Instruction Manual for BI-DNDCW

Differential Refractometer Software: Static Mode, Measuring dn/dc

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Please Read

This is your instruction manual for your Brookhaven BI-DNDC Differential Refractometer, Static Mode and BI-DNDCW software. Please read it carefully before making measurements. The "How To" section describes the installation of the software, the general plan of the manual, and a brief statement about its use. You may familiarize yourself with some of the features of this software by reloading data files (Files/Database). If you have any questions or suggestions, please contact Brookhaven Instruments.

This program requires Windows 9X or higher, a 32-bit operating system, and at least 32 Mb of RAM.

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.

Software License Agreement

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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.

Table of Contents

Section I: How to Use this Manual and Software

The differential refractometer software, BI-DNDCW, is a program that will allow you to collect and analyze the difference in refractive index between a solution and the corresponding pure solvent as a function of solute concentration. From this dn/dc, a parameter used in the calculation of molecular weight from light scattering data, can be calculated (Section VII). This software must be used with the corresponding BI-DNDC differential refractometer (Section II).

You will need the correct value for dn/dc when calculating molecular weight from light scattering data collected with Brookhaven's **BI-200SM** goniometer, Brookhaven's **BI-MwA** molecular weight analyzer, or with any other static light scattering instrument.

WARNING: Do not let any polymer or salt solutions dry in the instrument. Always flush with solvents. See the warnings in Section III.

When calibrating (Section IV), the solute is typically a salt and the corresponding solvent is DI water. We have literature values built into the program to assist you during calibration. When making a measurement (Section V), the solute is a polymer dissolved in any appropriate solvent. The program allows you to set the temperature, the time at which data is collected, and the number of data points. It retrieves from memory the refractive index of a number of common solvents at the instrument's wavelength and the set temperature. Or, you may enter the refractive index for a liquid not specified in the list of common solvents. The program also allows you to edit data points and select different plots of the raw and calculated data including: voltage vs. time, voltage vs. concentration, and Δn vs. concentration. The differential refractive index increment (dn/dc) also known as the specific refractive index increment (S.R.I.I.) is determined and displayed as well as the corresponding calibration constant. The units of dn/dc are mL/g.

Results can be printed, saved, and archived to, or reloaded from, the database (Section VIII) for future reference.

To install the BI-DNDCW software from floppy disk, insert installation disk #1 in the floppy drive. Click on **Run** from the **Start** menu in Win 9X. Type A:\SETUP into the dialog box and click on OK. Follow the instructions that instruct you to insert diskette #2. Installation from CD ROM requires inserting the CD ROM into the disk drive and double clicking on Setup.exe. When you finish, a BIC icon with the name Differential Refractometer Software is 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.

