Instruction Manual for BI-DNDC

Differential Refractometer:
GPC Mode
Measuring Concentration or dn/dc

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Please Read

This is your instruction manual for your Brookhaven BI-DNDC Differential Refractometer, GPC Mode. Please read it carefully before making measurements. If you have any questions or suggestions, please contact Brookhaven Instruments.

Manuals are 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.

Table of Contents

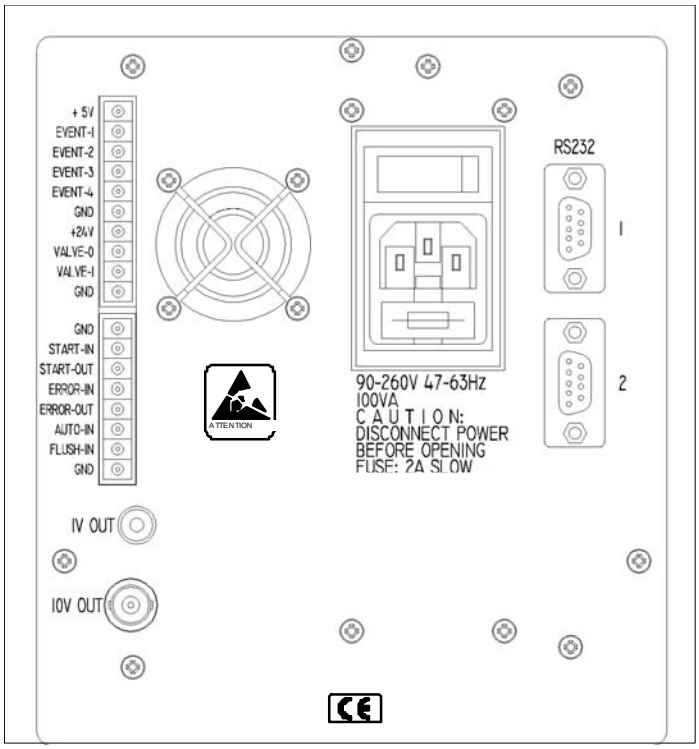
Section I: Connecting the BI-DNDC to a PC

Important Warning: DO NOT PRESS THE AUTOZERO BUTTON

If the Autozero button on the front panel is pressed at the wrong time, the zero glass could move far away from the correct position. In this case, it will be necessary to follow the instructions in Appendix D: Zero Glass Recovery. Autozero should only be pressed when not in flush mode and when both cells are equilibrated with the same solvent.

Analog Output

Analog signals are output via the two connectors on the back. The BNC connector labeled 10V OUT provides a voltage signal with a range from - 10 volts to + 10 volts. The voltage corresponds to that displayed on the front panel of the BI-DNDC. The RCA connector labeled 1V OUT provides a voltage signal with a range from - 1 volt to + 1 volt. The output voltage corresponds to 1/10 that displayed on the front panel of the BI-DNDC. For GPC-LS with the **BI-MwA** Molecular Weight Analyzer, connect the provided RCA cable to the 1V OUT connector. The flying leads on the other end should be connected to the **BI-MwA** Analog input #4. The black lead (ground) should be connected to the terminal labeled Ch04-. The red lead should be connected to the terminal labeled Ch04+.



Digital Communication

Typically, digital communication is only needed for reflashing the BI-DNDC firmware.

Use the provided cable to connect the top RS232C COM port, the one labeled “1” on the rear of the BI-DNDC, to the COM1 port on the PC. Then, turn on the BI-DNDC.

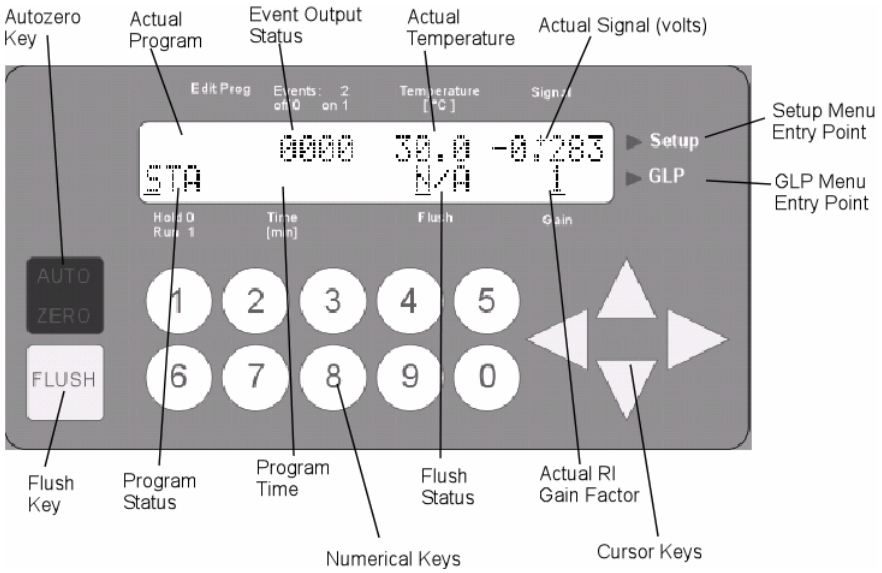
The standard communications protocol is the following: 19,200 Baud, 8 data bits, 1 stop bit, no parity, no handshake, and no X-on/X-off protocol.

If there are still no communications, locate the four cursor keys on the BI-DNDC membrane panel. Press the up arrow if the blinking cursor is not already on the top of the two-line display. Press the right arrow until the flashing cursor is over the voltage value. Press the right arrow one more time to enter the Setup Mode. Press the down arrow six times. You should see the following:

Control COM1: WinGPC

Baud Rate: 19200

If you see “WGE-EASY” instead of “WinGPC”, press the right arrow until the cursor is over the “W”. Press the up or down arrow until “WGE-EASY” appears. Press the right arrow until the cursor is over the first digit in the baud rate. Press the up or down arrow, cycling through the various baud rates, until 19,200 appears. Press the right arrow and the cursor is over the symbol “◆” in the lower left hand of the display. Press the left arrow to return to monitor mode.



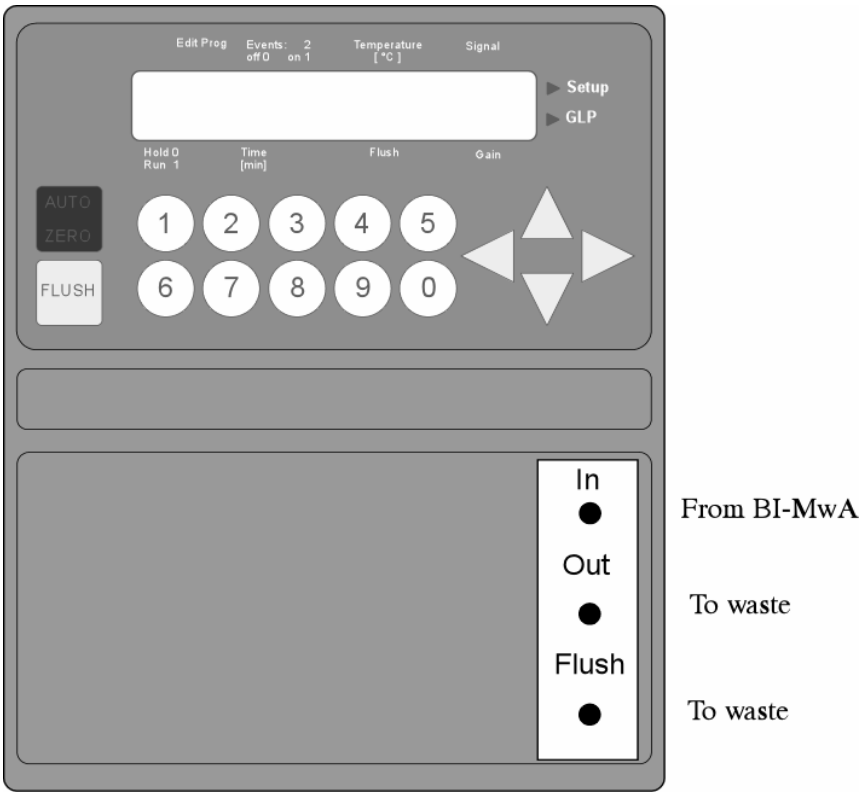
Section II: Plumbing Connections, Warm-up, Stability, and Other Tips of the Trade including IMPORTANT WARNINGS

