Instruction Manual for BI-ZPW

Zimm Plot Software for Molecular Weight Determination in Dilute Polymer Solutions

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Please Read

This is your instruction manual for your Brookhaven BI-ZPW Static Light Scattering Software for determination of M_w, R_g, and A_2 in dilute polymer solutions. Please read it carefully before making measurements. The How To section describes how to install the software and use this manual. 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.

This program requires Windows 3.X or higher and at least 8 Mb of RAM.

This program was designed for use with the BI-200SM motor-driven goniometer and the BI-9000AT digital autocorrelator or the BI-9025AT photon counter. Please read the goniometer alignment section in the BI-200SM manual **and** align the goniometer *before* using this software. You should consider using the BI-ISTW software to verify that the alignment is good. However, this software may also be used with a manually driven goniometer. Check with the factory for special modifications.

Software is never 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 cannot find the answer to your questions in the main part.

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.

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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: Introduction, Software Installation, & References

The Zimm Plot software is a program that will allow you to collect and analyze scattered light intensity as a function of angle and concentration from polymer solutions. The measured photon count rate is the intensity. The program allows you to control a motorized BI-200SM goniometer, to collect and to correct the measured photon counts, and to plot the results in a variety of ways. You can use the excess intensity above that scattered from the liquid, as is normally recommended, or you can select to ignore the solvent contribution. 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. A stability test allows you to check the laser, detector electronics, and main power supply for stable operation.

This type of light scattering is variously known as static light scattering (SLS), classical light scattering (CLS), and total or integrated light scattering (TLS or ILS). It has even been called TILS for total intensity light scattering. The integration here refers to the averaging over time. If measurements are made at a single angle it is called SALS for single angle light scattering. MALLS is an acronym for multi-angle laser light scattering. One “L” would have been good enough, because, before the introduction of lasers, other 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 ~ 10 ms is sufficient, even for the largest polymers encountered. Most intensity measurements are averaged for less than one second.

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 and polymer relaxation times due to various modes of motion.

Before proceeding, align the goniometer following the instructions in the BI-200SM manual. Check the alignment with the BI-ISTW software. Take your time. Do a thorough job. Then, follow the instructions in this manual.

To install the BI-ZPW software, place the installation disk in the floppy drive. Click on **Run** from the **File** menu. Run A:\SETUP. When finished, a BIC icon with the name Zimm Plot Software will be added to your BIC program group, or a group with this icon and name will be added. Double click on the icon to start the program.

References

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, Principles of Colloid and Surface Chemistry, Marcel Dekker publisher, 1986.

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. 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 the vertically polarized laser, for example. Nevertheless, these accounts serve as an easy introduction to the subject.

Section II: THEORY

The Basic Equation

In the limit of low concentration and scattering angle the data obtained from SLS measurements for a dilute solution homopolymer in a single solvent can be written as:

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

The optical constant H is given by:

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

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 I.
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 a HeNe laser it is 632.8nm, and for an Argon-ion laser it is either 514.5nm (green) or 488.0nm (blue). For frequency doubled, solid state laser it is 532 nm, and for diode lasers in the red it varies from 660 to 680 nm.. The classical Hg lines are 436nm and 546nm.
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.
M_w	is the weight-average molecular weight (molar mass). Values in the range from a few thousand to a few million g/mol (daltons) are measureable, more commonly 10^4 to 10^6.
$P_z(q)$	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.)

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. For unpolarized incident light the factor is 2.

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

$$P_z(q) = 1 - (R^2_g \cdot q^2)/3 + O(q^4) \quad , \qquad (2)$$

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.

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:

$$H \cdot c/\Delta R(\Theta,c) = [1/M_w] \cdot [1 + (R^2_g \cdot q^2)/3] + 2A_2 c \quad . \qquad (3)$$

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. A calibration constant is used to calculate excess Rayleigh Factors from measured intensities.

A calibration constant is obtained by measuring the intensity of light scattered from a standard. It is assumed that $R_c = k_c \cdot I_c$, where k_c is the calibration constant and R_c is the known Rayleigh Ratio. R_c is known for toluene and benzene at the laser wavelengths. These values are given in the BI-200SM manual. 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.

If the left hand side of Equation 3 is plotted against $\sin^2(\Theta/2) + k_p \cdot c$, 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. It is this parameter of a polymer distribution that is most often of interest. And 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

Rayleigh scatterers are defined such that their size is small compared to the wavelength of light. In this case the angular term in Equation 3 is negligible, and it can approximated as:

$$H \cdot c/\Delta R(\Theta,c) = 1/M_w + 2A_2c \quad . \quad (4)$$

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 is often a minimum and because residual dust or microgel contributions are usually insignificant at such a high angle.

Whenever the molecular weight is small, roughly 100K daltons and lower, consider collecting data for a Debye Plot. The measurement time is shorter; the interpretation of possible downward curvature at low angles is avoided. Downward curvature may be due to dust, microgels, colloids, or flare.

The BI-ZP 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. The converse is not possible: If you collect data at only one angle, you cannot make either Zimm or Berry Plots.

The Berry Plot

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 screen 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 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 use 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:

$$[1/M_w]\cdot[1+(R^2_g\cdot q^2)/3]\cdot[1+2A_2M_wc+3A_3M_wc^2+ ...] \quad . \qquad (5)$$

For hard spheres $A_3M_w = (5/8)\cdot(A_2M_w)^2$, 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,

$$H\cdot c/\Delta R(\Theta,c) \cong [1/M_w]\cdot[1 + (R^2_g\cdot q^2)/3]\cdot[1 + A_2M_wc]^2 \quad . \qquad (6)$$

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

$$H\cdot c/\Delta R(\Theta,c) = [1/M_w]^{0.5}\cdot[1+(R^2_g\cdot q^2)/6]\cdot[1+A_2M_wc] \quad . \qquad (7)$$

This equation is the basis of the Berry Plot. The square root of $Hc/\Delta R$ is plotted against $\sin^2(\Theta/2) + k_pc$.

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:

$$A_2 = \Gamma/M_w$$

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:

$$M_{w,app} = M_w \cdot [1 + (7/5)\delta^2] = M_w \cdot [(3 + 3\rho_v)/(3 - 4\rho_v)] \quad , \tag{8}$$

$$A_{2,app} = A_2 \div [1 + (7/5)\delta^2] = A_2 \div [(3 + 3\rho_v)/(3 - 4\rho_v)] \quad , \tag{9}$$

$$R_{g,app} = R_g \div [1 + (7/5)\delta^2]^{0.5} = R_g \div [(3 + 3\rho_v)/(3 - 4\rho_v)]^{0.5} \quad . \tag{10}$$

For R_{uv} measurements on rigid rod molecules:

$$R_{g,app} = R_g \cdot \{[1 - (4/5)\delta + (47/35)\delta^2]/[1 + (7/5)\delta^2]\}^{0.5} \quad . \tag{11}$$

For R_{vv} measurements on flexible chain molecules:

$$M_{w,app} = M_w \cdot [1 + (4/5)\delta^2] = M_w \cdot [3/(3 - 4\rho_v)] \quad , \tag{12}$$

$$A_{2,app} = A_2 \div [1 + (4/5)\delta^2] = A_2 \div [3/(3 - 4\rho_v)] \quad , \tag{13}$$

$$R_{g,app} = R_g \div [1 + (4/5)\delta^2]^{0.5} = R_g \div [3/(3 - 4\rho_v)]^{0.5} \quad . \tag{14}$$

For R_{vv} measurements on rigid rod molecules:

$$R_{g,app} = R_g \cdot \{[1 - (4/5)\delta + (4/7)\delta^2]/[1 + (4/5)\delta^2]\}^{0.5} \quad . \qquad (15)$$

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:

$$\rho_v = R_{hv}(90°) \div R_{vv}(90°) \, , \qquad (16)$$

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, it is extremely important to use the excess values.

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

$$\delta^2 = 5\rho_v \div (3 - 4\rho_v) \qquad (17)$$

The BI-ZPW software includes a routine for measuring ρ_v provided the optional BI-PA polarization analyzer is available. Alternatively, literature values of ρ_v can be input, and its effect on recalculated values M_w, R_g, and A_2 determined.

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 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, scratched cells, microgels, other supramolecular entities, and flare light at the cell-solution interface. 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-ZPW 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 supramolecullar entities (colloids, perhaps) are most likely the problem. Look at the number of repeats necessary to accept a data point. If 1 of 30 instead of 10 of 10 measurements are required, dust or its equivalent is most likely the cause. 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.

This warning is repeated in the data analysis section of this manual.

Section III: Setting Up the Measurement

Measuring A Signal

This section of the installation procedure takes you through the operations necessary to verify that you can measure a signal that is a result of scattered light. Omit this section entirely if you have already used the system to make measurements.

Your goniometer does not have to be fully aligned to make these basic tests. It should, however, be assembled to the point where you can measure a signal. (See the BI-200SM manual.)

1. Do not turn on the correlator at this time. If you have an external high voltage, do not turn it on either.

2. Connect the signal and voltage cables between the correlator and PMT or between the correlator and controller boxes, depending on the type of detector system you have. The connectors are all labeled and they are not interchangeable. Make sure the output signal of the detection system is connected to INPUT A on the correlator.

3. Turn on the computer. Click on the Zimm Plot icon to start the program.

[In what follows click on the command button labeled **INTENSITY** to measure a count rate in "cps", "kcps", or "Mcps" that stand for counts/second, kilocounts/second, or megacounts/second, respectively. The count rate is proportional to the signal strength that, except for dark count rate, is proportional to the scattered light intensity. Everything else is derived from this raw signal.

The random error of repeated measurements of the count rate is proportional to the square root of the count rate. Therefore, the percent random error is very high for low count rates (dark count rates) and is about 1% at 10 kcps. So, do not be surprised to see large percentage jumps at low count rates. That is normal.]

4. Click **INTENSITY** several times. With no high voltage attached, you should see zero counts. If counts are registering, contact the factory for advice. [Note: If you do not have an adjustable HV, or if you cannot turn the HV off, you may see counts at this point. There is nothing wrong.]

5. If you have a separate HV that is adjustable, with it off, connect the high voltage power supply to the HV connector on the rear of the detector system. Set the HV to -1,000 volts.

6. Turn off the room lights. Block the laser beam with the mechanical shutter. Set the filter wheel to one of the closed positions. They are marked

"C".

7. Turn on the HV. Click **INTENSITY**. There should be no counts. [Note: If your HV is not adjustable, then you will get dark counts immediately and you can ignore the remainder of this paragraph.] Lower the HV by 100-volt steps [-1,000, -1,100, -1,200 …]. At each level, measure the counts. When you get to the HV setting stated in the goniometer manual and repeated on the PMT label (typically between -1,600 to -2,000 volts), the counts per second should be approximately equal to the dark count rate. With PMT systems the dark count rate can be anywhere from ~ 50 cps to ~ 2,000 cps, depending on the particular PMT that was supplied. With the BI-APD the dark count rate may be higher. Look for information that came with the BI-200SM, typically in Section II: Configuration of the 200SM manual.

8. Turn on the room light. The dark count rate should not increase. If it does, check for light leaks around the detector system. Perhaps the screws holding the detector to the optics are not tight enough, allowing light to leak around the o'ring. Perhaps the o'ring is not properly seated in its track. Once you have found the source of the leak you can either block it with black electrical tape, or, better, turn off the HV and low voltage and then reseat the detector optics or the PMT. Make sure the o'ring or other seals are properly seated into their recessed tracks before fully tightening the screws. Do this with the HV off. Then recheck the dark count rate again with the lights on and off.

9. Turn the filter wheel to the wavelength of the laser. Measure the count rate. It should be the same, possibly a little higher depending on the aperture setting. The 100, 200, and 400 μ settings will result in little if any additional light reaching the detector. The 1, 2, and 3 mm settings may, depending on the lighting conditions in the room, show a few hundred counts/second more.

10. Now turn on the laser, unblock the beam, and provide a scatterer. Typically, the index matching liquid is sufficient for this purpose. Measure the count rate. It should be, typically, several thousand counts/second or more (it depends on the laser power) with the aperture wheel set to 2 mm. Do not be fooled by jumps in the count rate if dust has not yet been removed from the index matching liquid. Your purpose is to determine if the detector is responding to light.

