



Features Added to the Particle Size Distribution Software

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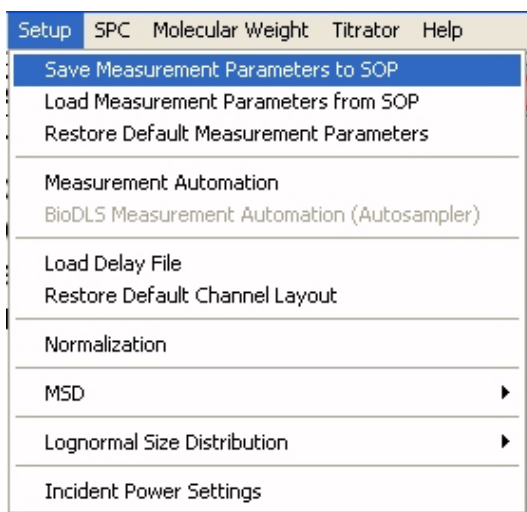
Features Added to the Particle Size Distribution Software

Saving/Loading SOPs, Standard Operating Procedures

Standard operating procedures are useful for enforcing uniformity in measurement conditions and data analysis. Pharmaceutical companies are particularly fond of SOPs. In the current context, procedures are the parameters (number of runs, duration, refractive index, etc.) that are used to make the measurement and to analyze the data. The software can create an SOP from whatever parameters are currently set.

Click *Setup/Save Measurement Parameters to SOP*. Name the file that will be created. It will be saved with the file extension *.sop*. It is saved in a subdirectory called SOP. You can create an SOP before you start a measurement, after it is finished, or by recalling a previous measurement. In all three cases, whatever parameters are currently set becomes the basis of the SOP.

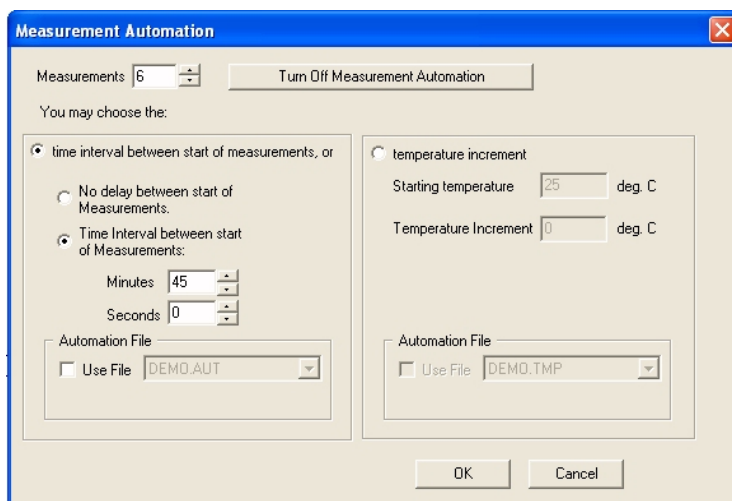
To use a particular SOP for a new measurement, click *Setup/Load Measurement Parameters from SOP*. Click on the filename from the list of SOPs displayed. Click *Open*. Change the Operator ID or any of the other values shown in the Parameters window. However, changing too many values defeats the purpose of the SOP. Click *Okay*. Click *START*.



Measurement Automation: Time and Temperature

Measurements consist of one to 10 runs. The number of runs and the duration per run are selected in the *Parameters* window. You can make automatic, unattended measurements to see either the effect of time or temperature.

Click *Setup/Measurement Automation*. Select either time interval or temperature measurement.



The time interval between the start of each new measurement must be greater than the number of runs per measurement times the duration per run. The time intervals selected this way are constant. If you require a selection of times that are not constant, you can create an *.aut* file, a simple ASCII file, and recall it when required. Such files are located in the 9kpsdw/data subdirectory. The default.aut file has instructions and an example of how to create such a file.

Suppose you are interested in the overnight stability of a suspension: Does it increase size over time? A measurement might consist of 3 runs, 3 minutes each, for a total of 9 minutes. Perhaps you will leave for the evening starting at 17:00 hrs and return the next morning at 09:00 hrs. If you set the time interval between start of measurements at 30 minutes, then including the measurement at 5 pm, you will have 33 measurements covering 16 hours.