Important Warning #1: NEVER ALLOW LIQUID TO DRY INSIDE THE INSTRUMENT

Never allow any liquid to dry inside the instrument. Always flush the solute or polymer with solvent. That is, do not allow polymer to stay in the cell (e.g., by stopping flow) for an extended period. If you are not going to work with the instrument for more than a month, flush both sides with compatible solvents (see end of this section) until you can replace the final liquid with isopropyl alcohol (IPA) or some other alcohol in which bacteria will not thrive. Then block off the inlet and the two outlets with the plugs that were included when we shipped the instrument to you. If you plan to work with the instrument over a several day period, flush the solute side with solvent by enabling the flush (see Flushing on page II—3) so that 20 mL of solvent flows through the instrument for each column. For example, if the flow rate is 1 mL/min and there are two columns, the instrument should be flushed for 40 minutes.

Plumbing Connections

A block diagram of the instrument face is shown below for reference.



The inlet is marked In. The internal, stainless steel tubing that connects the inlet to the sample cell has an inside diameter, I.D., of 0.25 mm (0.010", or 10 mil). Connect the inlet to the eluent from either the column or the BI-MwA. **Make sure that the DNDC unit is the last item in the GPC flow system before waste so that it experiences the lowest back pressure.** This is because the cell is fragile.

PEEK (polyetheretherketone) tubing is resistant to most but not all organic solvents. DO NOT USE any of the following acids with this tubing: HNO_3, H_2SO_4, HF, HB, and HI. HCl is normally okay to use. At room temperature, DMSO, methylene chloride, and THF are normally okay. However, due to possible swelling, avoid the use of PEEK tubing at higher temperature and pressure with these solvents. The maximum recommended operating temperature for this PEEK tubing is 100 °C. Since the BI-DNDC's maximum temperature is 80 °C, there is no problem.

Alternatively, use stainless steel tubing. To connect to the inlet, use Upchurch, Stainless Steel, Male Nuts, U-400, 1/16" O.D., 10-32 threads and U-401 Ferrules compatible with 1/16" tubing. When swaging the fitting for the first time, push the needle tip all the way into the inlet fitting, and tighten the nut by hand, pushing the ferrule as far as it will go. Use a small, ¼" wrench and make a ½ turn for the final swaging process. Do not over tighten. These stainless steel tubes are compatible with almost all solvents. These tubes should be swaged in place (SIP). Do not use pre-swaged connectors as they my not mate with the BI-DNDC properly. Bend the tubing about three or four centimeters from the tip by wrapping it around a 25 mm diameter cylinder. A gentle bend is best. Avoid a sharp bend.

Connect the Out and Flush ports to the waste. Tape or otherwise fix the Teflon tubing to the side of the instrument such that the end of the tubing sits inside a waste bottle. Place the tubing tip below the level of the waste so that no drips can be observed. Drips can show up in BI-DNDC data.

Connect the tubes to the Inlets/Outlet using an Upchurch, F-120, 10-32, Fingertight Fitting made of PEEK. When making connections, push the tubing 3/16" (~5 mm) past the end of the fitting. Let that protrusion bottom out on the mating part. While pushing firmly on the tubing, screw in the fitting. In this way, the dead-volume is minimized and the connection is leak-tight.

PEEK fingertight fittings and tubing are available from Upchurch Scientific Inc. Contact them at +1-800-426-0191, +1-360-679-2528, or http://www.upchurch.com .

Warm-up Time

After unpacking, let the instrument sit at room temperature for at least a day.

The temperature control works best from ~5 °C above room temperature (i.e., ~30 °C) to 80 °C.

Important Warning #2: DO NOT PRESS AUTOZERO

Pressing autozero on the front panel when both sides of the split cell are not filled with the same liquid will result in significant inconvenience.

For measurements made at 30-40 °C wait at least 1 hour after turning on the instrument. Increase the warm-up time for measurements made at higher temperature. It may take several hours, even overnight, to stabilize measurements made between 60 and 80 °C.

While the instrument is warming up you may perform some simple flow checks after installing the fittings, tubing, and syringes.

Initial Setup

We ship the instrument with 100% IPA (isopropyl alcohol) in the cell and all tubing. When you remove the acetal plugs it is normal to see a few drops of clear liquid and to smell the alcohol. **Do not discard the plugs**, as they will be useful. Simply flush the BI-DNDC with the DI water that you will use for GPC or THF if you are going to use an organic solvent for GPC. Flow 5 mL of water or THF into and out of the instrument. Press the flush button to route some flow through the reference cell. Repeat several times.

DO NOT EXCEED a flow rate of ~ 3 mL/min.

Look for any liquid leaking around the various fittings. Reposition and tighten if necessary. REMEMBER: When connecting PEEK tubing, poke the tubing 3/16" (~ 5 mm) beyond the screw-in fitting, and, while pushing the end of the tube against the mating part, screw in the fitting.

Flushing

Once the DNDC is connected, both sides must be flushed with eluent. In order to flush a short period, simply press the flush button on the front panel. Part of the flow entering the sample cell will be rerouted and pass through the reference cell before exiting the system at the waste port marked flush. This operation may need to be repeated a few times before a stable baseline is achieved. In order to flush a long period, use the arrow keys to move the cursor to just above the small flush label and press the number 5 button. Flush the instrument with 20 mL of eluent for each column during installation and whenever columns or eluents are changed. For example, if the flow rate is 1 mL/min and there is one column, flush for 20 minutes. Be careful to avoid flowing immiscible solvents. See the section on working with solvents for more details. Press the number 5 button a second time to stop flushing.

Stability Checks

When the system is completely stable and the same liquid is in each side of the cell, the voltage should be constant with a minimal drift. With even the slightest

differences in concentration, temperature, or pressure between the two sides of the cell, you will see a change in voltage on the front panel of the BI-DNDC.

When the system is completely stable the average temperature will be stable to ± 0.010 °C **at the cell**.

However, because the cell is so small and buried deep inside a large, thermal reservoir, it is difficult to monitor the temperature directly at the cell. So the temperature is monitored near the heating element and appropriate time constants are used to determine when to switch the heater on and off.

The oscillations of nearly 0.15 °C are normal at the heater. Deep within the thermal reservoir at the cell, the variations should be no more than ± 0.010 °C. If the variations were more than that, the voltage stability graph would show a drift. It is the more sensitive of the two methods for checking stability.

This temperature oscillation is also apparent on the BI-DNDC's front panel LCD and is normal.

Working with Solvents (and going from one solvent to another)

Methanol, ethanol, isopropanol, THF, and acetone are intermediate solvents that are miscible with water and with less polar solvents like ethyl acetate, chloroform, methylene chloride, toluene, and benzene. Isopropanol and acetone are also miscible with even more nonpolar solvents like heptane, hexane, and other alkanes. When changing from water to a nonpolar solvent, first flush the salt or polymer solution side with water until the signal is stable. Next, if the current solvent is not a pure solvent (that is, if the solvent is really a salt solution), flush both cells with a pure solvent such as pure water (see Flushing on page II—3). Then flush *both cells* very slowly with an intermediate solvent like isopropanol or acetone if needed. Check for signal stability again. Keep flushing until the signal is stable. Now flush both cells with the final solvent until the signal is stable. The reverse transition from nonpolar solvent to water also requires an intermediate solvent.

