured close to the laser wavelength is sufficient for many purposes.

Suppose you find a literature value of 0.1500 mL/g measured at 436 nm and you want to estimate the value at 660 nm. Aside from the fact that the literature value may correspond to a polymer/solvent system with different impurities or molecular weights than yours, this large wavelength difference cannot be ignored. In this case A = $0.1500 - 0.0035/0.436^2 = 0.1316$, and the estimated value at 660 nm is dn/dc = $0.1316 + 0.0035/0.660^2 = 0.1396$ mL/g. Now the difference between the two dn/dc values is 7.4%. This could lead to a 15% error in M_w in the batch mode, something that may not be acceptable.

Included in the table are two values that demonstrate atypical situations. First, note the negative value of A and B for poly(dimethylsiloxane) in toluene. This means that dn/dc is also negative. Negative values of dn/dc arise when the refractive index of the solvent is larger than that of the polymer. Since, typically, this is not the case, most dn/dc values are positive. Second, notice the rather small value of A and B for PMMA in dioxane. This means that dn/dc is also small and it arises because the refractive indices of polymer and solvent are similar. It also demonstrates the fact that there exist polymer/solvent combinations with near-zero dn/dc values. In these cases, the errors in light scattering measurements will be very large.

Appendix D: Refraction & Reflection Corrections

This discussion is only for those who need to understand the mathematics behind the relationship between the Rayleigh Ratio, R(θ), and the measured intensities I(θ) in the BI-MwA.

Rayleigh Ratio Definition

The definition of the Rayleigh Ratio is:

$$\mathbf{R(\theta) = \frac{I(\theta)}{I_{inc}} \bullet \frac{r^2}{V_{obs}}} \qquad \text{Eq. (1)}$$

where I(θ) is the scattered intensity at angle θ, I_{inc} is the absolute incident intensity, and r is the distance from the center of the observed scattering volume, V_{obs}, and the detector.

Calibration

It is very difficult to measure an absolute intensity. In addition, the cross sectional area of the focused beam in the cell is difficult to measure, and it will change depending on the refractive index of the liquid in the cell. For these reasons, the BI-MwA is calibrated using a liquid with a known R(θ), lumping the I_{inc}, the r^2, and the A_{cross} terms together. This approach is valid as long as the fiber's projected core diameter is always larger than the diameter corresponding to the cross-sectional area A_{cross}, independent of the liquid in the cell. In the case of the BI-MwA, this condition is always true.

With k_c as the calibration constant, using the Raleigh Ratio measured at 90° scattering angle for toluene (a well-known value), the equation becomes this:

$$\mathbf{R_{toluene}(90) = k_c \bullet I'_{toluene}(90)} \qquad \text{Eq. (2)}$$

Reflection Correction

The intensities that are measured are designated with a prime as $I'(\theta)$ to distinguish them from the actual scattered intensities I(θ). This situation arises because the light entering any fiber has small components from reflections back into the cell at the interface between the exit window and the liquid and again at the interface between the exit window and the air. The letter F designates this aggregate reflection correction.

An analysis based on this description yields the following:

$$\mathbf{I(\theta) = \left[I'(\theta) \cdot N(\theta) - F \bullet I'(180-\theta) \cdot N(180-\theta)\right] / (1 - F^2)} \qquad \text{Eq. (3)}$$

where,

$$F = t^2(\text{win-liq}) * f(\text{win-air}) + f(\text{win-liq}). \qquad \text{Eq.(4)}$$

The small f's are reflection coefficients between the exit window (coated) and air and the entrance window and the liquid. We have coated the exit and entrance windows such that f(win-air) = 0.25% = 0.0025. Uncoated windows have reflection coefficients closer to 4% and are a major source of error if unaccounted for.

For 90^o reflection, Fresnel's equation gives for a reflection coefficient between two media

$$\mathbf{f(win-liq)} = \left[\frac{\left(\mathbf{n}_{\mathbf{win}} - \mathbf{n}_{\mathbf{liq}}\right)}{\left(\mathbf{n}_{\mathbf{win}} + \mathbf{n}_{\mathbf{liq}}\right)}\right]^2 \qquad \text{Eq.(5)}$$

The refractive indices of the window and the liquid are wavelength dependent, and these are automatically calculated in the BI-ZPMwA software.