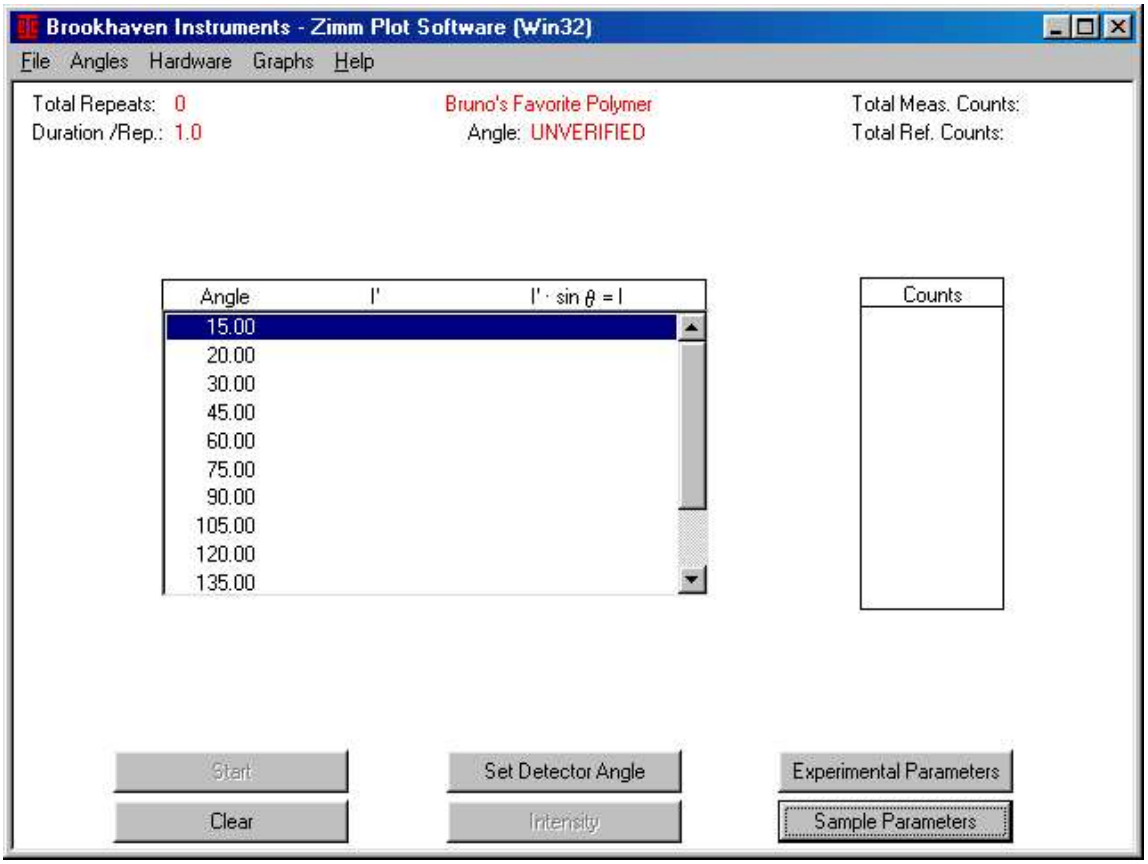
11. Set the aperture wheel to 1 mm. The rate should drop by approximately the ratio of the areas of the apertures, 1/4 in this case.

Congratulations. You have just determined that your dark count rate is reasonable and that your instrument responds to light. If the instrument will not be used imme-

diately, block the beam with the mechanical shutter. If the entire system will not be used for more than a few hours, turn off the laser and the high voltage. Block the beam to prevent dust from building up on the laser's exit window. Some solid-state lasers do not have long lifetimes compared to gas lasers. If a solid-state laser will not be used for than one hour, turn it off to increase its lifetime. Warm-up times for such lasers are typically less than 30 minutes.

Basic Settings

In the Menubar of the Main Window, click on **Hardware**, **Hardware Configuration**. Click on **Motor**, if you have a motorized goniometer. Click on **Manual**, if you do not have a motorized goniometer, or if you are using this program with a motorized goniometer other than the BI-200SM. Select either zero or one **Reference Detectors**. If you have a reference detector, plug the digital signal (TTL compatible) into input B on the rear of the correlator. The scattered light and reference intensities are collected simultaneously. A ratio is formed, after correcting both for their dark count rates and for the dead-time. This ratio will account for slow drifts in the laser output and absorption, if the reference detector is attached to the beam stop in the BI-200SM and a round cell is used. This latter case is quite rare. It is more common to find a reference detector placed between the laser and the input optics of the goniometer, accounting only for a slow drift in laser power.



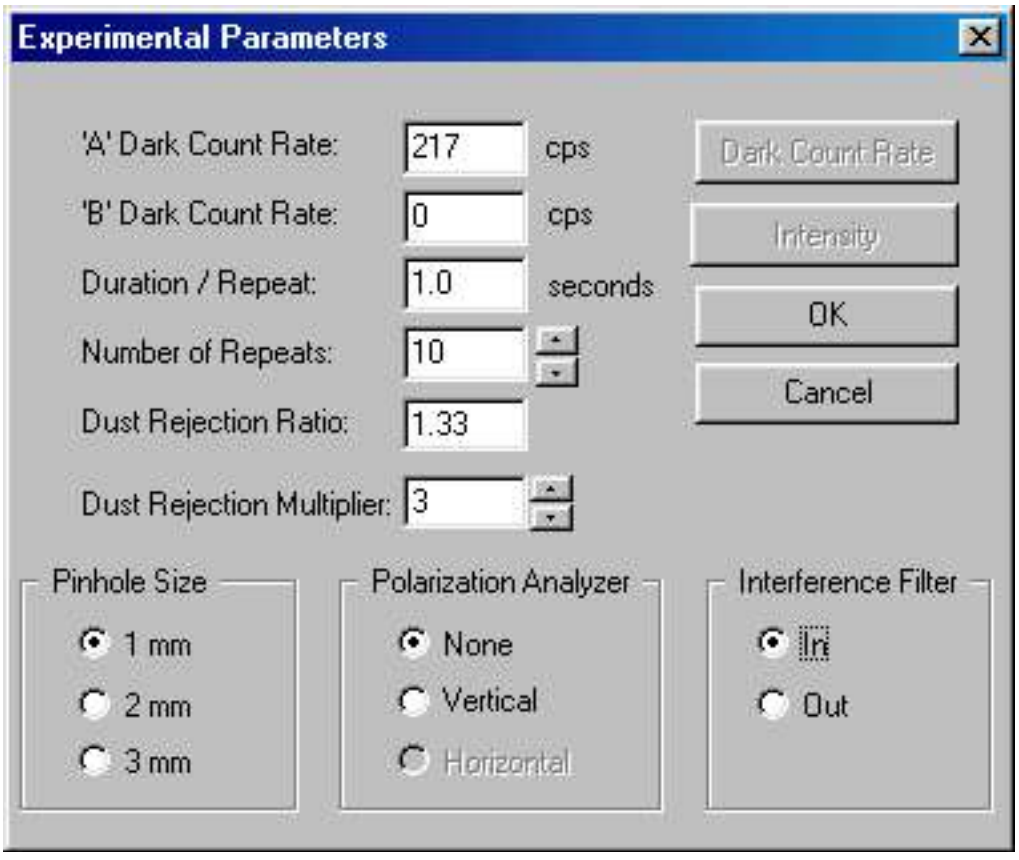
Manually set the goniometer arm to 90°. Click the **Set Detector Angle** command button and enter the angle as 90°.

Experimental Parameters

Click on the **Experimental Parameters** command button in the lower part of the screen. Block the light to the PMT by turning the neutral density filter wheel to the closed position marked "C" on the BI-200SM. Enter 10 seconds in the **Duration/Repeat** field. Click on the **Dark Count Rate** command button. The count rate is the total photons counted divided by the duration in seconds. With all light blocked, this is the dark count rate. The units are counts/second, abbreviated cps. Click the **Close** command button and notice that the measured dark count rate is automatically transferred to the appropriate field. Since dark count rates are normally small, the fluctuations are large. Therefore, counting for ten seconds yields smaller fluctuation, and it is easier to estimate the true dark count rate.

[If you use a reference detector, block the laser light and any other source of light reaching the reference detector before making the dark count rate measurements. The B input signal, assumed here to correspond to the B dark counts, is also automatically measured and transferred to the appropriate field. Close as above.

If room light contributes significantly to the dark counts registered at the reference detector, consider two options: either block the room light, or, if constant and small compared to the total reference counts, use the sum of the reference dark counts and room light as the apparent dark count rate of the reference detector.]



Select an appropriate Duration/Repeat. Select values in increments of 0.1 seconds up to a maximum of 10 seconds. One second is common, but not necessarily optimum. Photon counting follows Poisson statistics: The standard deviation of the distribution of repeated measurements equals the square root of the average. Here the measurements refer to the Total Measured Counts or, in the case of a reference detector, the Total Reference Counts. The intensity is the Total Counts divided by the duration (units of counts/second, cps, or kilocounts/second, kcps, or megacounts/second, Mcps). To repeat: Total measured counts follow Poisson statistics; count rates --counts/second-- do not.

Because of the square root relationship, the relative error (std. dev. ÷ the mean) is: 1% for 10,000 total counts; 0.32% for 100,000 total counts; and 0.1% for 1,000,000 total counts. Aim for 100,000 total counts. If this can be done in one second by increasing the incident laser power or selecting a larger pinhole (1, 2, or 3 mm), do it. If not, increase the duration/repeat. This will result in an increase in the total experiment duration; it will also increase the probability that a measurement will be ruined by dust. With weak scatterers and low laser power, it may be better to accept 50,000 or 20,000 or even 10,000 total counts rather than increase the duration/repeat. Experience will help you judge the best alternative.

The strongest scattering will generally come from the highest concentration sample at the lowest angle of interest. If you have not done so previously, move the goniometer to, and **Set Detector Angle** at, the lowest angle. Insert the highest concentration sample. Click the **Intensity** command button in the main screen or from within the Experimental Parameters window. The Total Measured Counts are shown in the upper part of the main screen. Adjust the laser power, the pinhole, or the duration/repeat until a suitable total measured count is achieved as described in the previous paragraph. In any case, it is best to keep the maximum count rate less than approximately 1.5 Mcps. At all other angles, the Total Measured Counts will generally be lower and the corresponding relative error higher. [The exception is when the size of the scatterer of interest is on the order of the wavelength and the lowest angle of interest happens to be near a minimum in the scattering pattern. In this case try a few different angles.]

NOTE: At the lowest angle you can expect dust is a problem, unless you have filtered the sample. Therefore, when estimating the highest count rate, do so several times, and use an average of several of the lowest values. The highest values are probably from dust floating through the beam.

From within the Experimental Parameters window, select a value from 1 to 10 for the **Number of Repeats** (N). This value is used to discriminate against dust as explained below. This value may be adjusted up or down, depending on the cleanliness of the sample, the duration/repeat, and the intensity of scattered light. If the sample has been well filtered, the laser power is low (< 20 mW), and the pinhole is maximum (3 mm), select 3 to 5 repeats and 1 to 5 seconds duration to achieve 10,000 to 50,000 total measured counts. If the laser power is high (> 50 mW), and the pinhole is set at 1 or 2 mm, select 5 to 10 repeats and 1 second duration to achieve 100,000 total measured counts. [If you

have the BI-APD detector, it is very sensitive at the red wavelengths. Therefore, the laser power statements above do not directly apply. You will have high count rates even with fairly low power lasers.]

With a well-filtered sample, enter 1.33 for the **Dust Rejection Ratio**. Values from 1 to 10 are allowed. Small numbers are best for clean samples [less dust]; somewhat larger numbers (up to 2 or 3) may be used if dust is difficult to remove. NOTE: Using larger numbers may reduce accuracy, especially at low angles, but may be necessary if dust cannot be removed. When fitting the data, look for abnormally high and erratic intensities at the lower angles. Delete them if necessary during fitting.

The **Dust Rejection Multiplier** (DR), a number between 3 (default) and 10, is a new feature added with the 32-bit software. Previously, the default of 3 was fixed. This number allows multiples up to DR times N, the number of repeats, from which intensity measurements are selected to provide for software rejection of high values (dust). The procedure is described in **Section IV: Making a Measurement** paragraph 4.

Fill in the remaining 3 boxes of information. None of the values are used in any subsequent calculation, yet they are valuable pieces of information when reviewing the experimental conditions. Select the **Pinhole Size** corresponding to the pinhole set on the detector optics. If you do not have the optional polarization analyzer attached to the front of the detector optic, the most common case, select "*none"* in the **Polarization Analyzer** box. If you have an analyzer, set it to accept vertical polarization to match the vertical polarization of the laser and so indicate in this box. To block room light use the snout provided with the BI-200SM and rotate the interference wheel until the filter number matches the wavelength of the laser used. Click on **Interference Filter "In"**.

Click the **OK** command button to save the changes and exit the Experimental Parameters window. NOTE: If you click on **Cancel**, none of the values entered will be accepted. Previously entered values will be used.

Sample Parameters

Click on the **Sample Parameters** command button in the lower part of the screen. Fill in the **Sample Identification**, **Operator Identification**, and **Notes** fields. The Sample ID is used in the Database section of the program for naming the files. Therefore, care should be taken in organizing files by the use of appropriate Sample IDs. Select a liquid from the pull down box or select Unspecified and then fill in the **refractive index** for the **Sample Liquid.** Fill in the values for the **Sample Cell (**typically ~1.5) and the **Vat Liquid**. If the vat liquid is decalin (cis-trans decahydronaphthalene), the refractive index is 1.474. If you click on **Apply Reflection Corrections**, then the refractive indices are used to correct for the fact that a measurement at any angle also includes a small fraction of the intensity measured at the supplement of that angle. This is due to the light being reflected at the cell/liquid and cell/vat-liquid interfaces.