If the flow path is plugged or material is dried within the instrument

If liquid (with or without solute or polymer) dries inside the instrument, the signal stability will suffer. The signal will drift continuously in one direction. You need to slowly flush the system with a solvent that will dissolve whatever has dried onto the walls of the tubing and cell. This may take an entire day or two. Pump solvent at a very slow speed.

If solvent will not dislodge the dried particles, consider using 0.1 molar nitric acid to chemically attack the particles. Wear gloves and safety goggles. You may have to repeat this several times to get even a small flow to the outlet. Given the partial restriction that still exists, pressure can increase rapidly and cause the cell to fail if too high a flow rate is used.

If bacteria in aqueous solutions sit in the instrument, the bacteria may adsorb onto the walls of the system and act like dried debris. This will also require slow flushing with alcohol or nitric acid solution and then water to remove it.

You can determine when the instrument is stable by monitoring the signal with solvent in both cells.

Section III: Making a Measurement

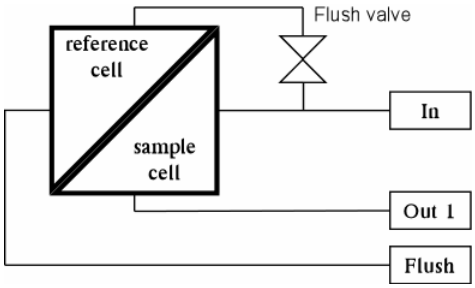
Measurement

Once the instrument is flushed, set the gain to 8 or 16. Use the arrow keys to move the cursor to above the gain label. Press “5” and the gain will double until the maximum is reached, at which time the gain will return to 1. Press “0” and the gain will halve until 1 is reached, at which time the gain will go to the maximum. Once a gain of 8 or 16 is selected, press the up arrow key to move the cursor. If you will work with low molecular weights or high concentrations, you may saturate the detector at gain 8 or 16, in which case, reduce the gain to eliminate saturation. Saturation of the detector will result in peaks with flat tops in the GPC elugram.

If the reading is greater than +1 volt, press the Autozero button to zero the instrument. The instrument will automatically adjust the zero glass to null the optics.

Section IV: Theory: Instrument

A simple flow diagram for the instrument looks like this:

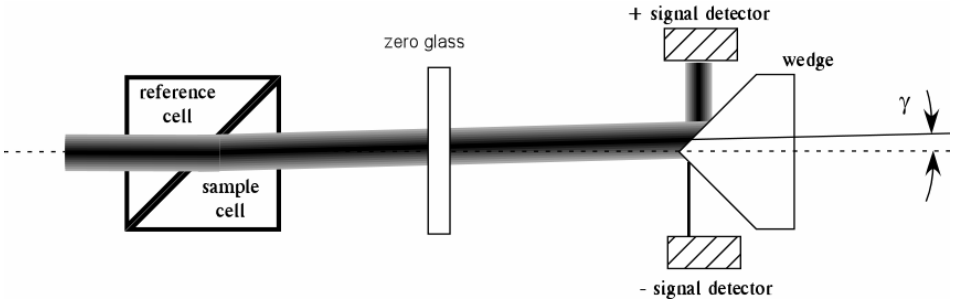


The optical configuration is shown below.

The incoming beam is defected slightly due to the refractive index difference between the reference and sample cells. The magnitude of the deflection angle, γ is obtained from the difference in signal between the two detectors shown, which is measured as a voltage. This voltage and angle is proportional to the relative amount of the beam entering each detector. Since the deflection angle is proportional to the refractive index, one can write

$\Delta V \sim \gamma \sim \Delta n$

$$\Delta n = k \cdot \Delta V_g$$



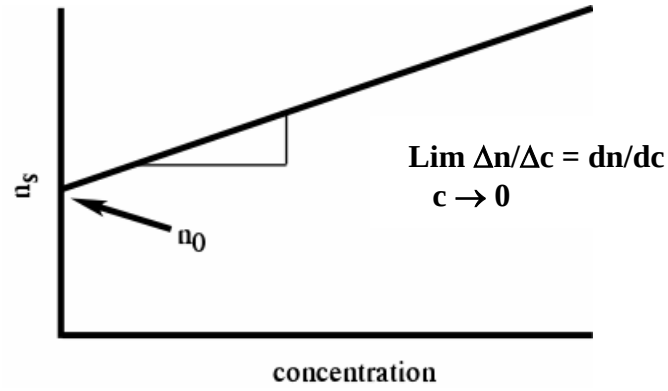
where ΔV is the voltage difference and Δn is the refractive index difference. At low concentrations, Δn is proportional to the concentration difference (through the refractive index increment, dn/dc). Thus, one can write $\Delta V = k\Delta c$ where Δc is the difference in concentration between the reference and sample cells. If the reference cell is filled with pure eluent (as is frequently the case), the concentration difference is the concentration in the sample cell. Thus, one can obtain the concentration of sample.

In addition, there is a plate of glass between the cell and the detector that can be used to change the beam direction slightly and optically zero the beam. This is the zero glass and it is manipulated when the autozero button is pressed.

Section V: Theory: dn/dc

Definition of dn/dc

The dn/dc is also sometimes referred to as the specific refractive index increment, or SRII. It is the change in the solution refractive index, n_s, with change in concentration in the limit of zero concentration. The solute may be a simple salt, as in calibration measurements, or it may be a polymer. In most polymer/solvent samples n_s vs. concentration is a straight line and the limiting slope is the same as the slope at finite concentrations, even up to 10's of mg/mL. Small salt molecules do not necessarily have a linear dependence at such high concentrations. The figure below summarizes this concept.



In practice, the variation in n_s with temperature is larger than its variation with concentration, and the above plot is nearly impossible to obtain with precision unless extreme measures are taken for temperature stability. This point is worth making quantitatively and is discussed in the section entitled **Temperature Dependence.** To avoid this problem, one must use a differential refractometer (or interferometer with dual or split cell design).

Differential Refractometer

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

Within an error less than approximately 0.1%, the signal in a differential refractometer is proportional to the displacement of a beam of light as it traverses the solution and solvents sides of the cell. The displacement is proportional to the difference in refractive index of the solution and solvent-sides of the cell.

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

Uses of dn/dc and Establishing Error Limits

The value of dn/dc can be used in two ways: either to calculate concentration (RI detector in GPC/SEC or HPLC) or as a parameter in calculating molecular weight of dilute polymers in solution. The instrument discussed in this manual is optimized for use as a concentration detector.

Static light scattering measurements on dilute polymer solutions yield the weight-average molecular weight (molar mass), $< M_w >$, the z-average radius of gyration (rms radius), $< R_g >_z$, and the second virial coefficient, A_2. [Note: Unless otherwise specified, M_w and R_g are also used to represent the average values without the brackets]. Of these quantities, only M_w and A_2 determinations depend on the value of dn/dc; **R_g determination does not depend on dn/dc.**

For example, a Zimm plot is used to determine these three quantities by making measurements of the intensity of light scattered by dilute polymer solutions as a function of scattering angle, θ, and polymer concentration, c. Subtracting the scattering from solvent without polymer forms the excess scattered intensity. Multiplying by a calibration constant determined from a sample with a known Rayleigh ratio determines the excess Rayleigh ratio, $\Delta R(\theta,c)$. From these measurements, the parameters that characterize dilute polymers in solution are determined.