You can use either the SPC feature or the *Log File Settings* (under *File*) feature to look at the data. With the SPC chart, it is assumed the data are linearly spaced. In this example they were; in other cases they may not be. With the *Log File Settings* you create a new ASCII file with a limited set of information from each measurement, the set being determined by highlighting only the data you want saved from a long list of choices. The information in this *logfile.txt* is located in the 9kpsdw/data subdirectory and can be imported into Excel or any other plotting or spreadsheet program. Note: Log file data use the mean values and not the combined values from each file.

Automatic temperature measurements require a start temperature and an increment. If the number of measurements times the increment plus the start temperature exceed the capacity of the temperature controller, the run sequence will be automatically terminated after the last attainable temperature. If you require a selection of temperatures that are not linearly related, you can create a *.tmp* file, a simple ASCII file, and recall it when required. Such files are located in the 9kpsdw/data subdirectory. The default.tmp file has instructions and an example of how to create such a file. Using the *Log File Settings* in combination with the automatic temperature measurements is a powerful way to collect temperature dependent size information without having to attend the instrument.

APD Detector

The avalanche photodiode detector has a much greater sensitivity (5-10 times) to photons in the red (620-680 nm, approximately) than a PMT. Thus, with weakly scattering systems, such as globular proteins, micelles, quantum dots, dendrimers, and other small nanoparticles and polymers, an APD has great advantages over an expensive, short-lived, higher power laser such as the frequency-doubled, 532 nm, and solid-state laser. Most 90Plus instruments are supplied with the BI-APD option.

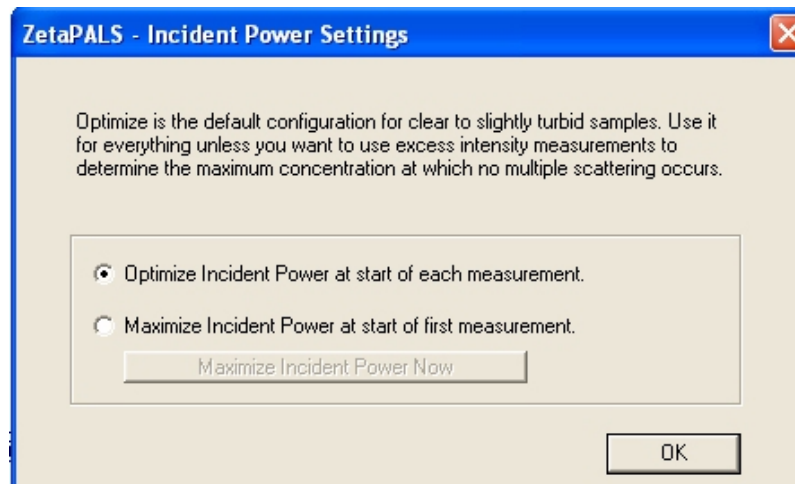
The APD, however, has a drawback. Due to its higher sensitivity, strong scatters can easily produce too many photons, resulting in distortion of the correlation function from which all size distribution information is ultimately calculated. To prevent this, a variable neutral density filter wheel is introduced and automatically varied to obtain a count rate less than 500 kcps. Note: With a PMT, the maximum, automated count rate is 750 kcps.

Optimize/Maximize Count Rate

For a long time this software had a feature that was applicable if the instrument possessed a variable neutral density filter. The feature was the ability to maximize the intensity by automatically rotating the position of a variable neutral density filter located between the laser and the sample. All instruments for zeta potential determination have such a filter. All particle sizing instruments with the BI-APD option have such a filter. All instruments with the 50 mW, 532 nm laser have such a filter.

An additional choice is now included: Optimize incident power at the start of each measurement.

To access either feature, click *Setup/Incident Power Settings*. Then choose either *Optimize Incident Power at the start of each measurement* or *Maximize Incident Power at the start of the first measurement*. Optimize is the default and should be used for particle size measurements except those where you are determining intensity vs. concentration in order to determine the range over which multiple scattering is a problem. In this case use the maximize feature.