Assuming the scattering is negligible compared to transmission and reflection, conservation of energy is used to relate the transmission coefficient, t, to the reflection coefficient, f:

$$t(\text{win-liq}) = 1 - f(\text{win-liq}) \qquad \text{Eq.(6)}$$

Two examples will suffice to show the range of values for F assuming λ_o = 677 nm for a particular BI-MwA: F(toluene) = 2.576e-03 and F(water) = 6.599e-03.

The worst case is for water since the difference between its refractive index and that of a quartz window is the largest amongst the common liquids used in light scattering. However, the light reflected back is only 0.66% for Rayleigh Scatterers. For Rayleigh scatterers, $I'(\theta)*N(\theta) = I'(180-\theta)*N(180-\theta)$. Therefore, the reflection corrections are negligible except for cases where R_g is large, say on the order of 100 nm or larger. Then $I'(35)*N(35)$ can be an order of magnitude larger than $I'(145)*N(145)$. Now there is a nominal 7% error unless the correction is made.

<u>Transmission Correction</u>

The incident intensity is the intensity inside the sample cell. It will vary slightly depending on the reflected light at the entrance window's face adjacent to the liquid inside the cell. Another way to express this is that the incident light intensity transmitted across the entrance window's face depends on the refractive index of the window and the liquid in the cell. The letter T designates this transmission correction.

In addition, when scattered light enters a fiber it crosses from the liquid into the fiber core. Since they have different refractive indices, there is also a common transmission correction

due to this effect. Since the calibration liquid is generally different from the solvent, a correction is necessary. The value of T is calculated as follows:

$$T = t(\text{win-liq}) * t(\text{core-liq}) \qquad \text{Eq. (7)}$$

By analogy to Eq. (6), t(core-liq) is calculated from f(core-liq) and it depends on the wavelength. The ratio of T for the calibration liquid, toluene for example, and T for water is 1.0059. This represents a 0.6% correction and, while small, is easy to account for.

Refraction Correction

The scattering volume is assumed to be the product of Z_{obs} x A_{cross}, where Z_{obs} is the length of the cylinder of light observed by a fiber and A_{cross} is the cross-sectional area of the cylinder. As explained previously, A_{cross} is absorbed into the calibration constant along with the incident intensity and r^2. Z_{obs} will depend on the scattering angle, θ, the fiber's core diameter, D_{core}, the cell's radius, R_{cell}, and the acceptance angle of the fiber, θ_a. The expression for Z_{obs} is:

$$\mathbf{Z_{obs} = (D_{core} + 2R_{cell}\tan(\theta_a)) \bullet \frac{1}{\sin(\theta)} \bullet \frac{\tan^2(\theta)}{\tan^2(\theta) - \tan^2(\theta_a)}} \qquad \text{Eq. (8)}$$

This formidable expression can be broken down into three manageable pieces for discussion sake.

First, imagine that the acceptance angle of the fiber was zero. Eq. (8) then reduces to $D_{core}/\sin(\theta)$. This expression should be familiar to anyone who has used a classic multiangle goniometer such as the BI-200SM. Using either pinhole or pinhole-lens optics, imagine that the observed length of the scattering region is D_{core} at 90°. At any other angle, the observed length increases. The increase is symmetric about 90°. It is easy to show that $1/\sin(\theta)$ is the factor that accounts for the observed length under these circumstances.

Second, due to the acceptance angle, the light received at 90° by the core is increased, and it depends on the cell radius and the acceptance angle. This is given by $D_{core}+2R_{cell}\tan(\theta_a)$. The value of this expression in toluene is calculated using 400 μ for the core diameter, 2.5 mm for the cell radius, and 4.24° for the acceptance angle. The result is 0.771 mm. For water, with 4.74° as the acceptance angle, the result is 0.815. As the ratio of these two numbers will become important when comparing measurements in water with a calibration in toluene, it is the ratio of these two numbers, 1.057, that will become important.

Finally, as the scattering angle θ changes, a small change in the length at 90° occurs due to finite acceptance angles. The complicated ratio of tangents squared accounts for this. Its value at 90° is unity, and it is a symmetric function. For water, with $\theta_a = 4.74°$, this function is 0.4834 at 35°; for toluene, with $\theta_a = 4.24°$, this function is 0.4848 at 35°.