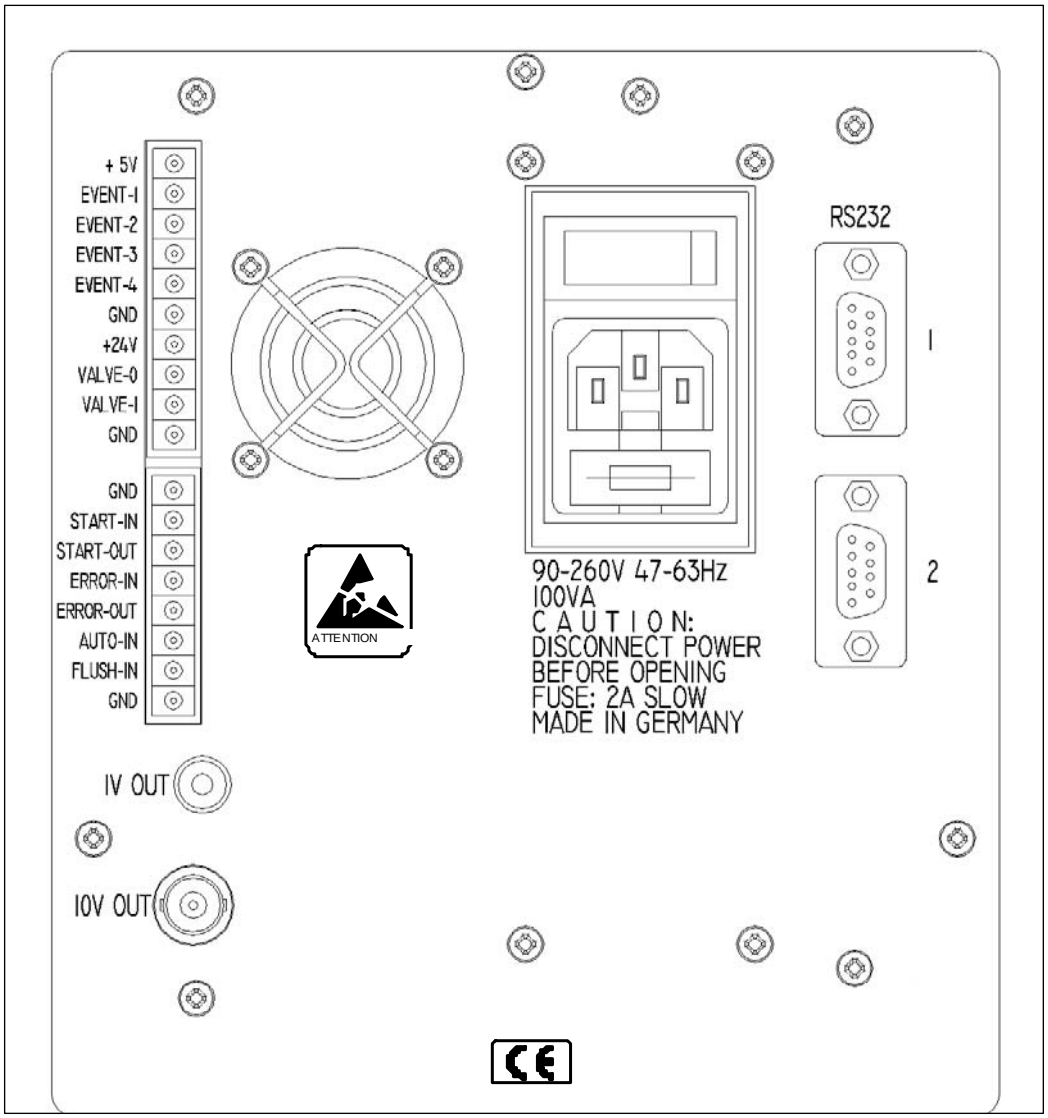
Use this program with the BI-DNDC Differential Refractometer when operated off-line in the static mode with syringes or a syringe pump. Typically you will measure dn/dc for use as a parameter in Zimm Plot determinations of molecular weight, $<M_w>$ and second virial coefficient, A_2. The data from which such a plot is made comes from a static light scattering instrument like the Brookhaven **BI-200SM** or the **BI-MwA** operated in the static or batch mode.

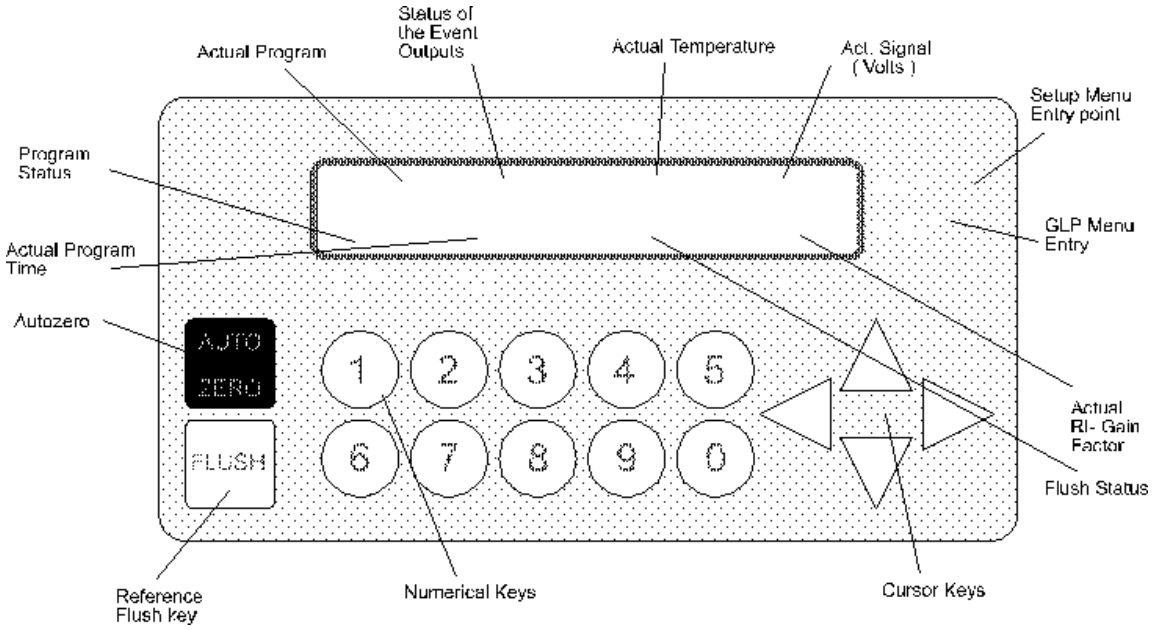
Section II: Connecting the BI-DNDC to a PC

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 default for the computer is COM1. If you change the computer port to COM2, you must change a parameter in the bi_dndc.ini file. This file is typically found on the path: C:\WINDOWS\bi_dndc.ini. Change the line that reads "CommPort=1" to "CommPort=2". Please note that there are no spaces before or after the equal sign. Also, follow exactly the spelling and capitalization.