For homopolymers in a single solvent, the most common form of the Zimm equation is as follows:

$$\frac{Kc}{\Delta R(\theta,c)} = \left(\frac{1}{M_w}\right)\cdot\left[1 + \frac{R_g^2\cdot q^2}{3}\right] + 2A_2c$$

Here q is the magnitude of the scattering wave vector and is given by

$$q = \left(\frac{4\pi n_o}{\lambda_o}\right)\cdot\sin\left(\frac{\theta}{2}\right)$$

For a vertically polarized laser of wavelength λ_o in vacuo, a solvent with refractive index n_o, where N_{avo} stands for Avogadro's number, the constant K, also called the Debye constant, is given by:

$$K = \frac{4\pi^2 n_o^2 (dn/dc)^2}{N_{avo}\cdot\lambda_o^4}$$

In a static measurement, the polymer concentration is determined gravimetrically (weigh the dry polymer) and volumetrically. The relative errors in M_w and A_2 due to an error in dn/dc are twice the relative error in dn/dc, because dn/dc is squared. The sign of the error is opposite: a positive error in dn/dc results in a negative error in M_w and vice versa.

It is now possible to establish acceptable error limits. In static mode, if you want to know M_w to 5%, then the error contributed by dn/dc must be less than 2.5%.

Literature Values of dn/dc

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

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.

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.

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

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

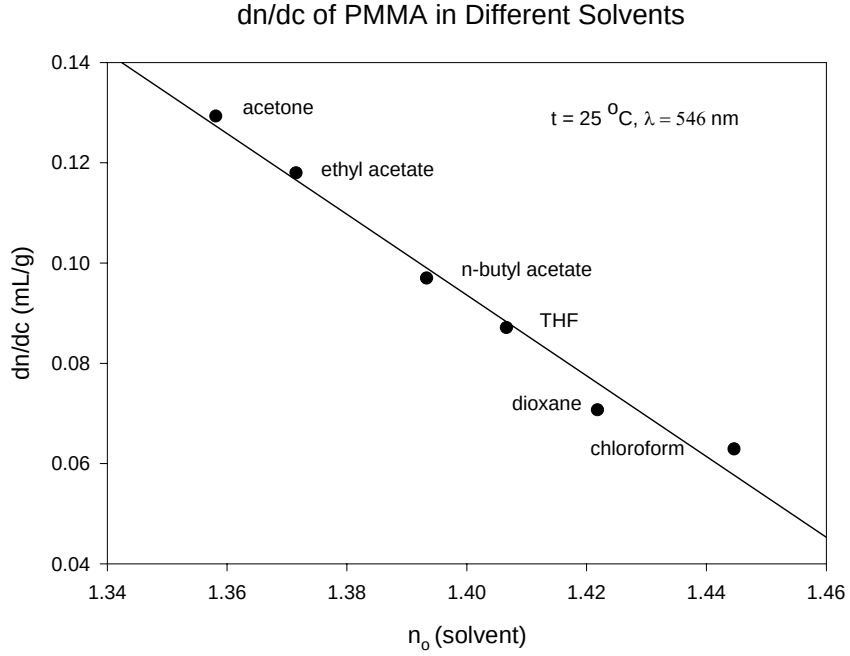
The classic values (before the widespread use of lasers, say 1970) are given primarily at 436 nm and 546 nm, the mercury lines. Hg-arc sources were the light sources used in differential refractometers and light scattering instruments before lasers and LEDs became widely available. As shown below (**Wavelength Dependence)**, fitting to $A + B/\lambda^2$ using values known at two wavelengths is reasonable for calculating at laser wavelengths of 488 nm and 514.5 nm (Argon-ion laser lines) or 532 nm (newer, frequency-doubled, solid state lasers). Using the fit to predict dn/dc at 632.8 nm (HeNe laser line) or 670 nm (“red”, diode lasers) is less acceptable, but may be necessary.

Without a direct measurement, literature values may be all that you have to work with. However, it is important to understand that dn/dc varies with the particular polymer/solvent solution, the wavelength, the temperature, and the molecular weight as well as the skill with which samples are prepared –both salt solutions for calibration and polymer/solvent solutions for measurement. Each of these will now be discussed.

Effect of Solvent’s Refractive Index

If a particular polymer can be dissolved in several different solvents, it is best to choose the one with the greatest difference in refractive index. In this case, dn/dc will be

a maximum, and errors in determining dn/dc will become less important. While the shape of the molecule (affecting R_g) and its interaction with the solvent (affecting A_2) may change from solvent-to-solvent, M_w will not. The variation of dn/dc with solvent refractive index is roughly linear. Here is an example.



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

Effect of Impurities in Solvent and Polymer

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

Another manifestation of this same problem may occur if the solutions and solvent have not been kept under the same conditions for both the dn/dc and light scattering measurements. Ideally, the exact same solutions should be used for both measurements, and the solvent used to prepare the solutions should be kept under the

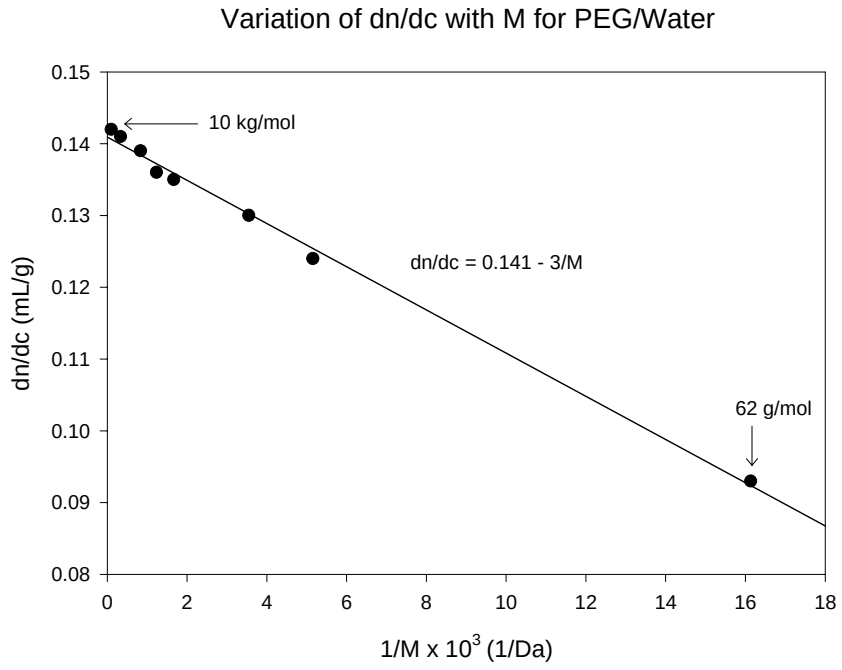
same conditions (sealed, no additional moisture or other impurities allowed in contact). Otherwise, the subtraction involved in determining the excess scattered intensity and that involved in determining Δn may be different.

It is not uncommon to prepare solutions for dn/dc, seal them, and work with solvent from a new bottle, not the one that was half empty, absorbing a bit of moisture, and used to prepare the solutions. If the solvent is strongly hygroscopic, a fresh bottle will have much less moisture than the original bottle and dn/dc will be affected.

Molecular Weight Dependence of dn/dc

Generally, dn/dc increases with molecular weight and reaches a plateau when the end groups are sufficiently few in number compared with midchain repeat units. 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 solvent, polymer shape and end-group contributions to the refractive index. Above this range it is, typically, constant.