The normalization process absorbs the 1/sin(θ) into its definition. It will also absorb the ratio of tangents squared term if and only if the liquid used for normalization is also the liquid used to dissolve the polymer. Then the acceptance angles are the same. In this case, only the first factor in Eq.(8) needs to be explicitly accounted for. In all further discussions, it is assumed that this is the case.

Acceptance angles are calculated from the numerical aperture of the fiber, N.A. = 0.11, and the refractive index of the liquid as follows:

$$\theta_a = \sin^{-1}\left[\frac{\mathbf{N.A.}}{\mathbf{n(liq)}}\right] \qquad \text{Eq. (9)}$$

Combining All the Corrections

With these considerations in mind, the Rayleigh Ratio is obtained by combining Eqs. (1), (2), (3), (7) and (8).

$$\mathbf{R_{liq}(\theta) = \frac{T_c\left(1+F_c\right)}{T_{liq}\left(1-F_{liq}^2\right)} \bullet \frac{D_{core}+2R_{cell}\tan\left(\theta_a^c\right)}{D_{core}+2R_{cell}\tan\left(\theta_a^{liq}\right)} \bullet \frac{R_c}{I_c'} \bullet \left[I'(\theta)N(\theta) - F_{liq}\cdot I'(180-\theta)N(180-\theta)\right]} \quad \textbf{Eq.(10)}$$

The subscript/superscript "c" refers to the calibration liquid, most often pure toluene. The subscript "a" refers to the acceptance angle calculated from Eq. (9) given the wavelength dependent refractive index of the liquid. The terms 1/sin(θ) and the tangent term in Eq. (8) is absorbed into the definitions for the N(q) values, here referred to as N(θ) values. The tangent term depends on θ_a. As long as one normalizes in the same liquid as used to measure the polymer, the tangent term cancels exactly.

In the literature, you will see a simplified version of this equation. The simplification arises by assuming the reflection coefficients F_{liq} and F_c are small, since, in the worst case, F_{water} = 0.0060. This author is reluctant to make that assumption because, as pointed out previously, for large polymer molecules the light scattered at low angles can be an order of magnitude larger than light scattered at high angles.

Equation (10) is the equation used in the BI-MwA for calculating the Rayleigh ratio for the polymer solution and for the solvent. The difference yields the value that is further used in Zimm, Debye, and Berry plots as discussed in Section II of this manual.

Appendix E: Rejection, Smoothing, Stability: Noise and Trend

Behind every accepted integrated intensity value are a sophisticated, multi-level set of criteria that minimize the effects of dust, bubbles, unusually large aggregates, laser drift, and random noise. Though it is not necessary for you to understand what these criteria are, you may be interested in knowing what goes on behind the scenes.

At the lowest level is the rejection of sweeps that goes on every second. Depending on the automatically set sensitivity (think of it as an integration time), a certain number of sweeps per second of the CCD detector is made. Typically, this varies from about 5 to 25 sweeps, with 100 being a maximum. Any value over the saturation limit (typically 80%) of the maximum value of 65,525 is thrown away. Then the remaining values are averaged and the standard deviation is calculated. Values higher than the average plus one standard deviation are rejected under the assumption they represent dust or bubbles in the scattering volume. Values below the average minus three times the standard deviation are also rejected under the assumption they represent dust or bubbles than can block incident or scattered light. If all values are rejected or greater than the saturation limit, the minimum of these value is accepted. Typically, about 70% of the values are accepted and fed into the smoothing routines.

The result of the rejection of sweeps is a value for each angle and the reference beam every second. A running average of seven seconds is used to smooth random noise. Until seven seconds worth of data is taken, the average of a point and the ones that precede it are used. Thus, the first point is not smoothed. The second point is the average of the first and second points. The third point is the average of the first three points. The eighth point is the average of points two through eight.

During a measurement the smoothed value for a particular angle is divided by the smoothed value of the reference beam intensity. The reference beam intensity is measured close to the laser's head. Thus, slow drifts in laser intensity are removed.

These smoothed values are then used to determine when stability has been reached after injection of a new sample or liquid. Two criteria must be satisfied. The first is called *Noise*. The second is called *Trend*. Both are based on a user-selectable number of *Stability Points*, N. By default N = 20. The user can select a different value by clicking **Setup** followed by clicking **Trend/Noise** and entering a different number of stability points. We have found 20 to be a reasonable value.

See Figure E-1.