Open the BI-DNDCW program by clicking on its name in the Brookhaven window. From within the program, click on **Instrument**, **Communications Monitor**, **Start Transmission**. If you see a stream of data with two columns: "Signal Volts = " and "Temperature = " then the connection is proper. In this case, click on **Stop Transmission** and exit the communications monitor window.





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 again to enter the Setup Mode. Press the down arrow six times. You should see the following:

Control COM1: WGE-EASY

Baud Rate: 19200

If you see "WinGPC" instead of "WGE-EASY," 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.

If you still cannot get communication between the PC and the BI-DNDC, please contact the factory for advice.

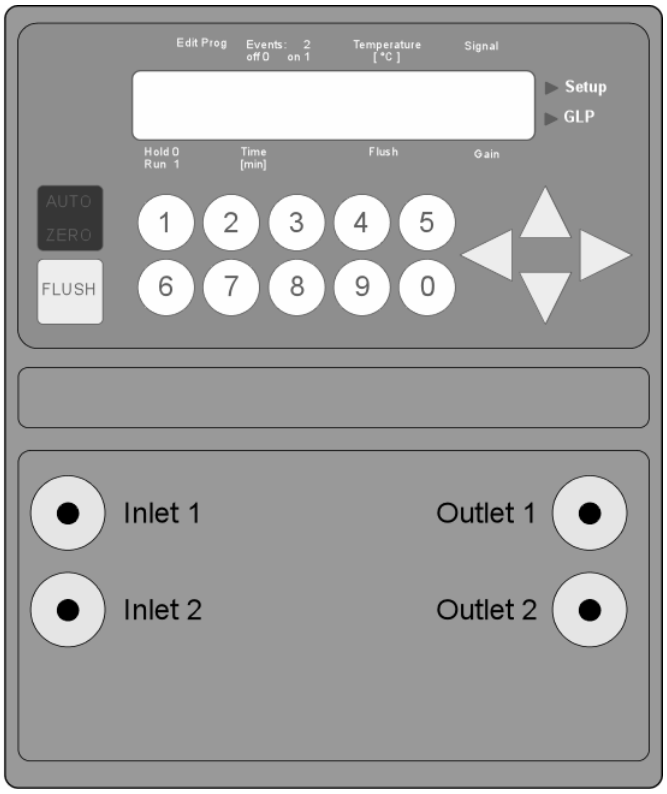
Section III: Plumbing Connections, Warm-up, Stability, and Other Tips of the Trade including IMPORTANT WARNINGS

Important Warning #1

Never allow any liquid to dry inside the instrument. Always flush the solute or polymer with solvent. If you are not going to work with the instrument for more than a day, 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 two inlets and the single outlet 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 before leaving it to sit for more than a couple of hours. Leave the syringes attached to both inlets to prevent entry of foreign matter.

Plumbing Connections

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



The inlets are marked as Inlet 1 and Inlet 2. The internal, stainless steel tubing that connects the inlets and outlets to the sample cell has an inside diameter, I.D., of 0.75 mm (0.030”, or 30 mil). Use Inlet 1 for the solutions and Inlet 2 for the pure solvents.

We recommend external plastic tubing as follows:
Inlet 1: ~12 cm, 1/16" O.D., of either orange, PEEK tubing of I.D. 0.50 mm (0.020") or green, PEEK tubing of I.D. 0.75 mm (0.030").
Inlet 2: ~12 cm, 1/16" O.D., blue, PEEK tubing of I.D. 0.25 mm (0.010")
Outlet 1: ~12 cm, 1/16" O.D. Teflon or Tefzel tubing of I.D. 1.00 mm (0.040", or 40 mil), translucent.
Outlet 2: ~12 cm, 1/16" O.D. Teflon or Tefzel tubing of I.D. 1.00 mm (0.040", or 40 mil), translucent.
PEEK tubing is polyetheretherketone, and it 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. When using a syringe, 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. You can purchase more PEEK tubing from Upchurch Scientific, http://www.upchurch.com.

Alternatively, use stainless steel syringe needles, 16 Gauge (1/16" O.D.), with Luer-lock fittings for easy connection to syringes. To connect to Inlets, use Upchurch, Stainless Steel, Male Fitting, U-400, 1/16" O.D., 10-32 threads and U-401 Ferrules compatible with 1/16" tubing/needles. 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. Also, consider Upchurch P-659 Quick Connect Luer Adaptors for 10-32 to female. These stainless steel alternatives are compatible with almost all solvents. You can also bend the needle such that when attached to the Inlets, the syringes are pointing upwards at 1 and 11 o'clock. When a syringe still has liquid but is not in current use, this vertical position with the barrel held down by gravity may prevent bubbles from forming in the syringes. Bend the needle 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.