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

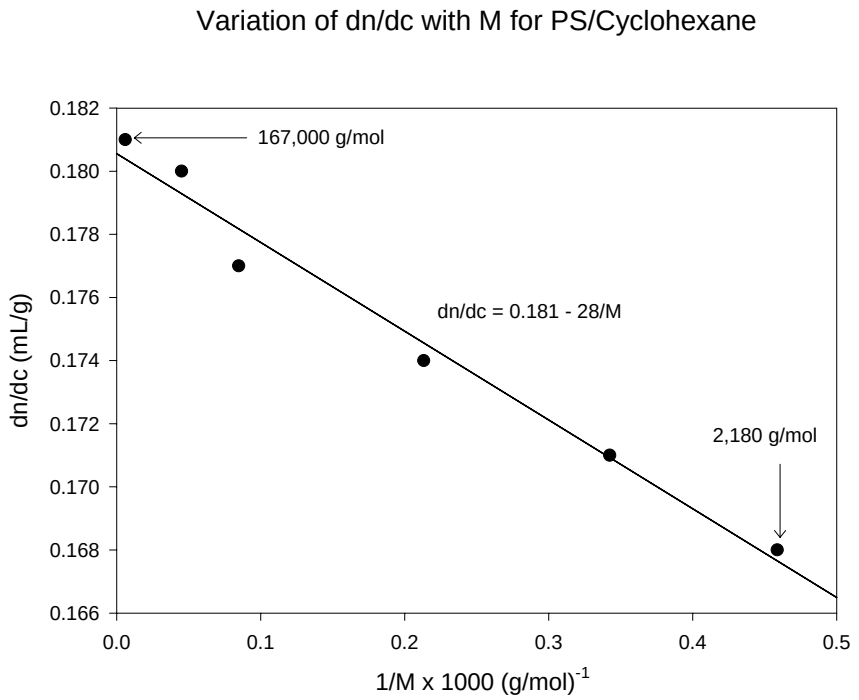


Generally, data like this fits reasonably well to $dn/dc = \alpha - \beta/M$, so when M is large enough, dn/dc doesn't change. Given the fit above, the change in dn/dc is less than 2% when M > 1,064 g/mol. This is an unusual case and may be due to the fact that both solvent and polymer have hydroxyl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

Polystyrene in cyclohexane is a more common case as shown in the next graph. Here the rate of change with M is 9 times as large and the change in dn/dc is less than 2%

when M > 7,750 g/mol. Both sets of data are tabulated in Chapter 6 of Huglin's 1972 book and in the original literature, the references for which are found in Huglin's book.

It is particularly important to realize that each set of data plotted here was measured by the same authors (Kratohvil, J.P. 1968 for PS/cyclohexane and Rempp 1957 for PEG/H_2O). So it is safe to assume that the samples and corresponding solvents in



each run were prepared under similar conditions for the different molecular weights.

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

Temperature Dependence of dn/dc

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

More quantitatively, let ν = dn/dc. Since, typically, | dν/dT | ranges from 1x 10^{-4} $mL \cdot g^{-1} \cdot K^{-1}$ to 5 x 10^{-4} $mL \cdot g^{-1} \cdot K^{-1}$, it is possible to estimate what temperature difference, ΔT is acceptable for a given level of error in M_w. The relationship is given by:

$$\frac{\Delta M_w}{M_w} = -2 \cdot \frac{\Delta \upsilon}{\upsilon} = -2 \cdot \frac{d\upsilon / dT}{\upsilon} \cdot \Delta T$$

For example, given dn/dc = ν = 0.14, and dν/dT = 3 x 10^{-4} $mL \cdot g^{-1} \cdot K^{-1}$, if the error from a difference in temperature between the light scattering and a static dn/dc measurement should be less than 5%, the ΔT should be less than 12 °C. It is for this reason that many people ignore the fact that the differential refractometer may be operating at 35 °C and the light scattering device may be operating at 25 °C.

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 2%. The difference in solution and solvent refractive index, Δn, at c = 1 mg/mL is therefore 1 x 10^{-4}. If you want to measure that difference to within 2%, you would need to measure Δn with a resolution of ± 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?

First, it means that the novice who uses even a very good Abbé refractometer that can measure refractive indices to 1 part in 10^6 will fail because the different polymer solutions as well as the solvent will not all be measured within 0.01 °C of each other. Second, it means that a differential refractometer is required (or an interferometer).

Wavelength Dependence of dn/dc

Experimental observations show that dn/dc decreases with increasing wavelength, with variations of 1 to 3% over the range of 436 nm to 546 nm. These two wavelengths, from a Hg-arc source, represent the two most common ones used in SLS measurements prior to the use of lasers. Since they span the 488 nm and 514.5 nm lines of an Argon-ion laser, and the 532 nm line of a frequency-doubled, solid state laser, one could interpolate (not linear interpolation, see equation below) values of dn/dc from those measured at the classical values. More uncertainty would accrue to values extrapolated to 632.8 nm, the wavelength of a HeNe laser, or to values extrapolated to ~670 nm, a common wavelength for a "red" diode laser. (Again, the extrapolation is not linear. See below.)

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

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

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

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

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

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

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

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

Suppose you measure dn/dc using the 620 nm BI-DNDC and find a value of 0.1500 mL/g for a random-coil polymer in an organic solvent and you want to estimate dn/dc at 632.8 nm, the wavelength of the laser you use in the light scattering experiment. Assuming B = + 0.0035, calculate $A = 0.1500 - 0.0035/0.62^2$. The result is A = 0.1409. Now calculate dn/dc at 632.8 nm. The result is $dn/dc = 0.1409 + 0.0035/0.6328^2 = 0.1496$. The difference in these two values of dn/dc is 0.3%. It is negligible. Even using the largest B value in the table yields a difference of 0.6%, also negligible. This case demonstrates why using the dn/dc values measured close to the laser wavelength is sufficient for most purposes.

Suppose you find a literature value of 0.1500 mL/g measured at 436 nm and you want to estimate the value at 632.8 nm. Aside from the fact that the literature value may correspond to a polymer/solvent system with different impurities or molecular weights than yours, this large wavelength difference cannot be ignored. In this case $A = 0.1500 - 0.0035/0.436^2 = 0.1316$, and the estimated value at 632.8 nm is $dn/dc = 0.1316 + 0.0035/0.6328^2 = 0.1403$ mL/g. Now the difference between the two dn/dc values is 6.5%. This could lead to a 13% error in M_w in the static mode, something that may not be acceptable.

Last, consider the case of the Brookhaven **BI-MwA**, a multiangle, static light scattering device that can be used for both static measurements of M_w as well as a GPC/SEC detector (dynamic mode). The laser used in this machine is a "red" diode laser and has, typically, a wavelength of 635 nm; whereas, the closest match is the 620 nm BI-DNDC. In this case the difference in dn/dc values is $B\cdot (1/0.62^2 - 1/0.66^2) = 0.306 \times B$. With B = 0.0035, the difference is 0.001 mL/g. Depending on the absolute magnitude of dn/dc, this is either negligible or an easy, first-order correction to make.

It is up to the user to decide if making a small wavelength correction is useful. If it is smaller than the precision of multiple dn/dc measurements, it is probably not worth the effort.

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

Appendix A: Effect of Source's Wavelength Distribution

The narrow-band, LED sources in the BI-DNDC are characterized by the modal value, λ_o, of the distribution of wavelengths, $S(\lambda)$. The current supply of sources allows a selection of modal values at either 470 nm, 535 nm, or 620 nm. However, since the source is not monochromatic, the following question may be asked: What is the average wavelength at which dn/dc is actually being measured? In other words, does the finite spread in wavelengths of the source matter?

To answer that question one must also consider the detector response $D(\lambda)$. A photodiode with a glass or quartz window has a linear wavelength response over the visible and may be represented by the following equation:

$$D(\lambda) = a + b\lambda$$

For the diodes used here, with λ in microns, a = -0.283 and b = 1.425 when the maximum relative response is defined as 1.000 and occurs at approximately 0.9 μm.

As shown in Section VII on the theory of dn/dc, its wavelength dependence is adequately described by:

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

Now averaging this over the wavelength dependence of the source and detector yields the following equation:

$$\langle dn/dc \rangle = \frac{\int (dn/dc) \cdot S(\lambda) \cdot D(\lambda)\, d\lambda}{\int S(\lambda) \cdot D(\lambda)\, d\lambda}$$

Substituting for dn/dc and $D(\lambda)$, the intermediate result is:

$$\langle dn/dc \rangle = A + \frac{B}{a + b\lambda_o} \cdot \left[a \cdot \langle 1/\lambda^2 \rangle + b \cdot \langle 1/\lambda \rangle \right]$$

where λ_o is the modal wavelength of the source, the wavelength at which the instruments are specified, and where the brackets represent an average over the wavelength dependence. In particular, the two averages on the right represent averages over $S(\lambda)$ as follows:

$$\langle 1/\lambda^n \rangle = \int \lambda^n \cdot S(\lambda)\, d(\lambda) \quad \text{where} \quad \int S(\lambda)\, d(\lambda) \equiv 1$$

Here n = -1 and -2, and the second integral represents the normalization condition.