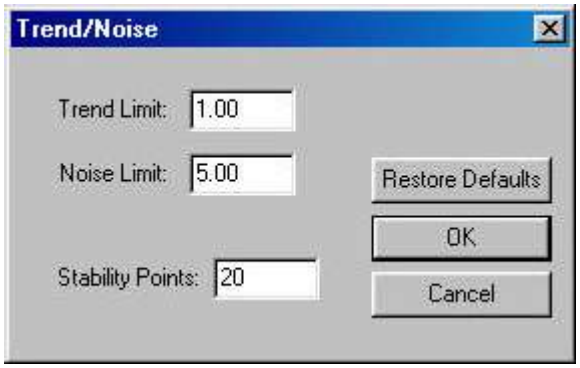


Figure E-1: Trend/Noise window.

Both the *Noise* and the *Trend* criteria are applied to the 90° data only, as it is least likely to be effected by flare light. In addition, it is relatively immune to dust that has not already been rejected.

After N smoothed values have occurred, they are averaged and the standard deviation is also calculated. If 100 times the ratio of the standard deviation divided by the average is less than or equal to the *Noise* rejection value, this criterion is satisfied. If the criterion is not satisfied, another smoothed value is taken and the test applied again. The default value for the *Noise* rejection criterion is 5, implying 5% random error is acceptable.

The value for the *Trend* is 100 times the smoothed slope over the N data points. The default value for *Trend* is 1. Thus, if the slope is less than or equal to 1%, the data are accepted.

In practice, both criteria must be satisfied. With filtering of clean liquids, they are often satisfied within the twenty to twenty five seconds after the intensity levels have reached what appear to be new, level values. In cases that take longer than N seconds, you may see two “traffic lights”, each with a green (accepted) and red (rejected) light. One traffic light represents the *Trend* criterion and one represents the *Noise* criterion. When both traffic lights are “green”, then both criteria are satisfied and the average of the preceding N integrated intensity values will be used in the equations to determine molecular properties.

Appendix F: Export File Format

From within the **Database**, after selecting a data file or files, click on **Export Selected Files**. If you select, but do not open, a folder, you can export its entire content of data files by clicking on **Export Selected Folder**. Data files are appended with the extension ".dat". When exporting more than one file, the program will automatically supply increasing numerical labels to the first file name you entered. The default name is zpmwa.dat. Here is an example.

PS/Tol 300K	Sample Identification
JM unit #3	Operator Identification
12:29:19	Time
12/31/01	Date
7	Number of Angles
35.00	Angle 1 in degrees
50.00	
75.00	
90.00	
105.00	
130.00	
145.00	Angle 7 in degrees
0.8690	Angle 1, N(q)
0.9480	
1.0590	
1.0000	
0.5800	
0.4580	
1.0120	Angle 7, N(q)
0.420352	Angle 1, solvent intensity
0.376666	
0.337224	
0.345317	
0.609100	
0.756338	
0.467470	Angle 7, solvent intensity
9.734000e-001	Concentration 1 in mg/mL
1.056881	Angle 1, Conc. 1, sample intensity
0.950632	
0.837888	
0.854778	
1.489699	
1.822834	
0.947042	Angle 7, Conc. 1, sample intensity
2.032000e+000	Concentration 2 in mg/mL

Appendix F-2

1.534584	Angle 1, Conc. 2, sample intensity
1.388508	
1.222983	
1.248872	
2.187801	
2.686702	
1.329601	Angle 7, Conc. 2, sample intensity
2.935000e+000	Concentration 3 in mg/mL
1.854573	
1.680304	
1.483791	
1.516179	
2.649858	
3.246703	
1.584461	
3.908000e+000	Concentration 4 in mg/mL
2.090453	
1.898851	
1.676363	
1.715649	
2.993627	
3.666229	
1.779201	
4.891000e+000	Concentration 5 in mg/mL
2.269535	Angle 1, Conc. 5, sample intensity
2.059145	
1.819331	
1.863775	
3.250375	
3.976539	
1.925473	Angle 7, Conc. 5, sample intensity
660.0	Laser Wavelength
1.488	Refractive Index Solvent
3.519000e-005	Calibration Constant
1.489	Refractive Index Calibration Liquid
1.182000e-005	Rayleigh Ratio Calibration Liquid
0.000	Depolarization Ratio
0.105	dn/dc in mL/g