Tape or otherwise fix the Teflon tubing to the side of the instrument in a loop such that the end of the tubing sits inside a 100 mL waste flask. Make sure the tip of the tubing is visible inside the waste flask so flow can easily be observed.

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 Fingertight. In this way, the dead-volume is minimized and the connection is leak-tight.

Luer-lock and Fingertight fittings of PEEK are available from Upchurch Scientific Inc. Contact them at +1-800-426-0191 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 (~30 °C) to 80 °C.

Important Warning #2

Always set the temperature using the BI-DNDCW program. Do not set the temperature from the front panel. If you use the front panel, the software, when running, will override it. Therefore, if this front panel temperature is set differently from the software setting, you may have to wait a long time to reach thermal equilibrium.

To set the temperature, click **Parameters** and fill in the desired value.

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 to 80 °C.

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

Initial Setup and Flushing

We ship the instrument with 70% 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. Simply flush that with DI water that you will use for calibration.

Do not discard the plugs. They are useful in a variety of ways as described in this manual.

Use a syringe to flow 5 mL of water into inlet 1 and out of the instrument. Use a different syring to flow 5 mL of water through inlet 2 and out of the instrument.

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

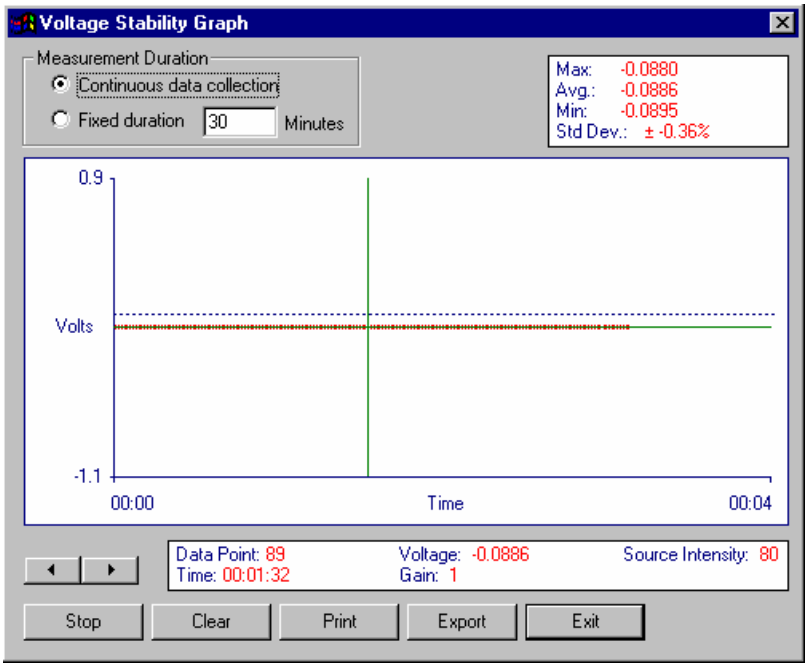
Look for any liquid leaking around the various fittings. Reposition and tighten if necessary. REMEMBER: When connecting PEEK tubing, poke the tubing 3/16” (~ 5 mm) beyond the screw-in fitting, and, while pushing the end of the tube against the mating part, screw in the fitting.

As you press on the syringe, look for droplets to form at the tip of the waste tubing. Push enough liquid through until you are certain there are no more bubbles and that you have replaced the previous liquid.

Check the voltage stability by running the program and clicking on Instruments/Voltage Stability. You should get a flat line. If not, slowly flush the system to remove air bubbles, solvent mixtures (IPA/Water), and any dust or bacteria attached to the walls of the system. BE PATIENT. The instrument is extremely sensitive to very small changes in refractive index on either side of the split cell.