Thin Shell Choice for Calculation of Mie Scattering Correction: Liposomes Primarily

In the Parameters window, on the right side, under "Refractive Index of Particles", there is now a new line. Choose "Uniform Spheres" and fill in the particle's real and imaginary refractive index (if it absorbs light at the laser wavelength), if the particles are uniform (homogeneous, i.e. constant composition throughout particle), and if and only if you want to calculate the volume-, surface-area-, or number-weighted MSD results. This is the standard choice as it has always been.

The new choice is "Thin Shells", also known as core/thin-shell. This model is appropriate for a liposome where the core of the particle has a refractive index equal or close to that of the liquid surrounding it and the shell's thickness is small compared to the diameter of the particle. In addition, this model is appropriate for diameters generally less than 300-400 nm. These assumptions are valid for most liposomes. When these assumptions are fulfilled, then the Mie scattering coefficient (same as the light scattering correction) is independent of the shell's refractive index and the calculation is made without reference to the particle's refractive index.

An interesting example of the change you will see can be seen in the Liposomal Formulation file in the Sample Data folder. Use the MSD, Uniform Sphere choice with the real part of the particle refractive index at 1.35, an average value between 1.33 for water that comprises most of the liposome and a higher value for the lipid layer that comprises a small fraction of the particle. Then recalculate selecting Thin Shell. The peaks stay in the same position but the fraction in each peak changes.

Parameters

Sample ID: Liposomal Formulation

Operator ID: Brookhaven Instruments

Notes: Transport vesical for solid cancer injection

Runs: 10 Batch #: 0

Temp.: 25.0 deg. C Run Duration: 5 Minutes

Liquid: 0.9% NaCl Seconds: 0

Viscosity: 0.890 cP Refractive Index of Particles:

Ref. Index: 1.332 Real: 1.420

Angle: 90.00 Imaginary: 0.000

Wavelength: 632.8 nm Uniform Spheres Thin Shells

Auto Save Results:

OK Cancel

Marquardt Baseline Algorithm

For noisy correlation functions, such as one might find with weakly scattering globular proteins, the measured baseline is sometimes hard to establish using our standard fitting routines. The Levenburg-Marquardt, often abbreviated Marquardt, algorithm makes it easier to establish the baseline. This improvement is now standard. It is transparent to the user. It works on all correlation functions, with or without noisy baselines.

Molecular Weight by MHS Equation: Main Menu Selection

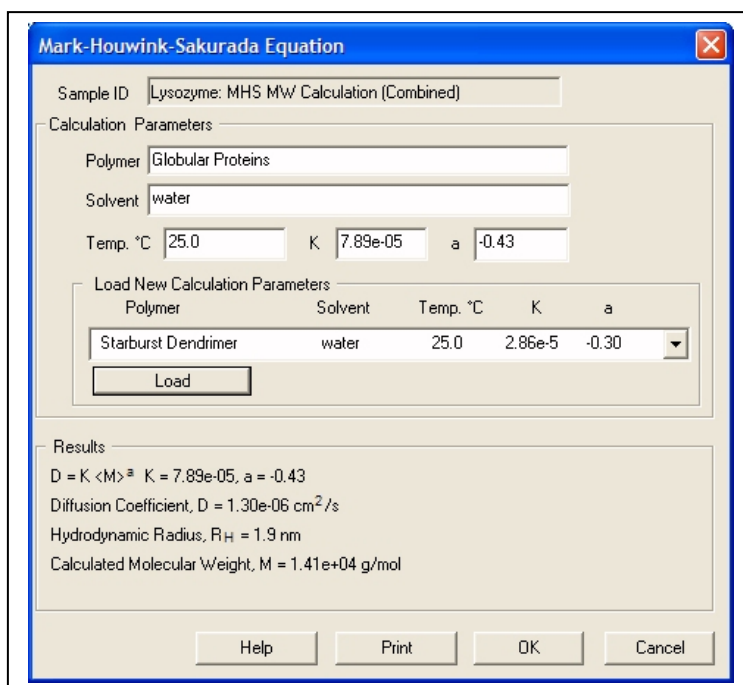
Over limited ranges of molecular weight, there is an empirical relationship between the translational diffusion coefficient and the molecular weight. It is known variously as a Mark-Houwink (MH), a Mark-Houwink-Sakurada (MHS) or a Mark-Houwink-Kuhn-Sakurada (MHKS) relationship:

$$D_T = K \cdot M^a$$

where {K, a} are a pair of constants and depend on the polymer, the solvent, and the temperature as well as the range of molecular weights examined. This equation has a more famous analogue where the intrinsic viscosity is related to molecular weight using a different pair of constants.