The fraction f is calculated from the refractive indices of the various interfaces involved. The fraction f is given by:

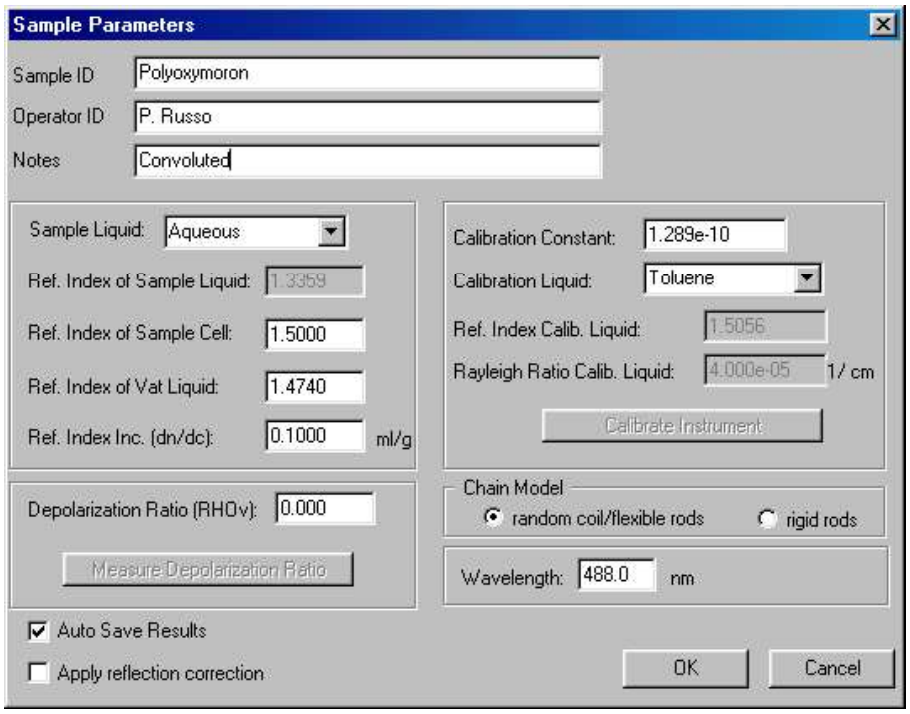
$$f \equiv f_{sl/c} + t^2_{sl/c} f_{vl/c}$$

$$t_{sl/c} = 1 - f_{sl/c}$$

$$f_{sl/c} = \left(\frac{n_{sl} - n_c}{n_{sl} + n_c} \right)^2$$

$$f_{vl/c} = \left(\frac{n_{vl} - n_c}{n_{vl} + n_c} \right)^2$$

where $f_{sl/c}$ is the fraction reflected at the sample liquid/cell wall interface, $t_{sl/c}$ is the fraction transmitted at the sample liquid/cell wall interface, and $f_{vl/c}$ is the fraction reflected at the vat liquid/cell wall interface. These f values are calculated assuming the angle of incidence is zero and Fresnel's law applies with n_{sl}, n_c, and n_{vl} the refractive indices of the sample liquid, cell, and vat liquid, respectively.



When the sample liquid and vat liquid are both organic liquids, with refractive indices in the range of 1.45 to 1.55, the reflection correction is typically insignificant. When the sample liquid is water (ref. index 1.33), the reflection correction is small, but worthwhile making. Because the reflection correction is small, it is not worth the effort to correct the refractive indices for wavelength or temperature. A refractive index at any wavelength in the visible portion of the spectrum is sufficient even if that wavelength is

different than the one used for the scattering experiment. Likewise, unless the temperature of the measurement is many tens of degrees different than room temperature, it is not worth making a correction. Use the refractive index at room temperature.

If the supplement of the scattering angle is not present in the list of angles measured, the nearest angle measured to the supplement is used. The error introduced by this is a second order correction to a small, first order reflection correction, and is, therefore, insignificant.

The application of the reflection correction is described in numbered paragraph 8 under the section entitled **Section V: Making a Measurement**.

Enter the refractive index increment, dn/dc, for the polymer/solvent/wavelength combination. For more information on dn/dc, see Appendix I in this manual.

Enter the depolarization ratio, ρ_v. For more information, see the discussion near th end of Section II of this manual. If in doubt, enter zero. If you have entered zero, then the choice of flexible coil or rigid rod does not matter. No changes occur. If you have the optional BI-PA polarization analyzer, click on **Measure Depolarization Ratio**. Follow the instructions for measuring the depolarization ratio. If the depolarization ratio is not zero, then the calculated results will depend on your model choice to describe the polymer. There are two choices in this software: flexible coil and rigid rod. Both are extreme cases of the worm-like chain model for polymers. For more sophisticated models, consult advance texts. This software does not support such models.

Enter the **Wavelength** of the laser.

Calibrating the Instrument

Select a liquid for calibration, typically toluene or benzene. Given the liquid and the laser wavelength, the “Rayleigh Ratio of Calibration Liquid” is automatically entered using the best literature values available. However, if you would like to use a different value, you can. Either modify the initialization file (contact the factory for advice), or modify the final “Calibration Constant”. Do this by multiplying it by the ratio of your preferred Rayleigh Ratio to the one we supply in the box next to “Rayleigh Ratio Calib. Liquid”.

The calibration is absolute. So use either triply distilled liquids or newly opened HPLC-grade pure solvents. Do not use bottles opened to the air that have been contaminated by moisture. Once you have a very pure liquid for calibration, it can last for years if sealed. You do not have to calibrate using the same type of sample cell as used in the solvent and polymer solution measurements. You do have to have the same laser power, pinholes, and filters to tie the intensity measurements to the absolute Rayleigh Ratio of the calibration liquid. Otherwise, M_w and A_2 are meaningless.

Click on **Calibrate Instrument**. Follow the instructions. Calibrate often. It is fast and convenient if you have previously prepared a sealed sample cell with a pure solvent like toluene. Since Rayleigh Ratios are on the order of 1 to 4 x 10^{-5} cm^{-1}, you can expect calibration constants on the order of 1 to 4 x 10^{-9} for a toluene count rate as low as 10 kcps and 1 to 4 x 10^{-10} for a toluene count rate of 100 kcps.
REMEMBER: Once you have calibrated the instrument, do not change laser power and pinhole or filter positions. If you do, the calibration is meaningless as are the results.

Click on **Autosave Results** to automatically save the final measurements. Otherwise, to save results for later recall, click on **File** and either **Save** or **Save As**. Data are saved in a binary format, as files in the open Folder. The Folder/File database is explained later in this manual.

Click the **OK** command button to save the changes and exit the Sample Parameters window. NOTE: If you click on **Cancel**, none of the values entered will be accepted. Previously entered values will be used.

Making a List of Measurement Angles

Click **Angles** in the Menubar at the top of the screen. Click **Edit Measurement Angles List**. For the alignment test click the **Default List** command button. This results in 12 angles ranging from 15^{o} to 155^{o}, a good selection for many initial measurements. The supplements of most of the angles are provided, and this allows for a simpler reflection correction if checked. Click on OK to implement the list and return to the main screen. If you click on **Cancel**, the last list, not the Default List, will be used in the main screen for measurements.

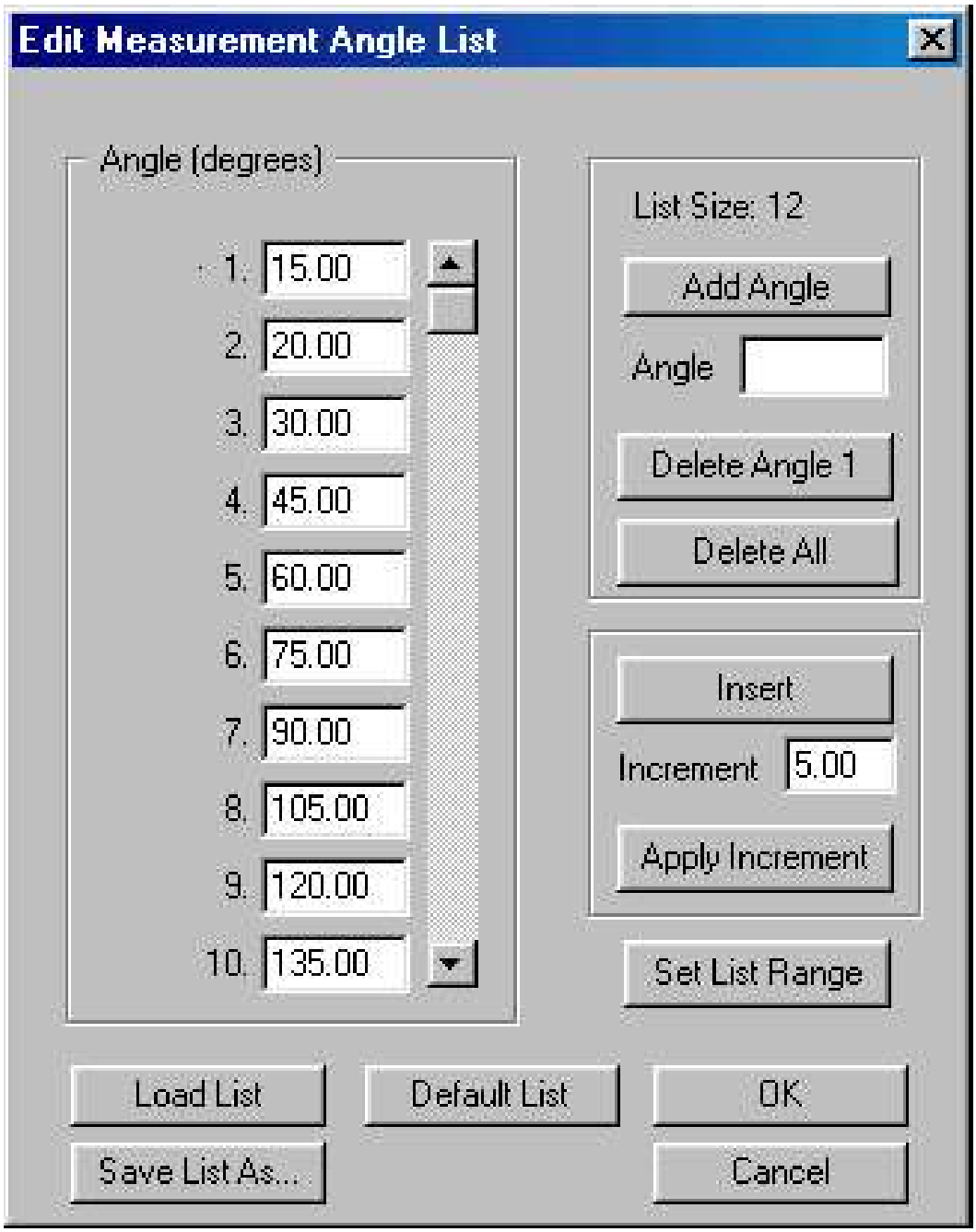
A list of measurement angles may be created in a number of ways from within the Edit Measurement Angles List window.

Click on the **Delete All** command button. Notice the flashing vertical cursor in the remaining edit box in the list of angles. Enter an angle between 10^{o} and 155^{o}. Click in the edit box labeled “Angle”. Enter an angle. Click on the **Add Angle** command button. Notice the angle is added to the list. Repeat the procedure to add more angles. The minimum allowed list contains five angles.

To add a single angle to a list of displayed angles, enter the new angle in the edit box labeled Angle and then click on the **Add Angle** command button. To change any angle in a list, click on it in the list and type over it. To delete an angle in a list, click on it and then click on the **Delete Angle** command button. Angles are automatically rearranged from lowest to highest.

Click on the **Increment** box and enter an increment in degrees. Click on any angle in the list. Click on **Insert Increment**. The new angle is inserted and the list size increases by one. If the increment is larger than the difference between the successive angles, only half the increment is added.

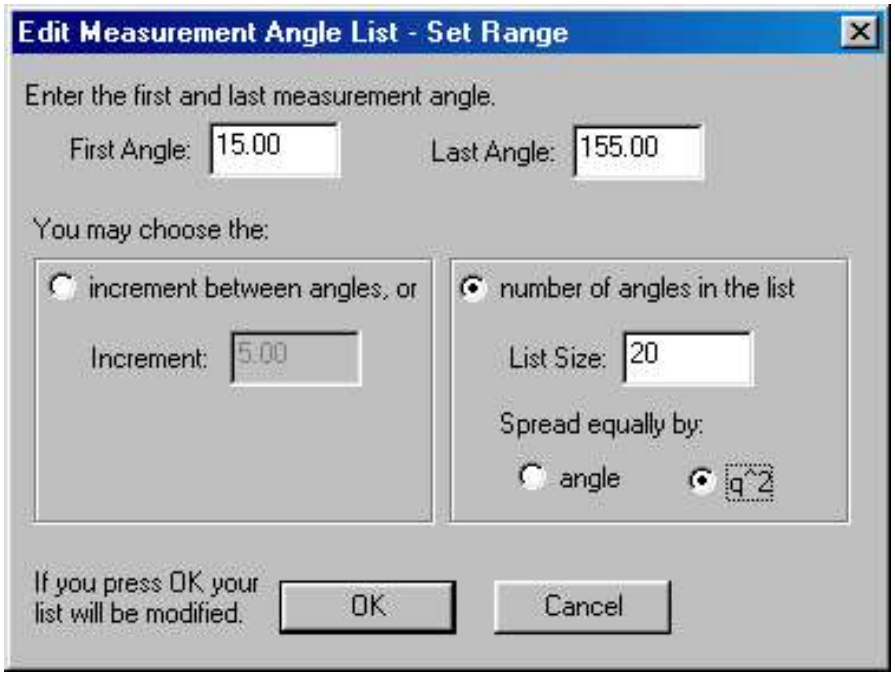
Click on the **Increment** box and enter an increment in degrees. Click on any angle in the list. Click on **Apply Increment**. The increment is applied from the current angle. The list size remains constant; however, the values of the angles after the current are different, with the increment applied consecutively.



To delete any angle, click on it. Then click on **Delete Angle**. Notice the List Size is decreased by one.

Click the **Set List Range** command button. Enter the **First** and **Last Angle**. Click on ***either*** the "**increment between angles"** or the "**number of angles in the list"**. If you selected "increment between angles", then enter the **Increment** in degrees. The new list will include the first and last angles you entered, with an equal increment starting from the first angle. The difference between the last angle and the one preceding it may not be equal to the increment entered. For example, if you entered 15° to 155° by 15°, the list will include 15°, 30°,..., 120°, 135°, 150°, 155°.