Stability Checks

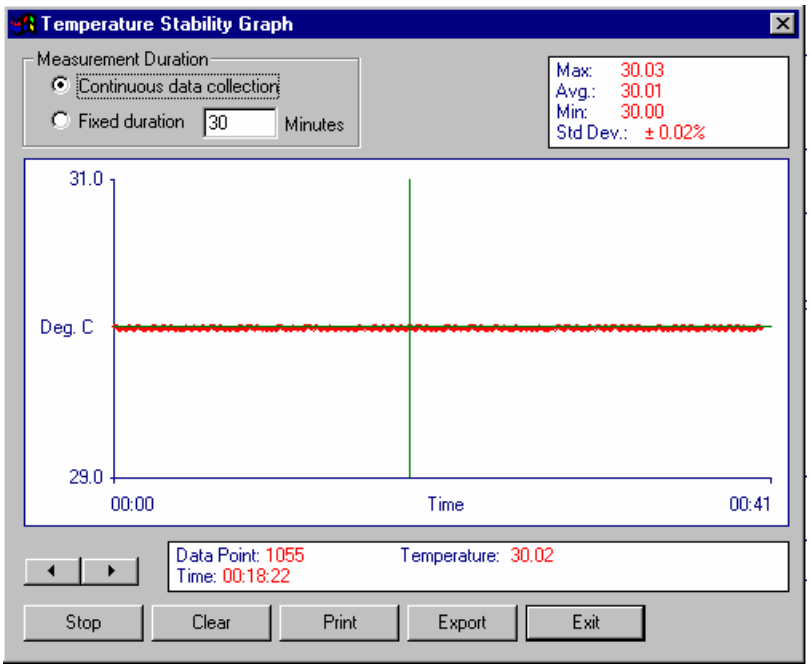
Click **Instrument/Voltage Stability**. You should see a window like the following. Here you monitor voltage vs. time. When the system is completely stable and the same liquid is in each side of the cell, the voltage should be flat to ± 1 mV. With even the slightest differences in concentration, temperature or pressure between the two sides of the cell, you will see a change in voltage.



The voltage you see here is the same as the one on the LCD display of the BI-DNDC. For the batch mode instruments discussed in this manual, the gain is kept at 1.

Click **Instrument/Temperature Stability**. You should see a window like the following. Here you monitor temperature (°C) vs. time. When the system is completely stable the average temperature will be stable to ± 0.010 °C **at the cell**.

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 temperature stability graph is showing the temperature near the heater vs. time.



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. Then flush *both inlets* very slowly with an intermediate solvent like isopropanol or acetone. Check for signal stability again. Keep flushing until the signal is stable. Now flush both inlets with the final solvent until the signal is stable. The reverse is also true.

By flushing the salt or polymer solution side first with the solvent, you prevent salt or polymer from precipitating, and then clogging the small-diameter tubing, in case they are not soluble in the intermediate solvent. For example, if you flush a KCl/H_2O solution used in calibration with acetone, the KCl will precipitate and clog the tubing. So flush first with water and then acetone, if that is the intermediate solvent. Also, if a salt solution, such as a buffer, is the reference solvent, the reference cell must also be first rinsed with water before flushing with another 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. Use a syringe pump and set it to a very slow speed. Alternatively, flush both inlets with 2 to 10 mL of solvent many, many times throughout the day.

If solvent will not dislodge the dried particles, consider using 0.1 molar nitric acid. Wear gloves and safety goggles. You may have to repeat this several times to get even a small flow to the outlet. Since the blockage is often near the beginning of the internal tubing, it often helps to flush the instrument in the opposite direction. Inject carefully the solvent into the Outlet Port. Do this slowly so as not to build up high pressure. This operation is **critical**; pressure higher than 0.5 MPa will damage the flow cell. Given the partial restriction that still exists, pressure can increase rapidly if you push too hard on the syringe barrel.

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 and then water to remove it.

You can determine when the instrument is stable by monitoring the signal with solvent in both cells. Click on **Instrument/Voltage Stability**.

Section IV: Making a Measurement: Calibration

Preliminary Comments

Always use the BI-DNDCW software when calibrating the instrument for static measurements and when actually making dn/dc measurements.

To determine dn/dc in the static mode, you must first calibrate the instrument with a solute/solvent combination of known dn/dc. Currently, we recommend KCl/H_2O. The values of dn/dc for this solution are stored in the BI-DNDCW software as a function of wavelength. To ensure accurate concentrations, unless the dry KCl is completely free flowing, heat the salt to ~ 80 ^oC for at least 30 minutes to dry the KCl. Let the salt cool to approximately room temperature. If you can do this in a vacuum oven, it is even better.

Do not make measurements unless the instrument is stable.

Always set the temperature using the BI-DNDCW program. Do not set the temperature from the front panel. If you use the front panel, the software, when running, will override it. Therefore, if this front panel temperature is set differently from the software setting, you may have to wait a long time to reach thermal equilibrium.

To set the temperature, click **Parameters** and fill in the desired value.

You can check the stability at any time. Warm up the BI-DNDC for at least an hour at 30 ^oC. Warm up the BI-DNDC for at least several hours, perhaps overnight, for temperatures in the range of 60 ^oC to 80 ^oC. Meanwhile, flush both inlet ports slowly with a liquid. If you have followed previous instructions, then the instrument has not been allowed to dry and it has not been left with water that may have become contaminated with bacteria. Most likely isopropyl alcohol (IPA) is in the lines. If, after you check stability, you intend to calibrate the instrument, use water for the stability test. If, after you check stability, you intend to make a polymer/solvent measurement, use the solvent for the stability test. Water is soluble in IPA; so is toluene, if the IPA is pure.