Such an empirical equation allows the calculation of a diffusion-coefficient averaged molecular weight if the pair of constants is known. The calculated molecular weight is empirical and not easily related to the weight- or number-averaged value obtained with static light scattering and osmometry, respectively, except for narrow distribution such as unaggregated globular proteins. This average value is convenient because the DLS instrument, unlike a static light scattering instrument, does not have to be calibrated nor does the differential refractive index increment, dn/dc , need to be known. In addition, a set of accurate polymer solution concentrations do not have to be prepared. A single, dilute solution will suffice. However, for large random coils, one has to watch out for modes of motion other than translational diffusion contributing to the correlation function.

To access this standard feature, click *Molecular Weight* in the main menu bar. For more information, read the *Help* command button in the MHS window.

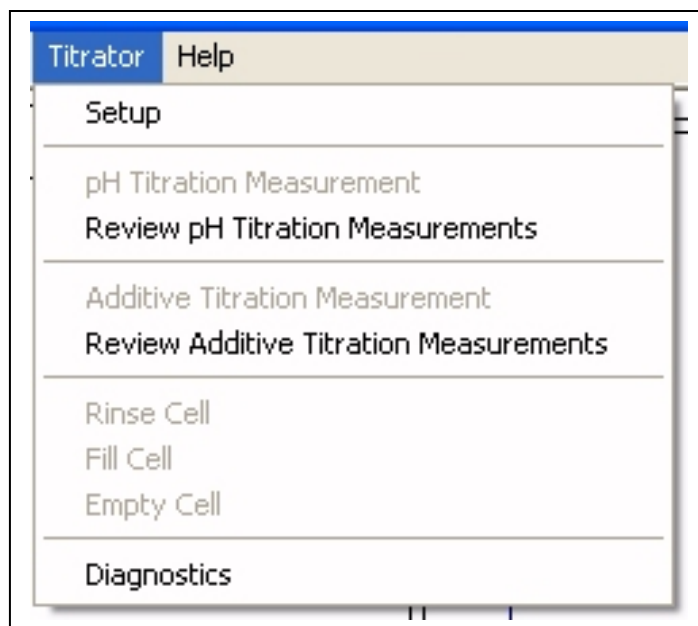


90PDP, Absolute Molecular Weights Using A Debye Plot: Refer to Separate Manual

For a more accurate determination of the well-defined, weight-average molecular weight of a polymer or protein in dilute solution, M_w , consider the 90PDP option that consists of a 40 μL flow cell and software. With this option, a so-called absolute Mw is determined if and only if the molecular size is small enough. Generally, this means radii of gyration less than about 12 nm, a criterion satisfied by globular proteins, highly branched polymers, dendrimers, and random coils up to approximately 100 kDa. A complete manual is available that describes the measurement and calculation that requires the value of dn/dc as well as a calibration, typically with toluene.

BI-ZTU Titrator Software Now Works with PSDW & Additives Other than Acids/Bases

The BI-ZTU, zeta titrator unit, is used primarily with the zeta machines (ZetaPlus or ZetaPALS or the BI-Zeta or BI-PALS options) and acids/bases to determine the isoelectric point. However, there are cases where one would want to automatically determine zeta potential or particle size as a function of pH, concentration of added surfactant, concentration of added salt, and in general the concentration of an added reagent that is expected to affect the surface potential. The latest PSDW software incorporates this software, though it is not enabled unless the BI-ZTU hardware is attached.