If you selected "**number of angles in the list"**, you may select equal spacing by angle or by q^2. Here q is the magnitude of the scattering wave vector, and it is explained further in Section II: Theory. A list of angles is created from the first to the last with appropriate spacing. The spacing is calculated as (First - Last)/(List Size - 1), where First and Last are either values corresponding to angle or q^2. From the equal spacing in angle or in q^2 the corresponding angle is calculated in order to compile the list of angles for the measurement.



At least 1 and no more than 50 angles are allowed. Click OK We recommend a minimum of seven angles are collected, however the data analysis will proceed with three or more.

After creating a specialized list of angles, click on **Save List As** and enter a filename up to 8 characters. The extension "ang" is automatically appended if you do not enter it. Click on OK.

[NOTE: To delete a file containing a list of angles, do so through the operating system. Open, C:\Bicw32\9kzpw\9kzpw32.exe locate the filename, and delete it.]

Click on **Load List** to reload a list of angles. Click on the filename of the list of angles. Click on OK. The angles in that list are now loaded.

CAUTION: Click on **OK** to implement the selected list of angles and return to the main screen. If you click on **Cancel**, the last list, not the selected list, will be used in the main screen for measurements.

Section IV: Sample Preparation, Cell Selection Considerations

Sample Preparation: Generalizations

This is the single most difficult part of making a good light scattering measurement. Most errors in a light scattering measurement—beyond those made while learning how to use an instrument—can be traced to poorly prepared samples.

References 3, 4 and 5 in Section I: Introduction, Installation, References each contain chapters devoted to sample preparation. The BI-200SM goniometer manual also includes a discussion on cleaning glassware and removing dust from samples. Please review that discussion, and read at least one of the references concerning sample preparation.

It is much easier to prepare samples in nonpolar liquids than it is in water.

Choosing Sample Cell Types

For low molecular weight, Debye plot measurements at 90° choose the least expensive cell such as the RC12 or RC27. The RC27s come with polyethylene screw caps and are, therefore, convenient, provided you have enough sample. You need a minimum of 10 mL to use the RC27 and 1.5 mL to use the RC12. If you purchased our BI-SFS sample filtration system, for closed-loop, multiple filtration, add another 0.5 mL for each 25 mm diameter filter you stack up in the clamp and 0.5 mL for the dead volume associated with the pump and tubing. NOTE: If you filter polymer solutions, make sure the concentration doesn't change.

Also choose either the RC12 or RC27 if your angular measurements in nonaqueous media covers the classical range of 30° to 150°.

For either low angle or aqueous measurements, choose either the largest diameter cell available (RC27 over the RC12) or the most highly polished (RC25). The RC25 cells are very expensive and should be handled with extreme care. The other cells are relatively inexpensive.

Measurement Schemes

There are several measurement schemes to choose from. Each has advantages and disadvantages. Pick the scheme that fits your requirements. In all the schemes you have to determine the concentration of polymer solution.

Gravimetric methods are more exact, even for the solvent volume (multiply by the solvent density determined to three significant figures including a small temperature correction). 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 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.

Separate Cells: You can use a separate cell for the solvent and each concentration. If one sample is contaminated or the concentration poorly known, it will not affect the others. This requires the greatest number of cells. If there are significant differences between the cells (typically scratches that result in flare light differences), then systematic errors in scattering may occur, most notably at the extreme angles.

With this method weigh the dry polymer. If possible weigh the solvent too and multiple by its density at the same temperature. However, if you have a calibrated pipette, you can use that too.

Single Cell: You can use just one cell. Fill it with the minimum amount of filtered solvent to make a measurement. Filter small amounts of stock solution into the cell to make different concentrations. Each sample is more concentrated than the previous.

With this method, you must rely on volumetric analysis to determine final concentrations. So, you must use a calibrated, automated pipette. Never rely on the markings of a syringe.

A minimum of a 4-fold change in polymer concentration from the least to the most concentrated is recommended, though it is possible to measure a higher ratio provided you have given due consideration to the maximum intensity (highest concentration, lowest angle, see Section III: Setting Up a Measurement). And, quite obviously, make sure you do not overflow the sample cell. If you get enough of the sample liquid into the index matching vat liquid, you may have to remove the vat, clean and dry it, then replace it and perform a shortened version of the instrument alignment. This is to be avoided.

This scheme is convenient because it uses just one cell. Errors due to cell differences cancel, especially when the cell is repositioned in the same place each time. If, however, the concentration changes significantly due to filtering, it is impossible to correct afterwards. Errors compound. Consider using this scheme whenever the polymer concentration can be shown to vary insignificantly after filtration. If it is possible to dry a solution without decomposing the polymer itself, or incorporating some of the solvent into the newly dried solution, then prepare a solution by weighing dried polymer. Then filter. Then dry the solution and see how accurately the two polymer weights agree. You should expect concentrations to be accurate and repeatable to better than +/- 1%. If not a significant amount of polymer is on the filter.

Two Cells. You can use just two cells. Make measurements on the solvent in one cell. Fill the other cell with the minimum amount of the highest concentration (about half the stock solution concentration) necessary to make a measurement. Make subsequent concentrations by filtering small amounts of solvent into this cell. Each sample is more dilute than the previous. A 4-fold change in polymer concentration from the most to the least concentrated is recommended. See the paragraph above under **Single Cell**.

The advantage in this scheme is that only solvent is filtered to make serial dilutions. Only the concentration of the stock solution is important. If significant change occurs upon filtration of the stock solution, only this final concentration needs to be determined as described below. Disadvantages include compounding errors if a mistake is made during one of the dilutions and

if there are differences in the two cells. If the scattering from the solvent is much weaker than the scattering from any of the solutions, then this later concern is not significant. If the solutions scatter weakly, then differences in the cell may be of concern.

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. Also, some polymers do not rapidly disperse when new solvent is added. You may have to wait, or, if you remove the cell and gently swirl it, you must be careful not to add dust. You must reposition the cell in the same place as before. If necessary, mark the cell and cell holder for this purpose.

Stock Solution Concentration

The total scattered intensity depends on many factors including: laser power, wavelength, angle, concentration, molecular weight, quantum efficiency (wavelength dependent) of the detector, detector pinhole diameter, dn/dc, and solvent refractive index.

There are so many variables that it is hard to predict, before a first measurement, what the optimum concentration should be.

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 when using either a 5 mW Argon at 488 nm with the 1mm diameter detector pinhole or a 10 mW HeNe at 632.8 nm with the 2 mm diameter pinhole.

Estimate the concentration of stock solution for other cases by scaling the above guess as follows: The intensity varies linearly with laser power, concentration, and molecular weight; with the square of dn/dc and the pinhole diameter; and inversely with the wavelength to the 4^{th} power.

If you have only a 5 mW HeNe and a polymer of 5,000 molecular weight, then the stock solution concentration will be 50 mg/mL. In addition, you will pick up another factor of 2 in scattered light by running the BI-200SM without the interference filter and the snout screwed to the front of the detector optics to block room light. In addition, the duration/repetition will be several seconds. This means that samples must be especially clean.

If you have a 35 mW HeNe, then you will have all the flexibility you need by changing either the pinhole diameter, working with/without the interference filter, or changing the polymer concentration.

If you have an Argon-ion laser, you will have to run it at 5 to 10 mW. Even then you will need to use the smallest (1 mm) pinhole suitable for SLS measurements. Work with the interference filter in place, and reduce the duration/repetition to 1 second or less. This allows for better dust rejection. It is an advantage unless your sample either absorbs or fluoresces strongly in the blue or green.

Ultimately you will compromise between speed of measurement (high concentration), minimum acceptable total photons counted during a repetition (1E4 to 2E4), total experiment duration, available polymer sample, and distortion in the Zimm due to interactions at high concentration (finite $3A_3c$ term).

Initially, try for at least 25 kcps for the pure solvent at 90° and no more than 2 Mcps for the maximum solution concentration at the lowest scattering angle. For a Rayleigh scatterer the intensity varies by a factor of 2 between 30° and 90° (4 between 15° and 90°). If you arrange the concentration to vary by a factor of 4 or 5, then the variation in the count rate will be roughly an order of magnitude.

An order of magnitude variation in the count rate is about all one can measure properly without either introducing calibrated filters or changing pinholes or suffering from high random errors (below 10,000 total counts) or dead-time effects (above 4 Mcps). **REMEMBER: Once calibrated, you should not change pinholes, laser power, or filter wheel settings. If you do the calibration and subsequent results are meaningless.**

Preparing a Stock Solution

This procedure assumes you have plenty of dry polymer. If you do not, change the proportions as necessary and use a smaller diameter sample cell. Consider using a small diameter filter, one of the newer types with dead volumes of 100 µL or less.

Prepare 20 to 50 mL of filtered solvent, using some of it to rinse a clean, dry bottle to remove dust on the bottle walls. Choose a filter suitable for use with the solvent: typically polycarbonate, cellulose, or Teflon in special cases. Filters with pore sizes in the range 0.1 to 0.45 µm are recommended, though newer ones at 0.020 µm are now available. With the later filter, use a smaller diameter syringe to increase the pressure; otherwise, it is very difficult to push the solvent through the filter. Rinse the filter several times with the solvent to remove residues from the filter manufacturing process **before filtering the solvent**. Always cap the bottle to prevent dust from falling into the bottle.

Alternatively, use the optional BI-SFS sample filtration system to prepare 25 mL of filtered liquid. It is automatic and, due to the multiple filtration, washes the dust off the inside of the cell.

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 for a solvent with a density of around 1 g/mL. Alternatively, use a **calibrated**, volumetric pipette to determine the volume of solvent.

Even if the solvent was well filtered, it often happens that dust is introduced into the solution from the dry polymer. Therefore, if you use the “Single Cell” or “Two Cell” methods described above, you need to filter the stock solution.

Filter the stock solution at least 2 or 3 times. After filtration, it is advisable to again measure the concentration to determine if the process of filtration removed some polymer. Concentration can be determined in one of the following ways: evaporation to constant weight; absorption at a wavelength where the extinction coefficient is known or measured; measurement of the differential refractive index where dn/dc is already known.

If you use the “Separate Cells” method, consider filtering the final concentration into each cell. Make sure the filter has been rinsed with solvent to remove residual scatterers on the filter surface. Use a small-dead-volume filter, or make sure that any solvent remaining in the filter is too small to significantly change the concentration. If you use the same filter, you must filter the lowest concentration first, proceeding to successively higher concentrations. This minimizes the cross-contamination effects.

[NOTE: Another technique for temporarily removing dust is centrifugation. Centrifugation requires special cells and holders to prevent damage. Subsequent handling is also important. While a good technique, it is not described further.]

During the preparation of the stock solution and in the subsequent handling of polymer solutions, remember that volatile solvents can cause concentrations to change. Also, minimize contact between filtered samples and lab air. It is laden with dust, and all your previous efforts can be 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 5 concentrations, though a minimum of 3 will give you a straight line.

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

Do not start with the most difficult sample you can find. Stop. Do not proceed with these notions. In particular do not try to measure that 30 million molecular weight DNA; do not try to measure sucrose in water (300 g/mol) down to 12.5° scattering angle; and do not try to measure the exotic polymer you just synthesized. 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.

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; dn/dc is approximately 0.105 mL/mg; and inexpensive reference standards are available that are good to about ±10 %. Check with a supplier of GPC/SEC standards for calibration of columns. They offer polystyrene “standards”.

Section V: Making a Measurement

Operational Steps

Assuming your samples, solvent, and calibration liquid are already prepared, the following sequence of steps takes you through a complete measurement.

Initial Setup

1. Test goniometer stability and alignment with the BI-ISTW program. See the BI-ISTW manual. If you do this then skip to step 4.
2. Set the current angle by clicking on **Set Detector Angle.**
3. Measure the dark count rate by clicking on **Experimental Parameters, Dark Count Rate** after you have rotated the aperture wheel on the detector optics to the "C" position. Use a 10 second duration to improve statistics. When finished, set the Duration/Repeat back to 1 second, the Number of Repeats to 10, the Dust Rejection Ratio to 1.33, and the Dust Rejection Multiplier to 3.
4. Estimate the maximum count rate (lowest angle, highest conc.). Click on **Intensity** a couple of times to make the estimate. Start with a duration of 1 second.
5. Adjust maximum count rate to be less than 1.5 Mcps using one or more of the following. If adjustable, change the laser power. If you have ND filters in front of the laser, select an appropriate one. Use the wavelength filter in the detector optics (~30% decrease over no filter). Change the detector optics aperture (1,2,3 mm).

Enter Parameters

6. Click **Sample Parameters** and enter Sample ID, Operator ID, and any Notes. Select the Sample Liquid from the list provided or type in the name. Fill in the Sample Liquid's refractive index if it is not amongst those listed. Fill in the value for dn/dc.
7. Fill in the laser wavelength. Select "Auto Save Results".