WARNING: Inexpensive IPA is a solution of up to 30% water. While this solution is suitable for killing bacteria, it is not entirely miscible with toluene. Therefore, if you use something other than pure alcohols like IPA when storing the machine, make sure to rinse the lines with pure alcohol before injecting toluene.

However, check that the liquid you will use for the stability test is miscible with whatever liquid is currently in the system. To check that any liquid is currently in the system, gently push air through one and then the other inlet and see if liquid emerges at the waste line. If the system is dry, or nearly dry, then use *pure* IPA to wet and flush. IPA is miscible with a wide variety of solvents. If the outlet liquid looks cloudy, chances are you have an oil/water or water/oil emulsion, and the liquids currently in the system

are not entirely miscible. In that case, flush with *pure* IPA or acetone until both outlets run clear.

For convenience, a list of solvent compatibilities is repeated here. Methanol, ethanol, isopropanol and acetone are intermediate solvents that are miscible with water and with less polar solvents like THF, 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, flush *both inlets* very slowly with an intermediate solvent. Check for signal stability. Flush again until stable. Now flush both inlets with the final solvent until the signal is stable. The reverse is also true: use an intermediate solvent. Be patient. Wait for stability.

Preparing KCl solutions for Calibration

Perhaps the most common error associated with static measurements is the accuracy with which concentrations are prepared and then handled. Prepare samples with concentrations accurate to at least +/- 0.5%; otherwise, you will discover that measurements do not fit to a straight line, as they should.

With volatile solvents, it is especially hard to maintain concentrations. Cover all liquid containers to prevent evaporation.

Using a single syringe with a succession of different solution concentrations may lead to cross-contamination unless you are careful. Expel the solution currently in the syringe. Rinse the syringe twice with the next higher concentration. Alternatively, rinse with pure solvent and dry the syringe before using it with the next concentration. Do not use a Luer-lock syringe without the needle. The Luer-lock mechanism has many hidden surfaces where a solution may dry, thus changing the concentration of the next liquid it contacts. Use the needle to withdraw solution from the sample bottle. Disconnect the needle and attach the syringe to the PEEK tubing fittings. Wipe the outside of the needle and flush it with air before putting it into the next solution.

Less error occurs if a small amount of low concentration solution contaminates a higher concentration than vice versa. Therefore, it is better to measure concentrations in ascending order.

It is better to prepare separate concentrations. However, if you do prepare a stock solution and dilute it, pay particular attention to details to avoid compounding errors. Serial dilutions are certainly convenient but are prone to greater error than the preparation of several separate concentrations.

See the attached guide, “Preparing Precise Polymer Solutions,” for details on solution preparation.

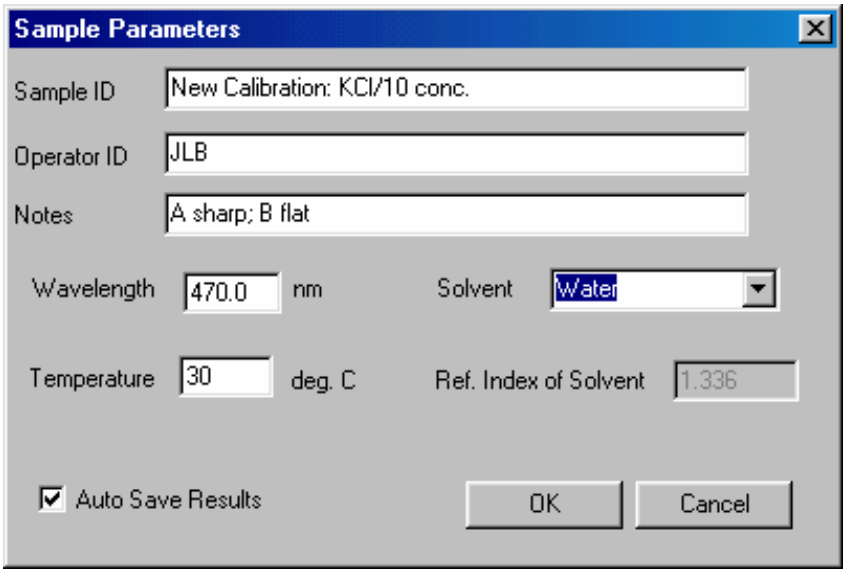
To prepare calibration solutions, add approximately the following amounts of salt to each dilution vial:

Nominal KCl Concentrations when making 20 mL of solutions.		
Vial	Wt. of KCl (mg)	Concentration (mg/mL)
1	20	1.0
2	40	2.0
3	60	3.0
4	80	4.0
5	100	5.0
6	120	6.0
7	140	7.0
8	160	8.0
9	180	9.0
10	200	10

These are ***nominal*** values. Do not struggle to weigh out exactly 10 mg of KCl. A value in the range of 9 mg to 11 mg is okay as long as it is known to ~ 0.1% accuracy. Add 20 mL of DI water and cover to prevent evaporation. Then weigh the solution and divide by the density of water to obtain the volume of solution. Finally, divide the exact mass of salt by the volume of solution to obtain the solution concentration.