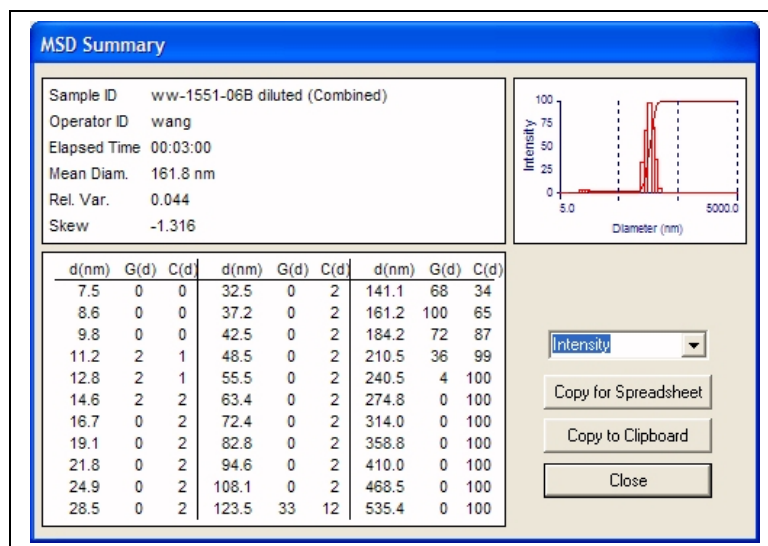
The *Setup* window is used to enter up to four different reagents, their names, concentrations and units. Pumps are assigned. In the “pH Titration Measurement” or the “Additive Titration Measurement” windows the number of points and desired concentrations are specified. After the measurements are made, the full curves of particle size vs. pH or additive concentration can be reviewed. For more detailed information, see the BI-ZTU manual.

Copy for Spreadsheet, Copy to Clipboard

There are times when the standard printouts are not enough. In these cases, you can take advantage of the clipboard to produce either a printable and editable picture of the displayed graph or a coma-delimited set of values suitable for re-plotting using a spreadsheet or plotting program.

In the example shown below, click *Copy to Clipboard*. Open the *Paint* application or another graphics program. Then *paste* the information in the clipboard. The result is the same as the image shown below without the copy and close commands. Such an image can be resized, printed, and even edited if you have the right tools.

Alternatively, you may want to plot the differential distribution, d vs. $G(d)$, or the cumulative undersize distribution, d vs. $C(d)$ on different scales or to label the axes differently. In this case, click *Copy for Spreadsheet*. Then *paste* the information into a text editor such as the Notepad program found in Windows. Save it as a .txt file. Use the *Import External Data* or equivalent tool in your spreadsheet or plotting program to separate the columns



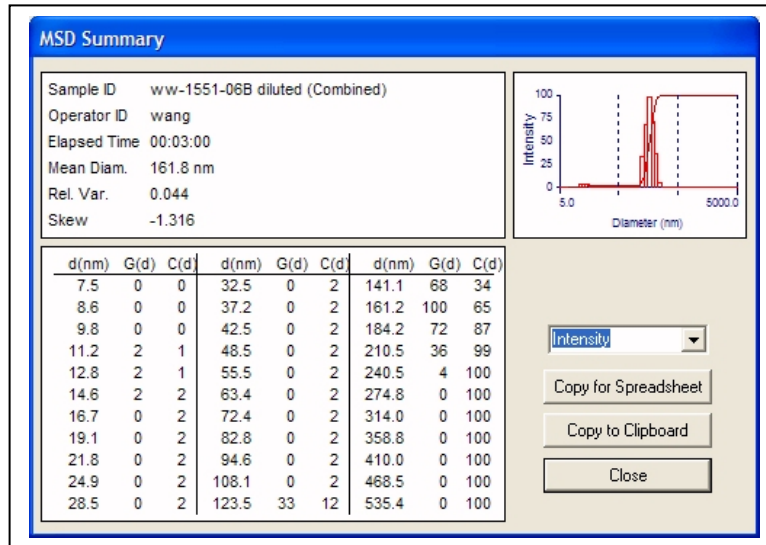
Using the Dust Cutoff to Improve Results

Dust is a generic term referring to a few, large particles that prevent a flat baseline. Without a flat baseline, it is difficult to properly normalize the data and therefore difficult to calculate good results. Dust can be a few, very large agglomerates coming from the sample itself, dust from the air or from cell and transfer vessel walls, or tiny bubbles. It is best to remove dust mechanically by filtration or centrifugation or by waiting for settling. A few seconds in a sonic bath is normally enough to drive air bubbles up and out.

However, there is usually residual dust and further procedures are necessary. One is an electronic filter known as the *Dust Cutoff* found in the *Parameters* window. This is a number used to filter out short-term spikes in the intensity data. Such spikes presumably come from rare, dust events. If the number is set high (100 or more), all data is accepted and none is filtered. If the number is set low (less than 5), real fluctuations might be clipped and the results distorted towards smaller sizes.