Calibrate Instrument

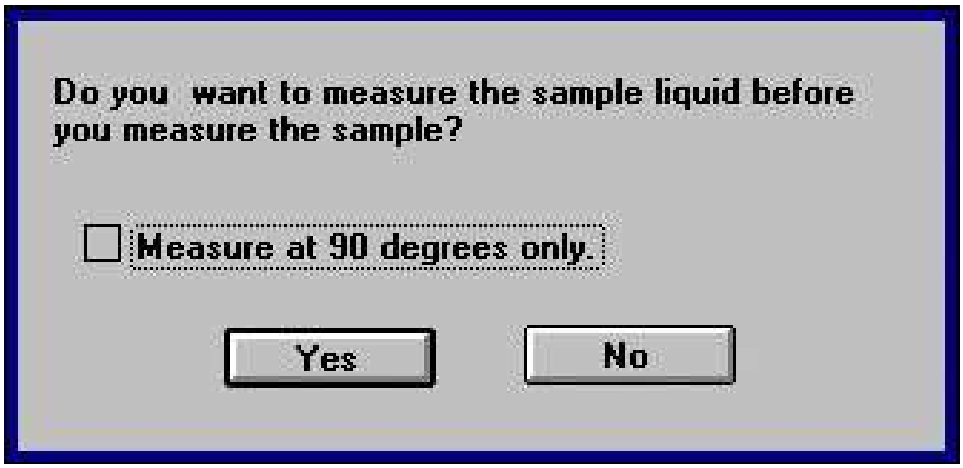
8. Select the "Calibration Liquid". If it is not toluene or benzene, fill in the Rayleigh ratio and the refractive index of the calibration liquid at the wavelength of the laser.
9. Click on **Calibrate Instrument**. Click **OK** to return to the main menu.

Initiate Run, Save Data

10. If you haven't already done so, click **Angles/Edit Measurement Angle List**, and select the angles as described in Section III: Setting Up a Measurement. Click **OK** to return to the main menu.
11. Click on **START** to initiate a measurement. Follow the instructions. We recommend at least seven angles and five concentrations are measured though a minimum of three each are required for data analysis.

12. When finished click on **Done** when asked for the next concentration.
13. Data analysis is described in the next section of this manual.
14. Make sure the data are saved by clicking on **File/Database**. If you do not see the Sample ID (**Auto Save Results**), click **Exit**. Then click **File/Save As** and enter a file description. When data is saved automatically, the Sample ID becomes the description.

When you click on **Start** to initiate a measurement, the following window appears:



Click on **Yes** to initiate a solvent run at the various angles. This is the recommended procedure since, by definition, the solvent intensities must be subtracted from the solution intensities to form the excess scattered intensity from which all data analysis proceeds. If you clicked "Yes", then you will notice during data collection a subscript "ex" attached to solution intensities. This signifies the solvent has been measured and subtracted. It is important to realize that solvent subtraction also is used to cancel any flare light. [Any flare light at 90° is also cancelled, except when calibrating. When calibrating there is no solvent to subtract. Therefore, of all the angles, 90° must be free of flare otherwise an accurate M_w is impossible. If your $I \cdot \sin(\Theta)$ plot is flat, this also suggests the flare light at those angles is minimized.]

Click on **No** if the scattering from the liquid is much lower than that from the sample **and** you have shown using the $I \cdot \sin(\Theta)$ plot that flare light at all the angles of interest is insignificant. In this case, there is no solvent measurement and no solvent subtraction.

There is a third alternative. Click on **No** and check the box marked **Measure at 90 degrees only**. The solvent is measured at 90° only; the solutions are measured at all the angles you have previously selected. A set of solvent values will be calculated from the 90^o value. To repeat: typically click on **Yes** and do a solvent subtraction at all angles.

When you measure any intensity—whether for calibration, solvent subtraction, or polymer solutions—the following sequence of events occurs:

1. In the box labelled “Counts” the total measured counts (raw, uncorrected counts) will appear, and repeat measurements will follow, up to the limit set by the Number of Repeats, 5 for example.

2. Dust discrimination is achieved using a software algorithm and is based on Poisson and Gaussian statistics as follows. The average and standard deviation of the set of N values are calculated as well as the square root of the average. The standard deviation is based on Gaussian statistics. Whereas, the square root of the average yields a standard deviation based on Poisson statistics. A dust event will increase the total measured counts during the measurement. Assuming most measurements are not affected by dust, this increase only modestly affects the average and the Poisson standard deviation calculated from the average. But it increases significantly the Gaussian standard deviation.

3. The ratio of the Gaussian and Poisson standard deviations is formed. If this number is greater than or equal to the Dust Rejection Ratio, the largest of the total measured counts in the set of N is rejected and another one is collected. The calculation is repeated with this new set of values. The rejection criterion is applied again, and, if necessary, the highest value is again rejected. The procedure is repeated until either a set of N values is found that satisfy the criterion, or a total of M·N measurements have been made, where M is the “Dust Rejection Multiplier”. Assuming this multiplier is set at the default value of 3, if after 3N measurements have been made, the remaining set of N values do not satisfy the criterion, the highest value is rejected and the remaining N-1 are tested against the dust rejection criterion. This procedure is repeated until the criterion is satisfied or the last, representing the lowest, total measured count remains.

4. This average (or lowest value) is displayed in the upper right hand corner of the main screen under the heading “Total Measured Counts”.

   With clean liquids, N values can normally be found to satisfy the criterion. At low angles, where dust scatters most, rejections will occur. If most of the measurements reach the limit of 3N measurements, consider aborting the run and cleaning the sample. If that is not convenient, change M from 3 to a higher value, up to 10. Measurements will take longer.

   This software criterion for rejecting residual dust works well when only a small amount of dust particles remain. It is no substitute for the mechanical removal of dust by filtration (most commonly) or, under special circumstances, centrifugation.

5. The average is divided by the duration to form the average, uncorrected count rate (an intensity).

6. From the average uncorrected intensity the dark count rate is subtracted. This is the measured, average intensity corrected for dark count rate.

7. The measured, average intensity corrected for dark count rate, I_m , is further corrected for the dead time, τ_d , to produce the true count rate as follows: $I_t = I_m/(1-\tau_d\ I_m)$. For the BI-9000AT, τ_d is 25 ns.

   The true count rate is always larger than the measured, since a finite dead time means that no more than one pulse can be registered in a given interval. For dark count rates, and for low count rates in general, this correction is insignificant. At 10,000 cps the result is only 3 cps, an insignificant 0.03% correction. At 100,000 cps the result is 250 cps, a small 0.25% correction. At 1,000,000 cps the result is 25,640 cps, a significant 2.6% correction.

8. The true count rate is finally corrected for reflection where

$$\mathbf{I(\theta) = I_t(\theta)\cdot\left[1 - f\cdot I_t(180-\theta)\right]/\left(1-f^2\right)}$$

   Here f is the combined reflection correction discussed in the Section III: Setting Up a Measurement.

   This average, corrected value is transferred to the column labeled I′ in the floating window in the middle of the main screen. It represents the intensity scattered by all the particles or molecules in the scattering volume as observed by the detector optics. If the solvent has been measured, then the excess scattering is labelled I'_{ex}.

9. To form the intensity scattered per unit volume, one has to account for the different volumes observed at different scattering angles. Focusing of a TEM_{001} laser beam produces a hyperbola of revolution; however, with a long focal length lens, such as that used in the BI-200SM goniometer, the shape is cylindrical, at least over a millimetre or two around the center of rotation. Therefore, at any angle the length of the cylinder is larger by $1/\sin(\theta)$. Thus, to compare the intensity per unit volume at various angles, the average, corrected value is *multiplied* by $\sin(\theta)$. The last column in the floating window shows a list of $I'_{ex}\sin(\theta)$ as a function of θ.

   [The effect is the same as looking at a railroad track through a hollow cylinder. Starting with the axis of the cylinder perpendicular to the track, rotate around a fixed point on the track. The length of the track seen varies as $1/\sin(\theta)$. In fact, this effect has given its name to the procedure in Brookhaven's alignment software, BI-ISTW.]

   Note: If you have designated a **reference detector**, the calculations are modified slightly as follows.

1. Pairs of Total Measured Counts and Total Reference Counts are collected simultaneously.

2. The dust rejection criterion is applied only to the Total Measured Counts. If rejected, the corresponding reference count is also rejected, and more pairs of measurements are made up to the limit of M·N pairs.

3. The Total Measured and Reference Counts are divided by the duration, producing intensities in counts/second.

4. The dark count rate is subtracted and the dead time correction applied to each one of the pair.

5. The reflection correction is applied only to the scattered intensity, not the corresponding reference intensity.

6. The ratio of corrected scattered intensity divided by corrected reference intensity is determined for the pairs, up to N, of those remaining after the dust rejection criterion was applied.

7. The average of the ratios is taken and multiplied by $\sin(\theta)$.

8. In the floating window in the middle of the screen, the following values are displayed at each angle θ:

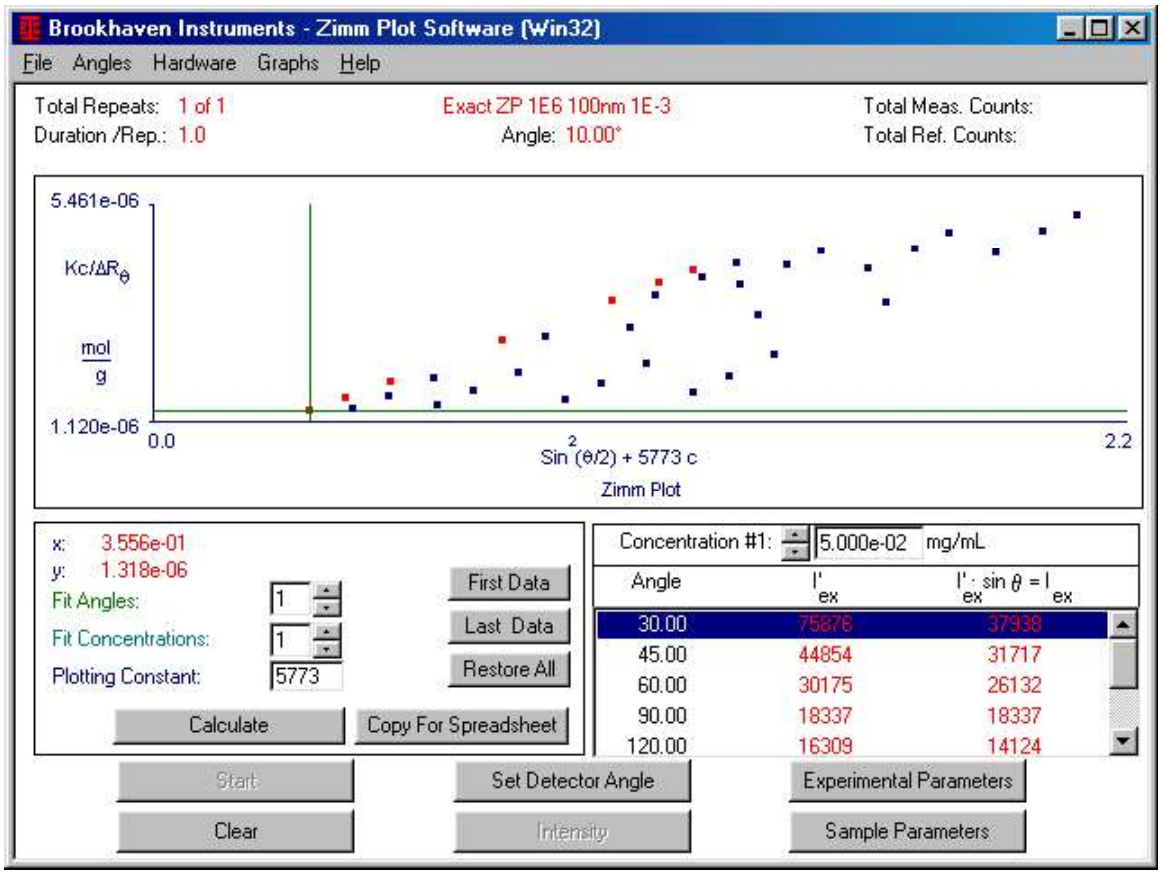
    a. The average of the corrected scattered intensities
    b. The calculated (see below) average of the corrected reference intensities
    c. The average of the corrected ratios as described in step 7.
    d. The average of the corrected ratios as described in step 7 multiplied by $\sin(\theta)$.

    The calculated average of the corrected reference intensities is obtained by dividing the average of the corrected scattered intensities by the average of the corrected ratios. It is the ratios that are important if the reference detector is of any value; however, the program also displays the average corrected intensities and reference intensities in order to determine the general magnitude of these values when data is later recalled.

    Note: One can turn the reference detector normalization on and off. For most modern lasers, the stability is very good over the many minutes required for most measurements. However, for older lasers, or in labs where the room temperature may change a few degrees over the time it takes to make a measurement, the reference detector can account for the drift in laser power that is related to slow temperature drifts or uncompensated power supply drifts. In SLS measurements that may take tens of minutes or longer, the normalization provided by a reference detector may prove even more useful. To test the stability of your laser and goniometer, use the stability test.

Section VI: Data Analysis

This is what you see after collecting data but before calculating any results.



Editing Data

You can click on any point in the plot. When you do, the cursors jump to that point and the concentration and angle are shown. Here it is 0.050 mg/mL and 30°.

You can delete any single point by doubling clicking on the reversed video, blue-highlighted line in the list of angles. The angle and the corresponding I'_{ex} and I_{ex} will turn gray while the point will disappear from the plot. Double clicking again will restore the data and plotting point. You can delete all the angles below the one selected on the plot by clicking on **First Data**; you can delete all the angles above the one selected on the plot by clicking on **Last Data**; you can restore all deleted data by clicking on **Restore All**.