To appreciate the meaning of this seemingly difficult expression for <dn/dc>, imagine that the source is monochromatic. In this case $<1/\lambda^2>$ and $<1/\lambda>$ would equal $(1/\lambda_o)^2$ and $1/\lambda_o$, respectively. In this simplifying case,

$$\langle dn/dc \rangle = A + \frac{B}{\lambda_o^2}$$

As expected for a monochromatic source, the wavelength-average value, <dn/dc> is the same as it is at its specified wavelength. Of course even for completely symmetric but finite distributions, $<1/\lambda^2> \neq (1/\lambda_o)^2$ and $<1/\lambda> \neq 1/\lambda_o$, though for very narrow distributions equality is approached.

Since, as shown below S(λ), is narrow, it is worthwhile writing the desired result as:

$$\langle dn/dc \rangle = A + \frac{B}{\lambda_m^2}$$

Then the relationship between λ_m and λ_o is

$$1/\lambda_m^2 = \frac{1}{a + b\lambda_o} \cdot \left[a \cdot \langle 1/\lambda^2 \rangle + b \cdot \langle 1/\lambda \rangle \right]$$

No further progress can be made without knowing something about the source distributions S(λ). These were measured using a spectrum analyzer. Each distribution is bell-shaped, though neither Gaussian nor symmetric, with a slight asymmetry towards higher wavelengths. For example, the 535 nm modal source has a half-width at half height of 20 nm towards the lower wavelengths and 24 nm towards the higher wavelengths as measured from the modal value.

Numerical calculations were performed with the following results: $\lambda_m = \lambda_o + 1$nm. Therefore, given the other experimental errors in determining dn/dc, the error in assuming the specified or modal wavelength of the source equals the average wavelength for the measurement of dn/dc is negligible.

Appendix B: Gladstone-Dale Equation and Predicting dn/dc

There have been many attempts to calculate dn/dc from other solution properties. Three are listed on Page 184 of Huglin's book (reference 1 in Section VII). One of these, the Gladstone-Dale (G-D) equation and its often-used, approximate form are given below.

$$\frac{dn}{dc} \equiv \upsilon = \vartheta_p(n_p - 1) - \overline{\vartheta_p}(n_o - 1)$$

$$\upsilon = \overline{\vartheta_p}(n_p - n_o)$$

Here n_p is the refractive index of the polymer in solution, n_o is the refractive index of the solvent, ϑ_p is the specific volume of the polymer (reciprocal of density, $1/\rho_p$), and $\overline{\vartheta_p}$ is the partial specific volume of the polymer in the solvent. This latter quantity represents the change in an infinitely large volume of solution when 1 g of solute is added. Partial specific volumes are described in most thermodynamic textbooks with chapters on solutions. The G-D equation is based on an empirical observation that for molecules of very different size but similar chemical composition, thermodynamic properties of solutions may be obtained as a weighted sum over individual properties, the weighting factors being the volume fractions instead of the more familiar mole fractions.

Now the approximate form of the G-D equation assumes additivity of volumes, and this implies ideal solution behavior. Neither electrolyte nor polymer solutions are ideal. Therefore, the approximate form is not exact. However, since dn/dc is required as a limiting quantity at zero concentration, the approximation is somewhat better than if it were required to be true at finite concentrations.

The approximate form, while normally not suitable for calculating dn/dc, may be used to justify several of the experimentally observed variations of dn/dc with solvent refractive index, wavelength, and temperature.

For example, it is clear why choosing a solvent with the largest difference in refractive index compared to the polymer results in a greater dn/dc. Also, if $n_p < n_o$, then dn/dc is negative. Since this is the exception rather than the rule, it is clear why most dn/dc values are positive. The approximate form also suggests that when $n_p = n_o$, **dn/dc = 0**. In fact, there are some polymer/solvent solutions where dn/dc is so close to zero, light scattering measurements are useless, or nearly so.

According to both the full and approximate forms, a plot of **dn/dc vs n_o** should be linear with a negative slope equal to the partial specific volume. In Section VII of this manual, a plot of **dn/dc vs n_o** for poly(methylmethacrylate) [PMMA] in solvents ranging from acetone to chloroform was indeed linear with a negative slope. This suggests that the G-D equation is reasonable and that the partial specific volume is equal in all these solvents. Yet, the partial specific volume is a measure of the size of the polymer in the solvent. Since different solvents will allow a particular polymer to expand or contract

differently, it is a little surprising that a straight line occurred. This is only one example, and should not be taken as generally representative.

Continuing with the PMMA/Solvent plot in Section V, the slope of the line is - 0.806 mL/g. Therefore, the partial specific volume is + 0.806 mL/g. If the approximate form of the Gladstone-Dale equation is correct, then the specific volume of PMMA is also + 0.806 mL/g. Finally, if all this were true, then the density of PMMA in solution is 1/0.806 = 1.241 g/mL. The density of bulk PMMA is 1.188 g/mL at 25 °C, about 4.5% lower.

The agreement is reasonable in this particular case. However, agreement is not always this good. For example, the density of bulk polystyrene is 1.051 g/cm^3. Therefore, the specific volume is 0.951 mL/g. As above, if we assume this is equal to the partial specific volume, we can calculate dn/dc = 0.951·(1.590 – 1.490) = 0.0951 mL/g (at 632.8 nm). The literature values center around 0.106 mL/g at this wavelength. The error in dn/dc using these approximations is low by 11%. An M_w calculated in the static mode would be high by 22%, clearly an unacceptable result. Generally, one cannot use the approximate form of the G-D equation to predict quantitatively dn/dc values.

According to the G-D equations, the temperature variation of dn/dc is given by:

$$\frac{d\upsilon}{dt} = \frac{d\vartheta_p}{dt}\left(n_p - 1\right) + \vartheta_p \frac{dn_p}{dt} - \frac{d\overline{\vartheta_p}}{dt}\left(n_o - 1\right) - \overline{\vartheta_p}\frac{dn_o}{dt}$$

$$\frac{d\upsilon}{dt} = \overline{\vartheta_p}\left(\frac{dn_p}{dt} - \frac{dn_o}{dt}\right) + \frac{d\overline{\vartheta_p}}{dt}\left(n_p - n_o\right)$$

where the second equation is for the approximate form of the G-D equation. It is apparent that the temperature coefficient of dn/dc can be positive, negative or zero depending on the relative values of the other temperature coefficients. This is in accord with the experimental observations referenced in Section VII.

Finally, one can use the G-D equation to predict the form of the wavelength dependence of dn/dc. The refractive index of the polymer and the solvent will follow Cauchy's equation as long as there is no absorption in the wavelength range of interest,

$$n = A + \frac{B}{\lambda^2}$$

Since specific and partial specific volumes have no wavelength dependence, the G-D equation can be written as:

$$\frac{dn}{dc} = \vartheta_p\left(A_p + \frac{B_P}{\lambda^2} - 1\right) - \overline{\vartheta_p}\left(A_o + \frac{B_o}{\lambda^2} - 1\right)$$

$$\frac{dn}{dc} = \left[\vartheta_p\left(A_p - 1\right) - \overline{\vartheta_p}\left(A_o - 1\right)\right] + \frac{\vartheta_p B_p - \overline{\vartheta_p} B_o}{\lambda^2}$$

Thus, like the individual refractive indices, dn/dc can also be written as $A + B/\lambda^2$.