When the dust filter is on, the percent data retained is displayed to the right of the baseline index. The dust filter is toggled on and off using the command button in the main window.

Start with a dust cutoff value of 30. The optimal value of the dust cutoff is actually related to the expectation of outliers in intensity, which is related to the size of particles being measured. For particle sizes on the order of 10 nm, use a value of about 15. For particle sizes on the order of several 100 nm, use a value of 30. For particle sizes on the order of 1 micron, use a cutoff value of 80. To truly optimize the dust cutoff value, lower the dust cut-off filter until about 98% of the data is retained.



Definitions and Calculations in the MSD Summary Window

The mean diameter is defined as the weighted sum over the diameters, d , with the weighting factor $G(d)$. Thus,

$$\text{Mean Diameter in MSD Window} = \bar{d} = \frac{\sum d \cdot G(d)}{\sum G(d)}$$

It is a measure of central tendency for the distribution.

A relative measure of the width is the square of the standard deviation of the distribution, the variance, divided by the mean diameter squared.

$$\text{Relative Variance} = \text{Variance}/(\text{Mean Diameter})^2$$

$$\text{Variance} = \frac{\sum (d - \bar{d})^2 \cdot G(d)}{\sum G(d)}$$

Narrow distributions have smaller relative variances than broader distribution. A mono-disperse distribution would have a zero relative variance.

Peaks can be skewed to the left (negative skew) or to the right (positive skew) of the modal value. A relative measure of skew is obtained by dividing the third weighted moment about the mean diameter [the weighting is once again $G(d)$] by the square root of the cube of the variance. This relative measure, like the relative variance, has no units.

$$\text{Skew} = \frac{m_3}{\sqrt{(\text{Variance})^3}}$$

$$m_3 = \frac{\sum (d - \bar{d})^3 \cdot G(d)}{\sum G(d)}$$

The cumulative undersize distribution, $C(d)$, is the percent of the size distribution at or below the diameter d . It is calculated by dividing the sum of the $G(d)$ values at and below d by the total sum of $G(d)$. The differential size distribution is a continuous curve connecting the values of $G(d)$. Thus, $C(d)$ is the integral of $G(d)$ and $G(d)$ is the differential of $C(d)$.

Weighting: Intensity, Volume, Surface Area, and Number

The MSD feature takes the accumulated, combined, autocorrelation function and uses the NNLS, non-negatively constrained least-squares, algorithm to produce an intensity-weighted differential size distribution: $d, G_I(d)$. When these values are used to produce a mean, relative variance, skew, and cumulative undersize distribution, it should be understood that these results are all intensity-weighted. Intensity weighting favors larger particles except in those rare cases where the light scattering coefficient of the larger particles is close to zero.

The volume-weighted differential size distribution $G_V(d) = G_I(d)/d^3M$, where M is the light scattering coefficient, also called the Mie scattering coefficient. M is a function of d , wavelength, scattering angle, and the refractive index of the particle (real and imaginary if it absorbs at the wavelength used) and the suspension liquid. For $d < 25$ nm, $M = 1$. This defines a Rayleigh particle, one with a diameter much smaller than the wavelength of light. It scatters equally in all directions. M decreases smoothly with size until the size approaches the wavelength of light. Then oscillations can occur.

With d and $G_V(d)$, the mean, relative variance, skew, and volume-weighted cumulative undersize distribution are easily calculated. Similarly, surface-area weighted size distribution information is obtained by noting that $G_{SA}(d) = G_V(d)/d$. Finally, to obtain number-weighted distribution information, $G_N(d)$ is calculated from $G_V(d)/d^3$.

While these definitions are straight forward and the calculations of $G_V(d)$, $G_S(d)$, and $G_N(d)$ are trivial, one should not assume the final results are accurate or meaningful unless they are repeatable and even then are in agreement with other information about the size distribution. Since all the calculations depend on the accuracy of G_I , look for repeatability in the intensity-weighted distribution before proceeding. Artifacts at low values of d in $G(d)$ due, typically, to noise in the accumulated function, dominate the other differential distributions because of the division of d raised to higher and higher powers.