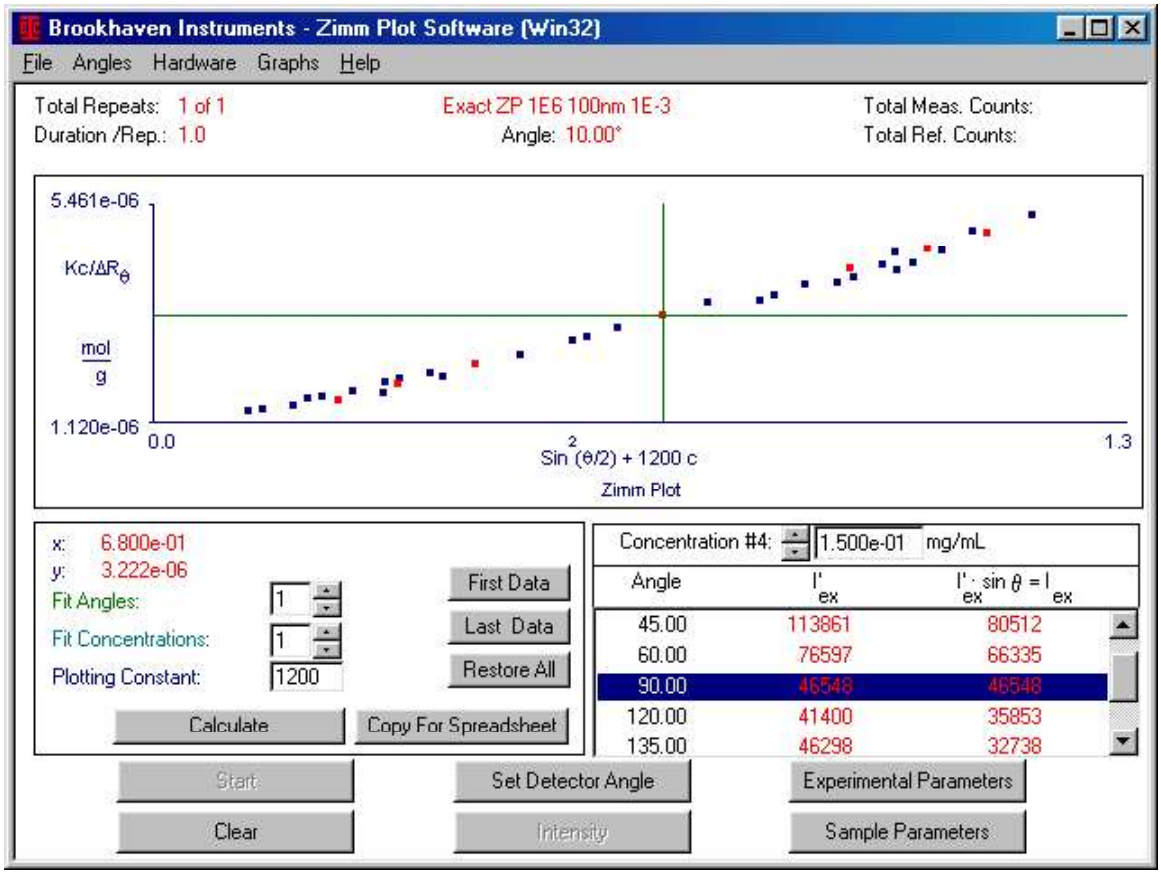
You can edit the concentration simply by overwriting it in the list box. Be sure to save any changes by clicking on **File/Save** or **File/Save As**. Use "**Save**" when you are

sure the edited concentration is correct. Use "**Save As**" to maintain both the new and old data until you are sure which one is correct.

While two points determine a straight line theoretically, the program will not fit data unless there are at least three points. For higher-order fits, there must be at least one more data point than the number of fitting parameters.

Plotting Constant

The plotting constant is chosen automatically. Here it is 5773. It does not effect the final calculations; however, a poorly chosen plotting constant obscures what otherwise might be good data. Plotting constants can be small or large, positive or negative. Type a new plotting constant in the list box, and click anywhere on the screen to see the graph rescaled. Here is an example:



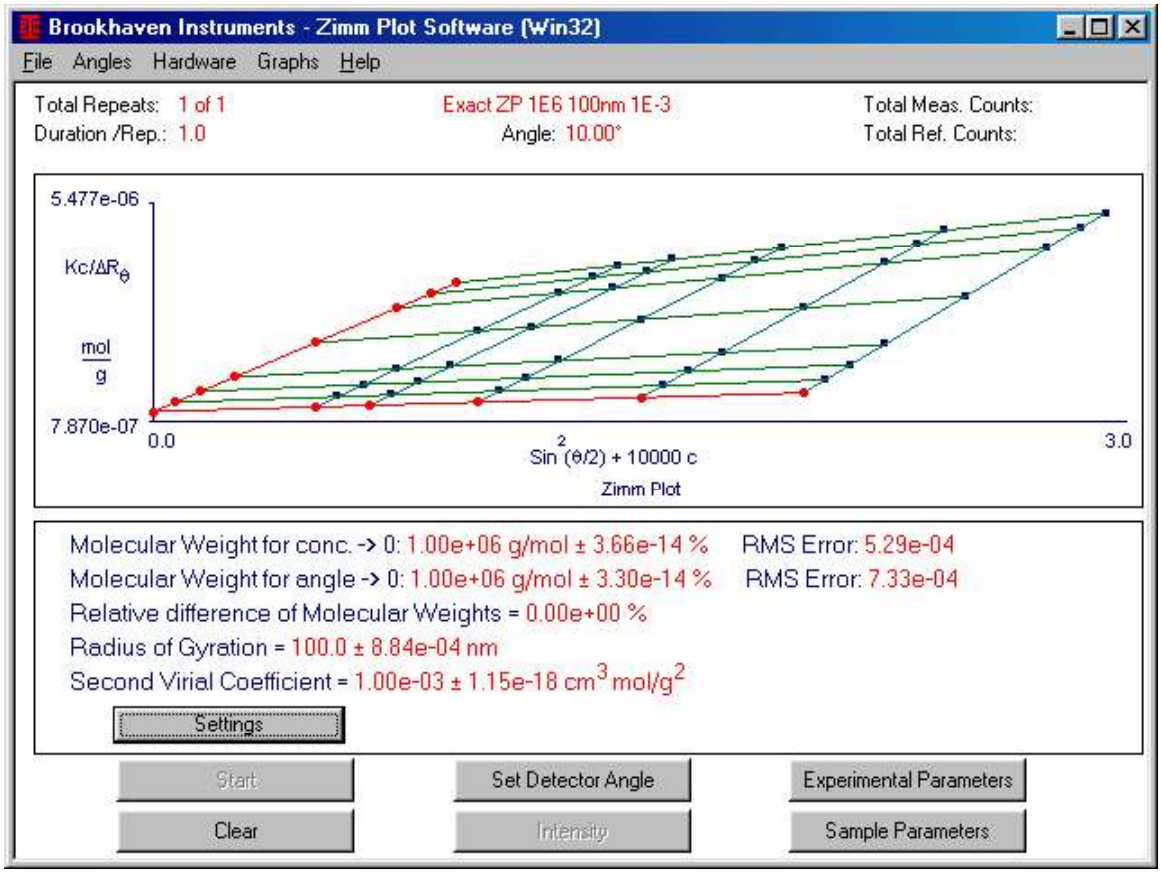
Here the plotting constant was overwritten as 1200. It is impossible to determine which data points are good and which are bad using this plotting constant. Clearly, the original plotting constant is better than 1200.

You can restore the original plotting constant simply by typing it in the list box and clicking anywhere else on the screen. If you have forgotten the default constant, click on **File/Database** and double click on the data file of interest.

The results described below were obtained using the original, default plotting constant of 5773.

Fitting Angle and Concentration

To fit data, click on Calculate after having selected the fit order for both constant angle and constant concentration lines. The fit orders are numbers from 1 to 5 shown in list boxes labeled “Fit Angles” and “Fit Concentrations”.



You can fit constant angle and constant concentration data to straight lines or to polynomials up to 5th order. In this example, all 5 constant concentration lines and all 7 constant angle lines were fit with straight lines.

Each fit to constant angle data is then extrapolated to zero concentration, and each fit to constant concentration is then extrapolated to zero angle. The extrapolated points—shown as red circles—are then fit with the same polynomial as originally chosen. These lines are shown in different colors on the screen, and they are shown in this black and white reproduction as the 6th (left most) line of concentration—actually, zero concentration—and as the 8th (lowest) line of angular data—actually, zero angle.

The weight-average molecular weight, $<M_w>$, is calculated from the intercept of each of the extrapolated lines with the y-axis. In a good measurement, both values $<M_w>$ agree to within 1%. [NOTE: The data shown here were generated and, therefore, show low fitting errors.]The radius of gyration, R_g, is calculated from the limiting slope of the extrapolated line at zero concentration. The second virial coefficient, A_2, is calculated from the limiting slope of the extrapolated line at zero angle. In case of a quadratic or higher order fit, the limits are taken at zero angle and concentration, respectively.

From the slope R_g, the radius of gyration, can be determined. R_g is given by:

$$R_g^2 = \sum_i m_i r_i^2 \div \sum_i m_i$$

were m_i is the i*th* mass element of the scatterer and r_i is the distance from the center of gravity to the i*th* element. The interesting thing about this definition is that it is independent of the shape of the scatterer, although to interpret the measured R_g further requires a model. For spherical scatterers $R_g^2 = 3/5\ R^2$ where R is the radius of the sphere and for long, thin rods $R_g^2 = L^2/12$ where L is the length of the rod. For other shapes consult specialized texts (Burchard , Static and Dynamic Light Scattering from Branched Polymers and Biopolymers, in Advances in Polymer Science, Vol. 48, Light Scattering From Polymers, table 3, pages 74-75, Springer-Verlag, New York, 1983).

Please note that R_g is also and more properly called the root-mean-square radius. Unfortunately, the vast majority of texts and publications use the term radius of gyration or root-mean radius of gyration, and it is difficult to break the habit. [Witness: molecular weight vs. relative molar mass.] In addition, for a polydisperse system, $R_g = <R_g^2>_z^{1/2}$, the z-average, root-mean-square radius. Z-averaging is a result of intensity-weighting, and has some interesting consequences which will not be explored further. Contrast this with the weight-averaging of the molecular weight also obtained from static light scattering.

The second virial coefficient is not a straightforward average. It is related to excluded volume effects and polymer-solvent, polymer-polymer, and solvent-solvent interactions. Consult one of the references for more information on this advanced topic.

To return to the main menu, click on **Settings**.

To print the results, first make sure the **Print Plot Data** box is checked in the **File/Report Print Options** box. You can view the two-page report before printing it by clicking on **File/Print Preview**.

Over Fitting Data

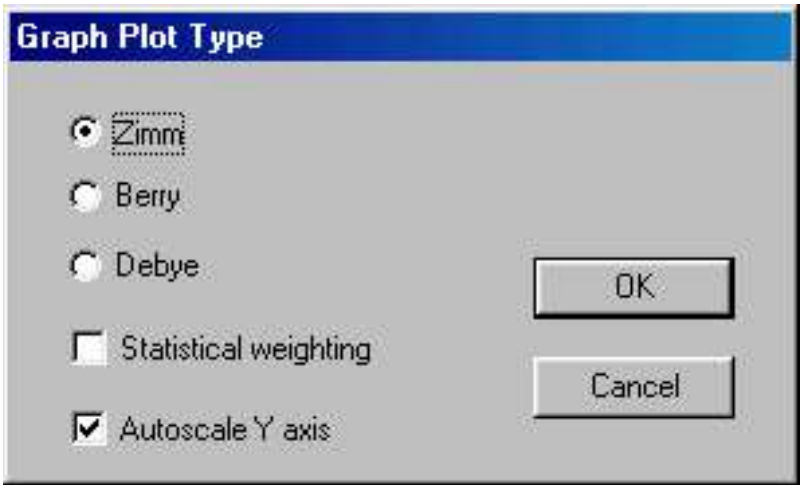
There is a temptation to over fit data by using a higher order polynomial. The fit is always better. But are the results any more meaningful? Was the downward curvature

due to artifacts—dust, flare light at low angles, microgels, etc.—or was it real? That is up to you to decide by repeating the measurements or from other evidence. WARNING. Do not overfit the data. Remember that 6 data points can be fit perfectly with a 5th order polynomial. (The constant term in the polynomial is the 6th fitting parameter.) But it is highly doubtful the intercepts and limiting slopes will be physically meaningful. Use the linear and quadratic fits mainly. Use the higher order fits with great caution.

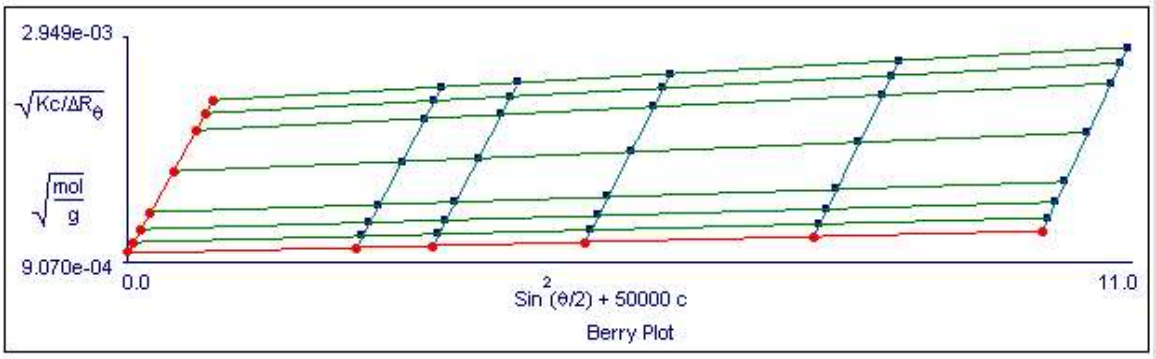
The effect of using a higher order polynomial than the data can support is to lower or raise the intercept. This lowers or raises the $<M_w>$. In addition, the limiting slopes, from which R_g and A_2 are calculated can show even greater errors due to overfitting.