Appendix C: Tubing Coding

1/16’’ OD tubing color coding

Upchurch and Alltech (striped or not) have the same PEEK color coding.

Stainless Steel (SS) color coding differs from manufacturers.

Valco doesn’t have color coding on PEEK or SS

ID (in)	PEEK	SS Upchurch	SS Alltech
.005	Red	Red	Red
.007	Yellow	Black	Black
.010*	Blue	Blue	Blue
.020	Orange	Yellow	Green
.030	Green	White	N/A
.040	Natural	No color	N/A

*GPC/SEC’s preferred size. N/A the company doesn’t offer this size ID.

Appendix D: Zero Glass Recovery

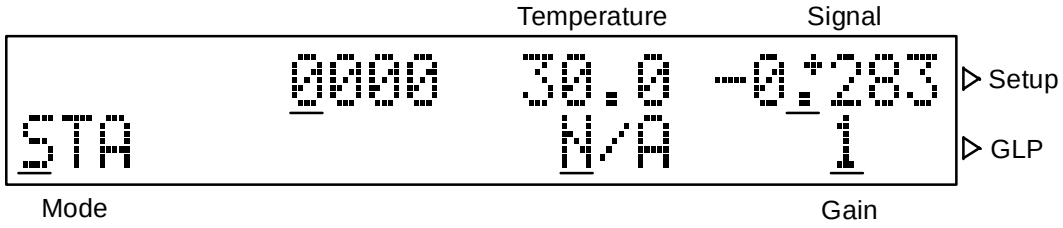


Figure D-1 : BI-DNDC front panel display.

IMPORTANT NOTICE

Before attempting a zero glass recovery, try to determine if the problem is with the zero glass. These next few steps walk the user through the setting of the BI-DNDC and the test required to check if the zero glass is at fault.

BI-DNDC Setup

With the unit switched off, unplug the RS-232 cable (signal cable) from the rear of the BI-DNDC. Then switch on the instrument. From now on, work only with the front panel of the BI-DNDC for the test of the zero glass and the zero glass recovery. Get familiar with the display on the front panel. It should look like figure D-1 above. In figure D-1, the cursor stops are indicated by the 5 underscores. The UP-DOWN-LEFT-RIGHT keys are the keys to the right of the numbers, located under the right portion of the display. Note also that the numbers 1 through 5 are located on the top row of numbers and 5 to 9 and 0 (zero) on the bottom row.

Before testing the zero glass, make sure the Gain is set to 1 on the front panel display. To do this, press the UP key 6 times, then the LEFT key twice. The cursor should be on the left-most zero of the top row of the display. Press the DOWN key once to go to the second row. Press the LEFT key 3 times, then the RIGHT key twice. This should bring the cursor to the Gain value. Pressing any number from the panel top row (numbers 1 to 5) will increase the gain value, any number in the bottom row will decrease the gain value. Set the gain value to 1, and then press the UP key to register the value.

The next operation consists of setting the signal polarity to positive. Press the RIGHT key once to put the cursor on the decimal point of the Signal. Pressing any number on the front panel will toggle the polarity as indicated by the small plus (+) or minus (-) sign on top of the decimal point. See figure E-1 above. Set the signal polarity to positive.

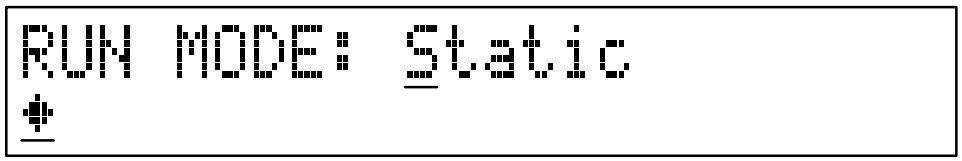


Figure E-2 : BI-DNDC setup mode.

The last step consists of putting the BI-DNDC in the static mode. With the cursor on top of the signal's decimal point, press the RIGHT key to go to the setup menu. Press the UP key until the display reads RUN MODE as shown in the illustration above. The cursor will be blinking on the diamond. Press the RIGHT key to move the cursor to the run mode setup. Pressing the UP key will toggle the run mode between static and dynamic. Set the mode to static then press the LEFT key twice and the UP key once to return to the main display. This leaves the BI-DNDC with the correct setup to test the zero glass.

Except for the temperature and signal values on the front panel display, the reading should be similar to the one on figure E-1. If the mode doesn't read STA, or the gain is not 1, or the signal polarity is not positive, please redo the setup.

Testing the Zero Glass

If the clear tubing is not already connected to the bottom port (outlet), do it now. Place the other end of the tubing in a waste beaker. Plug the top port using the provided plug. If the blue PEEK tubing is not already connected to the middle port, do it now. Using the provided syringe, push 2 ml of water through the middle port. This has filled the reference cell.

Remove the fitting attached to the blue PEEK tubing from the middle port and replace with one of the plugs. Remove the top plug and replace with the fitting attached to the orange PEEK tubing. Using the provided 2 cc syringe, push 4 ml (2 times 2 ml) of water through the top port at a high flow rate. You should be able to push the full 2 ml in 2 to 4 seconds.

If a change of signal greater than 0.1 occurs when 2 ml of water is rapidly pushed (2-4 seconds) through the top port, adjustment of the zero glass is NOT necessary. DO NOT PROCEED, your zero glass is positioned correctly.

If there is minimal signal change, try pushing air through the top port. If there is less that 0.3 signal change, adjustment of the zero glass is necessary. Before proceeding, push 4 ml more of water in the top port

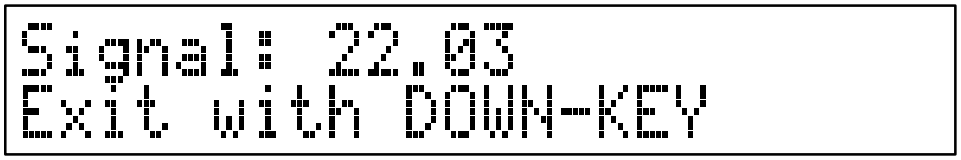


Figure E-3 : BI-DNDC in zero glass recovery mode.

Zero Glass Recovery Procedure

STEP 1: Switch off the BI-DNDC, wait at least 5 seconds.

STEP 2: Switch on the BI-DNDC.

STEP 3: Press and hold the UP key on the front panel of the BI-DNDC until the display reading is similar figure D-3.

STEP 4: Press the RIGHT key on the front panel. The signal will "swing" back and forth from 300 to -300 to 300... Take a good look at the signal and try to understand how the signal varies. Some BI-DNDCs will swing from 1000 to -1000 or other values depending on the wavelength of your BI-DNDC, the power of the source, the solvent used and other factors.

STEP 5: Stop the signal **on the way from +300 to -300** by pressing the UP key. After pressing the UP key, note the signal for the next 5 to 10 seconds. It should decrease. If it increases, repeat the procedure starting with step 4. (The zero glass was stopped too late, try pressing the UP key a bit earlier).

Note there is some lag time in between the time the UP key is pressed and the time the signal stops.

The signal value should be between –1000 and 1000. If it is outside these values, repeat from step 4.

STEP 6: WHEN SUCCESSFUL with step 5, press the DOWN key.

STEP 7: Press the Auto-Zero button on the front panel.

STEP 8: Push 2 ml of water quickly (2-4 seconds) through the top port. If the signal value changes by less than 0.1, the glass was not successfully stopped in the right range. Possibly the glass stopped on the way from negative values to positive. Repeat from step 1.

If the signal change is greater than 0.1 when pushing 2 ml of water fast through the top port, the zero glass has been successfully recovered.

Reconnect the signal cable at the back of the BI-DNDC and start the BI-DNDCW software to work with the instrument.

What Was that Procedure?