Calibration Measurement

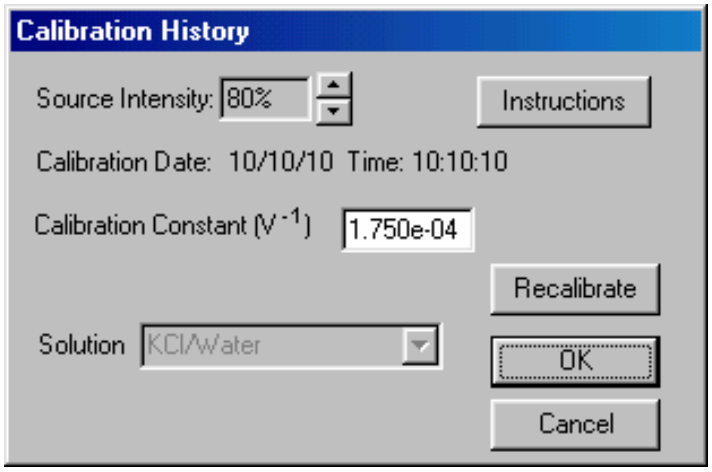
With the instrument warmed up and stable, click on **Parameters**. A window like the following will appear:



Fill in the *Sample ID, Operator ID* and *Notes* fields. [Note: Your notes need not be as melodic as the ones shown here.] Take care in selecting the sample identification. It is used in the database to identify files. A carefully chosen name will make it easier to spot the data file in the future. The wavelength is determined automatically, and it should agree with the instrument you purchased (470, 535, or 620 nm). Note that if the instrument is not yet hooked up to your computer, the displayed wavelength will default to 470 nm. If this occurs, make sure the instrument is connected to your computer and turned on. Then, exit the software and restart. The correct wavelength should be displayed. Select the solvent to match the one you will use for calibration. For calibrating in the static mode, it is water. The program automatically calculates the refractive index of the solvent given the wavelength and temperature from the common liquids listed. If your solvent isn't listed, select *Unspecified*, and then type in the name. In this case, you may also type in the refractive index. The refractive index of the solvent is unnecessary for the dn/dc measurement. This field exists for user convenience.

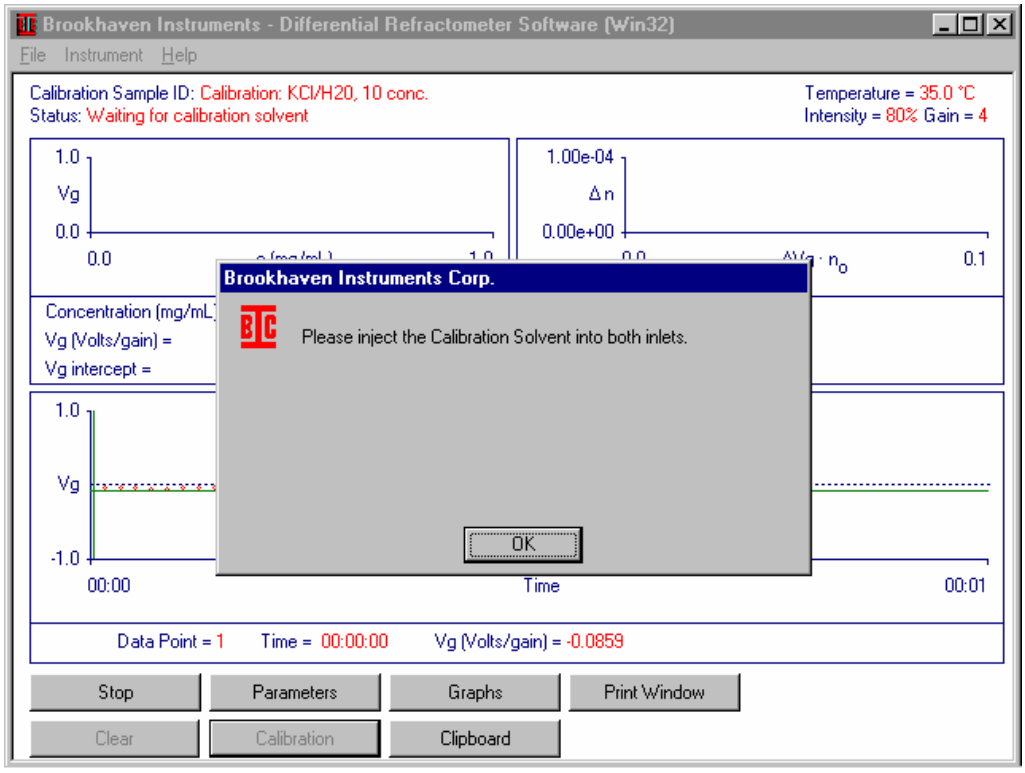
The temperature should already have been set. If not, set it now. If it is substantially different from the value on the LCD display of the BI-DNDC, then wait until thermal equilibrium is reached.