Plot Types

The current plots available are obtained by clicking **Graphs** in the Menubar of the Main Window.

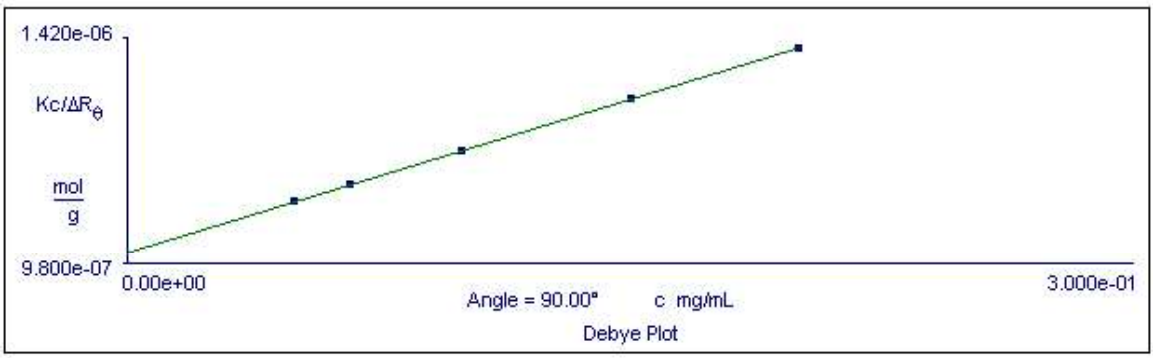


Here is an example of a Berry plot:



Notice the square root of the y-axis values. This is the essence of the Berry plot and is a first-order attempt to account for a finite third virial coefficient term, $3A_3c^2$. Sometimes you can get an equally good fit by using a quadratic fit to the concentration data in a Zimm plot. If the resulting set of $<M_w>$, R_g, and A_2 agree with the linear Berry Plot results, chances are it was a good fit.

Here is an example of a Debye plot:

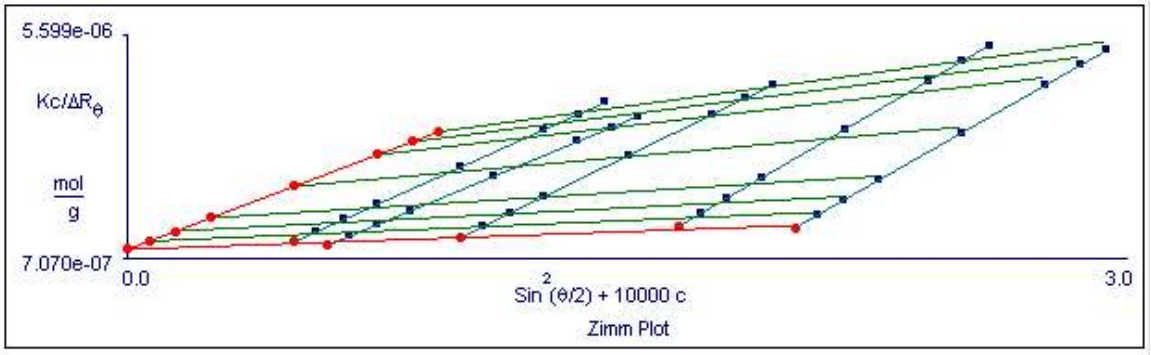


Molecular Weight for conc. -> 0: 1.00e+06 g/mol ± 5.31e-14 % RMS Error: 5.29e-04
Second Virial Coefficient = 1.00e-06 ± 1.67e-20 $cm^3\ mol/g^2$

Please notice that only the slope of the constant angle data (here 90º) and the intercept are obtained. The slope yields A_2 and 1/intercept equals $<M_w>$.

Common Error: Wrong Concentration

When one or more of the concentrations is wrong, the results look like this:



Notice that the lines of constant concentration are shifted and distort the otherwise regular grid pattern. This is typical. Concentrations should be accurate to ± 1%. A calibrated pipette can be made that accurate. An automatic pipette is also accurate, but only if calibrated. However, a syringe can never be that accurate. It is for this reason that dilutions using a syringe are not recommended.

It should be easy to weigh dry polymer to within ± 1%, unless you are trying to weigh extremely small amounts. Consider making a 1 mg/mL polymer solution that is 10 mL in volume. You need to weigh 10 mg of dry polymer. With a 1% error constraint, you must weigh to ± 10 μg . Most common lab scales weigh to ± 100 μg, so be sure to use one that is capable of a higher sensitivity.

What causes concentration errors? Perhaps evaporation is the cause; perhaps when diluting an error was made; perhaps during filtration some polymer was retained on the filter. Whatever the reason, the plots will look like that above.

Statistical Fits: RMS vs. χ^2

An RMS error is reported when you choose not to click on **Statistical weighing** in the **Graphs/Graph Plot Type** box. This is the equivalent of assuming equal weighting for all retained data points. The RMS errors, one calculated for the zero angle and one calculated for the zero concentration extrapolations, are measures of the random error in the fitted data and the systematic error in assuming the particular fitting order you selected. It is not a measure of the absolute accuracy of the result unless all other sources of systematic error are insignificant. In particular, if the calibration is wrong, or dn/dc is wrong, or the concentrations are wrong, then the calculated values of $<M_w>$ and A_2 are also wrong, even though the plot may look very good.

The advantage of using weighting is that the reduced χ^2 (reduced chi-square) not only is a relative measure of goodness-of-fit (lower values mean better fits as with RMS values), but that a good fit should result in a reduced χ^2 of 1 to 2. Smaller values are

probably due to statistical fluctuations and not better fits, while significantly larger values indicate one of two possibilities. Either the equation chosen to fit the data is not appropriate, or the errors (systematic like flare or dust, and the random errors) are too large. Again, there is no way to determine if the calibration or dn/dc values or depolarization ratios, if nonzero, are correct.

Whenever a data point is deleted or undeleted, a fit is changed (1, 2, ...5), or a change in the weighting is selected, this forces a new least squares fit to the data with changes in the results.

We use a least-squares fitting routine, where, when "Statistical weighting" is selected, we calculate the weights as the reciprocal of the variance in each Y-axis value. This variance must be calculated for the actual quantity plotted [$1/\Delta R_{ex}$, or $(1/\Delta R_{ex})^{1/2}$] taking into account that the variance for a counting experiment is equal to the square root of the total counts. In the table below the Y-axis variable, the X-axis variable, and the propagated statistical weight is shown for each case.

Weighted, Linear Least Squares Fitting

Type	Y-Axis Variable	Statistical Weights
Zimm	$1/\Delta R_{ex}$	$\sin^2\theta\cdot((I_s-I_t)^4/(I_s+I_t)$
Debye	$1/\Delta R_{ex}$	$\sin^2\theta\cdot((I_s-I_t)^4/(I_s+I_t)$
Berry	$(1/\Delta R_{ex})^{1/2}$	$\cdot\sin\theta\cdot(I_s-I_t)^3/(I_s+I_t)$

The weights are inversely proportional to the propagated variance of the Y-axis variable, where the propagation is initiated assuming the variance in the total counts of either polymer solution or solvent is equal to the total counts. This follows from Poisson statistics.

The weighted, least-squares fitting routine, using a straightforward matrix calculation, returns the intercept, the slope, the variances in each, the covariance between the two parameters, and the reduced χ^2 also designated χ^2 /(N-p) in some texts on data reduction and error analysis. Here N is the number of fitted data points and p is the number of fitted parameters. For polynomials p = n + 1, where n is the order of the polynomial fit. When the reduced chi-square is close to 1, the fit is good *if and only if* the data used in the fit contain only statistically weighted random errors and the equation chosen for the fit is appropriate. Values much greater than 1 indicate a poor fit. Please consult standard statistical textbooks for more information on the use of chi-square.

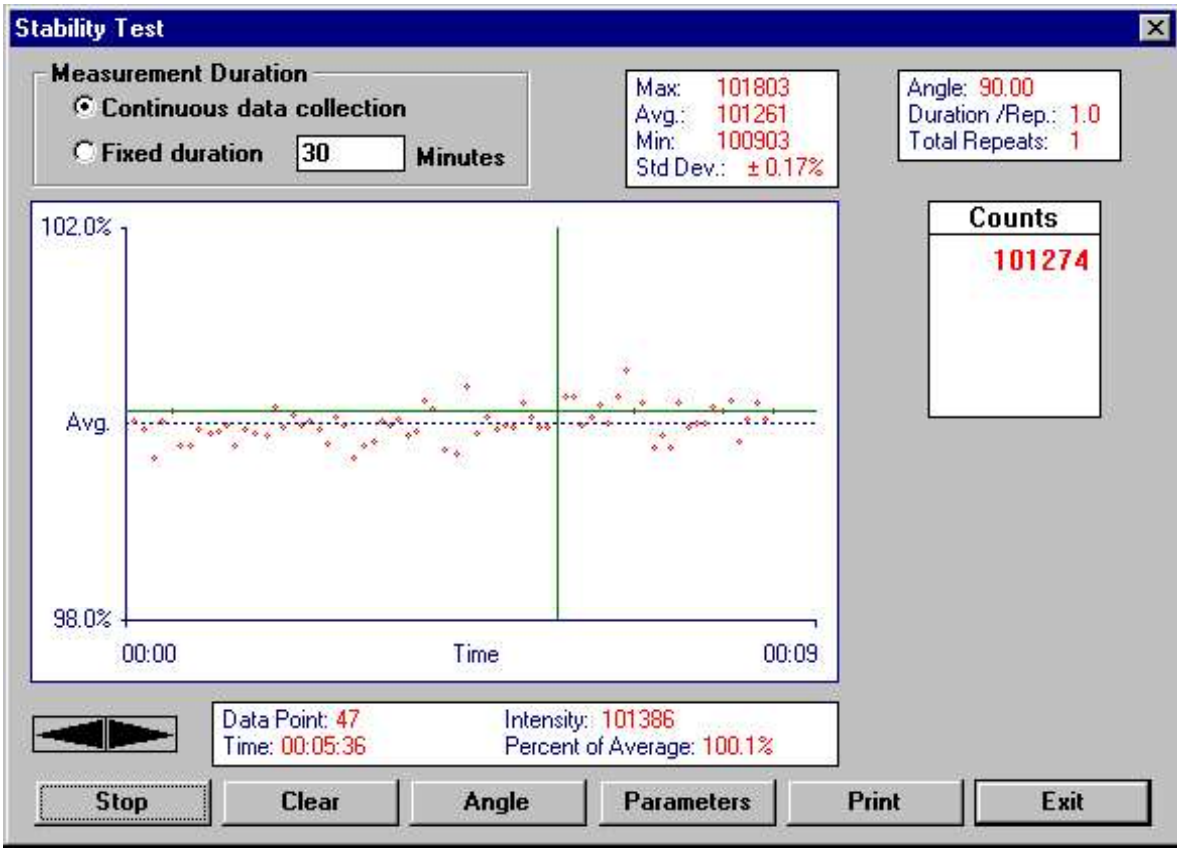
Systematic Errors

When we report $<M_w>$, R_g , and A_2 we also report the propagated and random error from the fit. However, do not place undo emphasis on statistical weighting. It is less important than obtaining good data and choosing the appropriate fit. There is no substitute for making repeat measurements on fresh samples and comparing results to determine if the statistical error from a single fit, using weights determined by the measured intensities and the propagated errors, is about equal to the error from multiple determinations. If it is, you can place a great deal of confidence in the answers. If it is not—perhaps due to concentration errors or flare light—you will have to judge the quality of the data in some other way.

Typically, the calibration error is about 1% and the error in dn/dc is approximately 2%. This gives rise to 5% errors in the final answers (double the dn/dc error because it is squared) without even considering concentration and statistical counting errors. Therefore, absolute accuracies of better than ±5% are not to be expected, though with practice, you may get the random errors below 2—3%.

Section VII: Stability Test

Occasionally stability is a problem. This may arise from an unstable laser, unstable detector electronics, or a problem in the laboratory's main power supply. It may occur if the mechanical parts are not secured properly, causing them to slowly move with time. If the stability is a problem, then do not expect static light scattering measurements results to be good.



You can check the stability at any time. Warm up the laser, filter the vat liquid, and adjust the detector optics such that the total measured counts at any angle (90° is convenient) is 100,000. The statistical repeatability should be less than or equal to approximately +/- 0.32%, for reasons explained earlier. If there is a nonrandom trend in the counts as a function of time, a trend that is significantly larger than the statistical result, then search for the cause of the instability before spending time making static light scattering measurements.