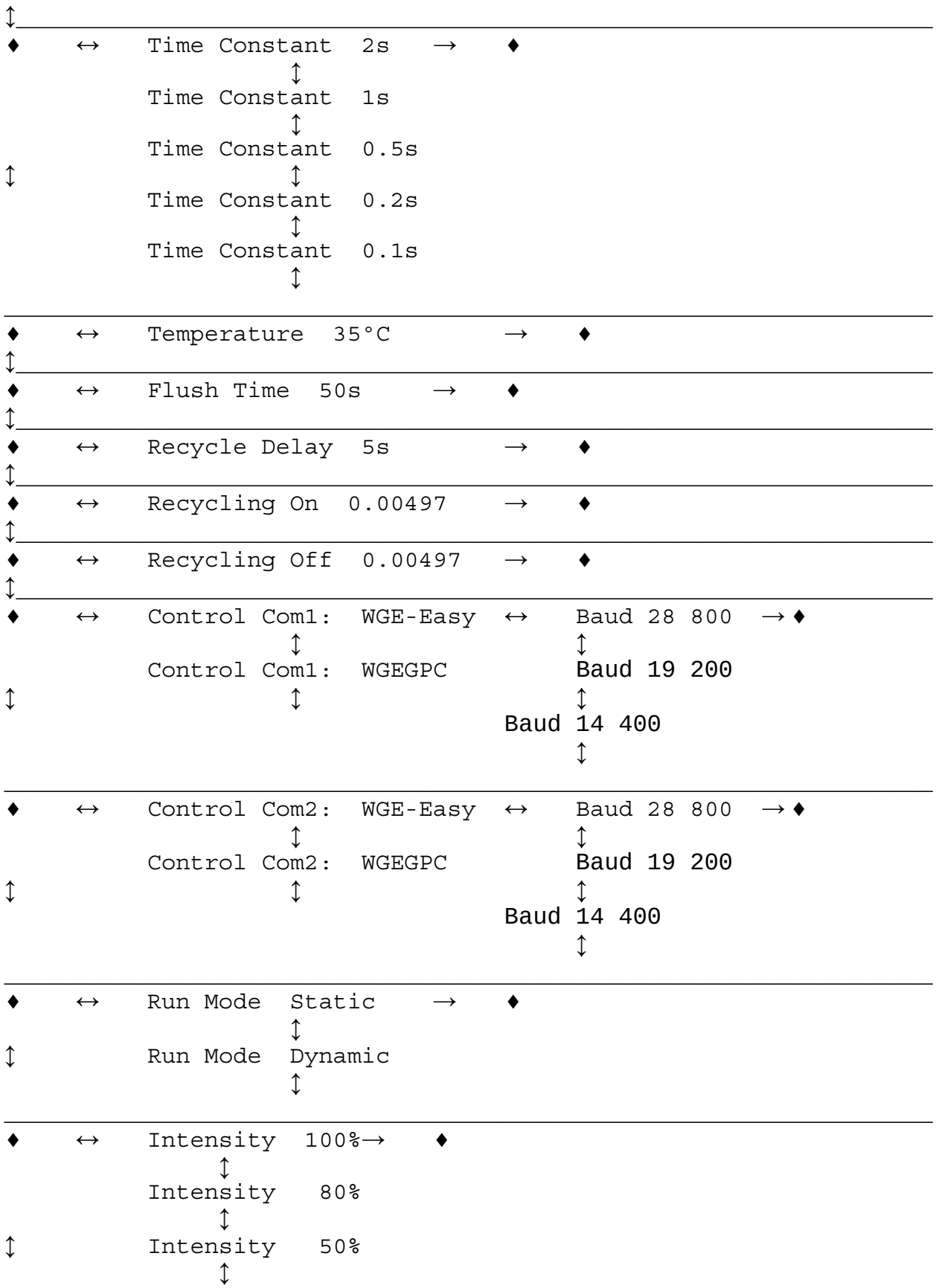
The figure on page VI-1 of this manual shows the optical arrangement of the instrument. Between the sample cell and the mirror on the right is the zero glass, a rectangular plate of glass. Tilting the glass will shift the beam slightly. Pressing autozero will cause the BI-DNDC to tilt the glass and shift the beam until a null signal (zero) is obtained. In general, the zero glass will be nearly perpendicular to the beam. The zero glass can rotate freely 360 degrees. If the zero glass is spun 90 degrees from the indicated position on the figure, the signal deflection for any solvent in the BI-DNDC will be close to zero. If the refractive index of the contents of each cell side differ substantially, such as when each side of the cell is filled by a different solvent, the autozero procedure will rotate the glass too far. When this occurs the zero glass has to be

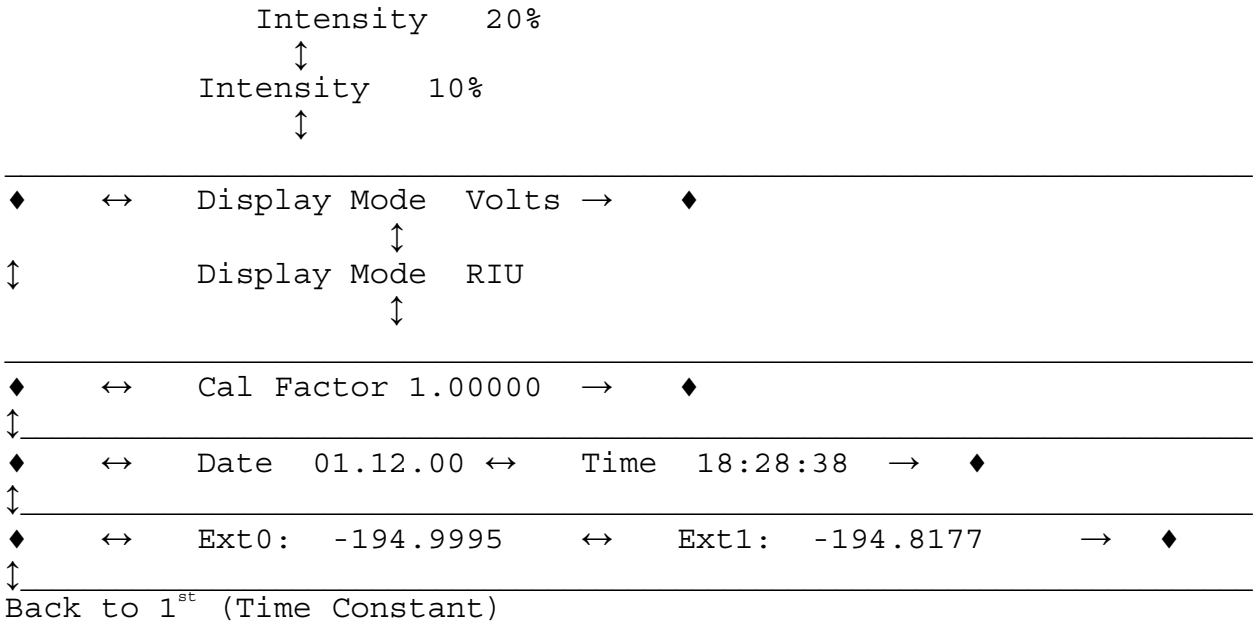
repositioned almost perpendicular to the light beam. This is why it is so difficult to stop the zero glass in the right position (step 5 of previous page).

How Can I Avoid Having to Recover?

As stressed in the manual (page I-1 and III-3) do not press the auto zero button on the front panel, or auto zero through the software unless both inlets have been flushed with the same solvent and the instrument is at thermal equilibrium. The zero glass might rotate perpendicular to the beam if the instrument is auto zeroed when the signal displayed is “over”, meaning the signal is smaller than –10 volts or greater than 10 volts.

Appendix E: BI-DNDC Setup Menu




Intensity 20%
Intensity 10%
Display Mode Volts
Display Mode RIU
Cal Factor 1.00000
Date 01.12.00
Time 18:28:38
Ext0: -194.9995
Ext1: -194.8177
Back to 1st (Time Constant)