To access the stability test window, click on **Hardware**, **Stability Test** in the menubar of the Main Window. Change the experimental parameters from within this window (**Angle**, **Parameters**). The intensity will, given enough time, show some trends,

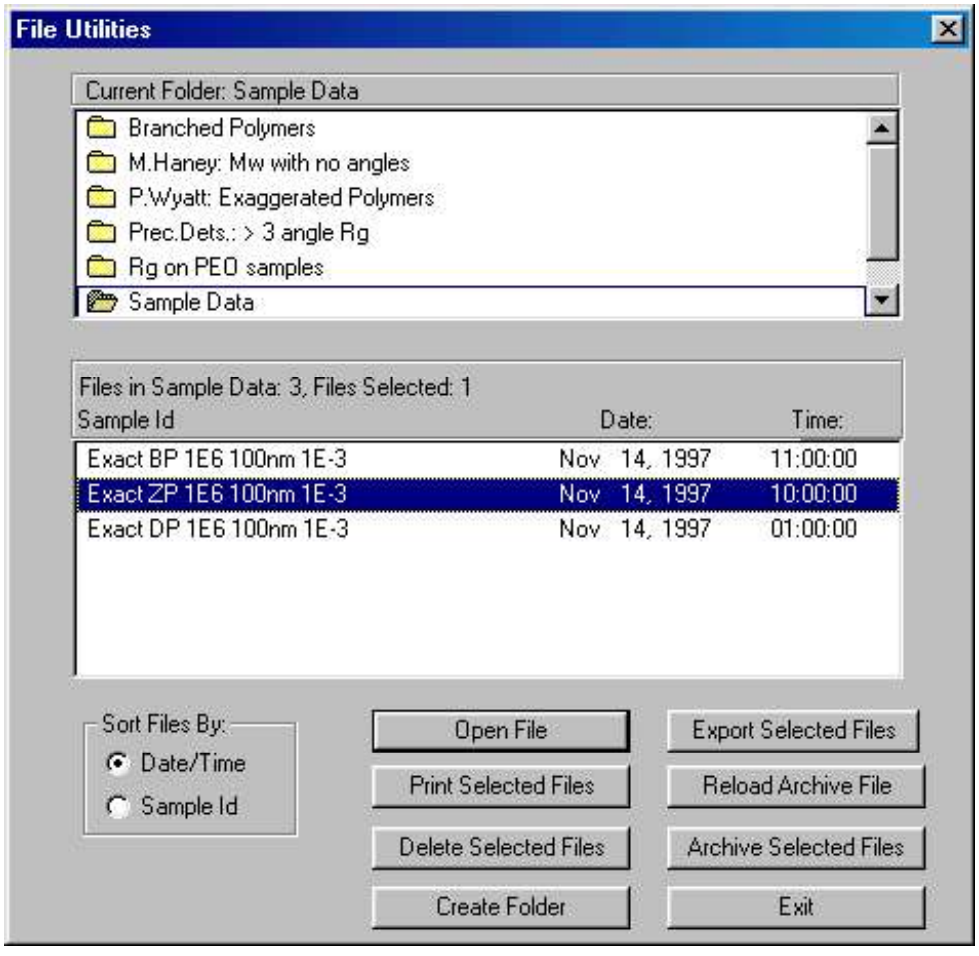
perhaps cycling very slowly with room temperature. As long as the results remain randomly flat, with deviations less than or not much greater than the expected statistical results, over times longer than the duration of measurements you will make, the stability is sufficient.

If the intensity vs. time plot shows no obvious trends, then use the *Std. Dev*. in the box at the top of the *Stability Test* window as a measure to compare against the expected statistical result.

Section VIII: Database and ASCII File Format

Results are saved in the currently opened **Folders** as **Files** using the **Sample ID, Date,** and **Time** as the file identifiers. Files are automatically saved when the **Auto Save Results** box, under the **Sample Parameters** command button, is checked. If not checked, you can still save folders using **Save** or **Save As** under the **File** menu.

To create folders, click on **File/Database**. Click on **Create Folder**. Enter a folder name; it can be more than 8 characters; it may include spaces and punctuation. Double click on the Folder 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**.



Select a single file 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)**.

Files are **archived** in a binary format in the drive\directory path c:\bicw\9kzpw\data 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\9kzpw\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**.

If you click "Copy for Spreadsheet" the X&Y values for the currently displayed plot are copied into the clipboard. If you open a spreadsheet or plotting program, you can paste these values and then create whatever type of plot you desire. Alternatively, you can create ASCII files suitable for use with spreadsheets and plotting programs. These ASCII files contain a lot more data in addition to the X&Y values for graphing. To create an ASCII file, first select a file, then click on **Export Selected File**. The format of exported files is as follows:

Sample ID
User ID
Measurement start time
Measurement date
Number of angles measured, N
Angle 1
Angle 2
.
.
.
Angle N
Yes/No (liquid intensity measured?), Yes/No (reflection correction applied?)
Liquid Intensity at angle 1 (only if "Yes", liquid intensity measured)
Liquid Intensity at angle 2
.
.
.
Liquid Intensity at angle N

First concentration in mg/mL
Intensity at angle 1
Intensity at angle 2
.
.
.
Intensity at angle N
Second concentration in mg/mL
Intensity at angle 1
Intensity at angle 2
.
.
.
Intensity at angle N
.
.
.
Last concentration in mg/mL
Intensity at angle 1
Intensity at angle 2
.
.
.
Intensity at angle N
Wavelength
Sample liquid refractive index
Sample cell refractive index
Vat liquid refractive index
Calibration Constant
Calibration liquid refractive index
Rayleigh Ratio of calibration liquid
Dark count rate
Differential refractive index increment, dn/dc
In/Out (interference filter)
N/V/H (polarization analyzer)
1/2/3 (pinhole size in millimetres)
Number of repetitions
Duration per repetition

The intensities are not the excess intensities. You will need to form those in your external program by subtracting the liquid intensities at the corresponding angles. However all intensities in the data file have already been corrected for dark count rate, dead-time, and averaged to reduce dust using repeated measurements and the dust rejection ratio.

APPENDIX I: Specific Refractive Index Increment, dn/dc

Introduction

In order to calculate M_W and A_2 from measurements of the scattered light, the specific refractive index increment, also known as SRII or dn/dc, must be known. The SRII is usually determined by a differential refractometer and, occasionally, an interferometer. We recommend the use of the Brookhaven BI-DNDC differential refractometer for this purpose. (The radius of gyration, more formerly the rms radius, R_g, is independent of both dn/dc and the calibration constant.) Since the SRII is raised to the second power in the calculation, a 1% error in dn/dc gives rise to a 2% error in M_W and A_2 for static measurements. For dynamic measurements, however, where polymer solution concentration is determined by the same differential refractometer, a 1% error in dn/dc gives rise to a 1% error in M_W and A_2.

[NOTE: The terms static and dynamic may be confusing, especially when compared to dynamic light scattering. Here a static measurement refers to static light scattering, variously called classical light scattering, total intensity light scattering, and integrated or averaged (over time) intensity light scattering. Such a measurement determines static properties of the polymer such as M_W. The intensity of scattered light is integrated (averaged) over time scales greater than about 100 milliseconds. However, one can make such a measurement on flowing (dynamic) or nonflowing systems (static). When light scattering is used as a detector for SEC/GPC fractionations, the solutions and solvents are flowing and this type of measurement is a dynamic measurement. But don't confuse that with dynamic light scattering that is used to determine dynamic properties of the polymer such as its translational diffusion coefficient. Here a digital autocorrelator, such as the Brookhaven BI-9000AT, is used.]

The SRII is defined as the slope of the polymer solution refractive index as a function of polymer concentration, dn/dc. In principle the slope could vary as a function of concentration; in practice no concentration dependence of dn/dc is observed for polymer concentrations up to 20 to 50 mg/mL. Since typical SLS measurements are made with concentrations well below these values, a straight line is observed, though measurements at several concentrations are recommended to minimize the error in determining the slope. [To calibrate a differential refractometer one uses salts with known values of Δn vs. concentration, and these plots aren't linear until the concentrations are below ~ 10 mg/mL.]

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.

Wavelength Variation

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, one could interpolate values of dn/dc from those measured at the classical values. More uncertainty would have to be attached to values extrapolated to 632.8 nm, the wavelength of a HeNe laser. Other common laser wavelengths of interest include the 532 nm line of the frequency doubled, solid state laser and the nominal 675 nm line of the common, red, diode lasers.

Except near to an absorption peak in the spectrum of either the solvent or the polymer, the variation in wavelength is given by:

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

If dn/dc is known at two wavelengths, this equation can be used to calculate it at a third wavelength. However, it is much better if three measured values fit well to a straight line.

This equation was derived from the Gladstone-Dale expression given below.

Temperature Variation

The refractive index of common organic solvents varies with temperature from about -3 x 10^{-4} mL/g•K to about -5 x 10^{-4} mL/g•K. The value for water is of -1.1 x 10^{-4} mL/g•K. The temperature coefficient for dn/dc 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/g•K. Thus, a few degrees difference in the absolute temperature between the measurement of dn/dc and the light scattering experiment is not significant. This should not, however, be interpreted to mean that the value of dn/dc can be determined without regard to temperature. This apparent paradox is explained by considering the magnitude of the difference of the refractive index of solution and solvent.

Consider the following numerical example. Assume dn/dc is 0.1 mL/g, and that you wish to measure it to within 1%. The difference in solution and solvent refractive index, Δn, at c = 2 mg/mL is therefore 2 x 10^{-4}. If you want to measure that difference to within 1%, you would need to measure Δn with a resolution of $\pm$ 2 x 10^{-6}. If you measured the refractive index of the solution and solvent in separate experiments and then formed the difference, you would typically need to keep the absolute temperature difference within 0.01 °C. This is not easy to do. What does all this mean?

One must use a differential refractometer. In a differential refractometer, the solution and solvent are maintained at the same temperature in the same cell separated by a partition. It is not important to know the exact temperature, only that the temperature difference is

small, ~ 0.01 °C. This is not a particularly stringent condition. It can be accomplished in a small cell by waiting for thermal equilibrium or by controlling the cell temperature to 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 temperature variation of dn/dc can be estimated by differentiating the Gladstone-Dale equation below. The result is:

$$\mathbf{d(dn/dc)/dt = (n_p - n_s)\cdot d\bar{\nu}_p/dt + \bar{\nu}_p\cdot(dn_p/dt - dn_s/dt)}$$

Here n_p and n_s are the polymer and solvent refractive indices; dn_p/dt and dn_o/dt are their temperature coefficients; and $\bar{\nu}_p$ and $\mathbf{d\bar{\nu}_p/dt}$ are the polymer partial specific volume and its temperature coefficient. These parameters are discussed below.

Molecular Weight Variation

Generally, dn/dc increases with molecular weight but becomes constant when the end groups are sufficiently far apart. Below very roughly 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 shape and end-group contributions to the refractive index. Above this range it is, typically, constant.

Theory

The refractive index of a solution is equal to the weighted sum of the refractive indices of its components. The appropriate weighting function for a polymer solution is the volume fraction, Φ, of each component. For a two-component system this can be written as,

$$n_{soln} = n_s + (n_p - n_s)\cdot\Phi_p$$

The volume fraction of the polymer can be written as

$$\Phi_p = c\cdot\bar{\nu}_p \quad ,$$

where c is the polymer concentration and $\bar{\nu}_p$ is the partial specific volume of the polymer. Substituting and taking the derivative, one obtains the Gladstone-Dale rule for calculating dn/dc:

$$dn/dc = \bar{\nu}_p\cdot(n_p - n_S) \quad .$$

Although n_S, the refractive index of the solvent is often available in the literature, values of n_p are less well known, and the partial specific volumes in various solvents of interest are the least well known. For these reasons, the Gladstone-Dale rule is rarely used to calculate dn/dc. It is better to measure it with a differential refractometer. However, the Gladstone-Dale rule is useful in several ways.

One can predict from the G-D rule that dn/dc is proportional to the difference in refractive index of the polymer and solvent. Therefore, the greatest scattering occurs when one can dissolve a polymer in a solvent with a large difference in refractive index. If the partial specific volume of a polymer is known for one solvent, one can assume it is roughly the same as in the solvent of interest, provided the solvents are roughly similar in chemical structure and the concentrations of interest are low. This leads to errors of roughly 1% to 3% in dn/dc and either 2% to 6% in M_W in static measurements or half these values in dynamic measurements.

In an ideal solution, the specific and partial specific volumes of the polymer are equal. Furthermore, since the specific volume is the reciprocal of the polymer density, one can then estimate the partial specific volume given these assumptions and the polymer density. This leads to errors of roughly 3% to 5% in dn/dc and either 6% to 10% in M_W in static measurements or half these values in dynamic measurements.

Of course polymer solutions are never ideal because of the spatial extent of the polymer. Using the G-D equation with the reciprocal density of the polymer in place of a partial specific volume may well lead to errors in dn/dc of 10% or more with 20% errors or more in static measurements of M_W.

For example, the density of polystyrene is 1.051 g/cm^3, its refractive index is 1.59, and that of toluene is 1.49. From this one can estimate dn/dc as 0.095. Measurements give 0.106 g/cm^3 at 633 nm (HeNe laser). The error is 10.4% in dn/dc and 20.8% in a static measurement of M_W.

By taking the derivative of the G-D rule with respect to wavelength, temperature and molecular weight, one can derive equations relating the variation of dn/dc with these variables. The ones for temperature and wavelength are given above.

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 (< ~ 1970) are given primarily at 436 nm and 546 nm, the mercury lines. Linear interpolation to 488 nm or 514.5 nm is acceptable in lieu of direct measurements. Linear extrapolation to 632.8 nm is somewhat less acceptable, but may be necessary.

Dust is the largest source of random error in SLS measurements. Flare light is a major source of systematic error in SLS measurements. The uncertainty in dn/dc is another major source of systematic error in SLS measurements.

With modern differential refractometers, the leading cause of errors in dn/dc measurements is the ability of the user to accurately determine and maintain low polymer concentrations, especially with highly volatile solvents. Great care must be taken when making concentration measurements and when flushing out the instrument between calibration and solvent measurements and from one solution concentration to the next.

In summary, it is best to make a measurement of dn/dc using a differential refractometer. If none is available, use literature values. If necessary make small, first order corrections for wavelength and temperature. The least acceptable method is a calculation of dn/dc from molecular composition using the G-D equation, though such a calculation can at least provide a crude estimate.