Click on the **Calibration** command button. If the **Source Intensity** box is not showing 80%, select it using the arrows. If you change the source intensity, you must recalibrate. Usually, you will use the 80% setting. Click on the **Instructions** button to learn more about the source intensity setting.

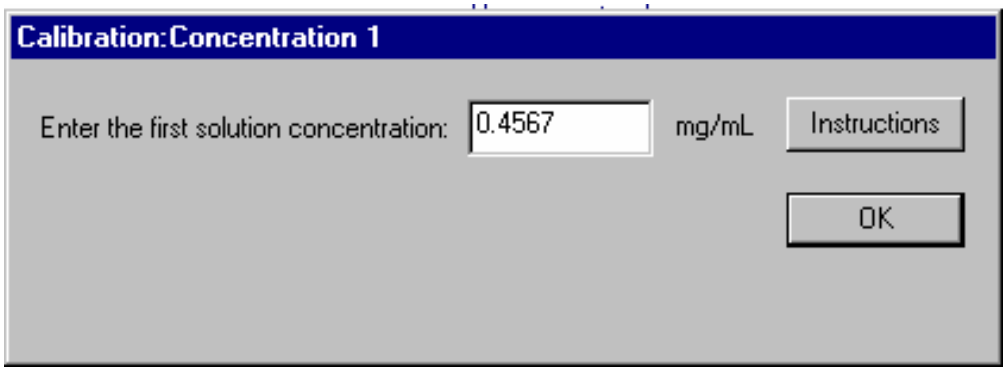


If the instrument has been calibrated, then the date and time of the last accepted calibration are shown as well as the calibration constant. Calibration constants vary from machine-to-machine and will drift slowly over time. Generally, they are in the range of 0.7e-04 to 7e-04. The text file c:\bicw32\dndcw\data\clblog.txt contains a log of all previous calibrations.

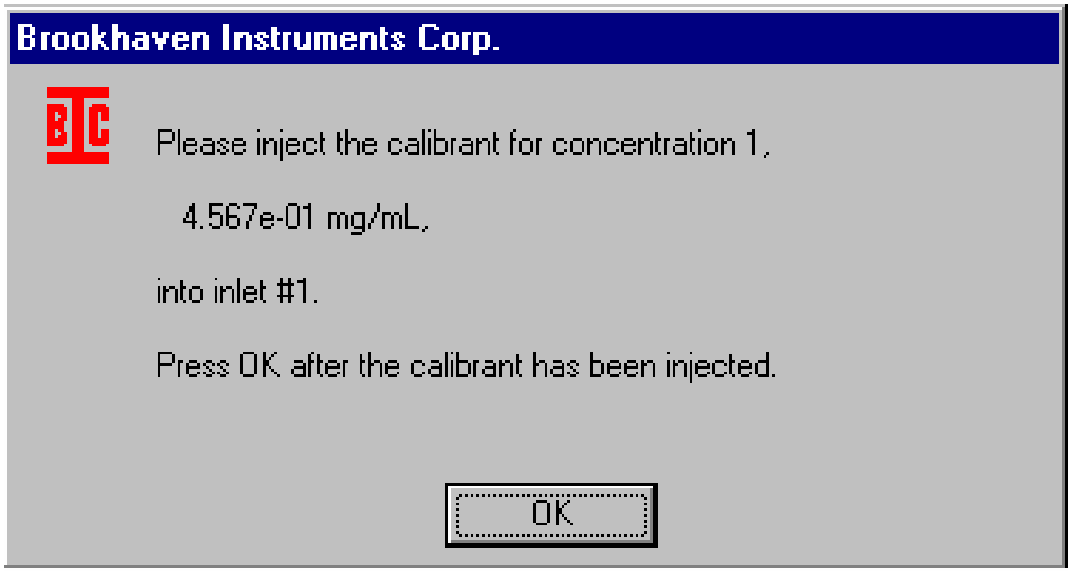
To initiate a new calibration, click on **Recalibrate** and follow instructions. After a few seconds, you will see a display that looks like the following:



If, during the warm up period, you did not inject the calibration solvent (typically water) in both inlets, do so now. Then click **OK**. After a few seconds you will see:



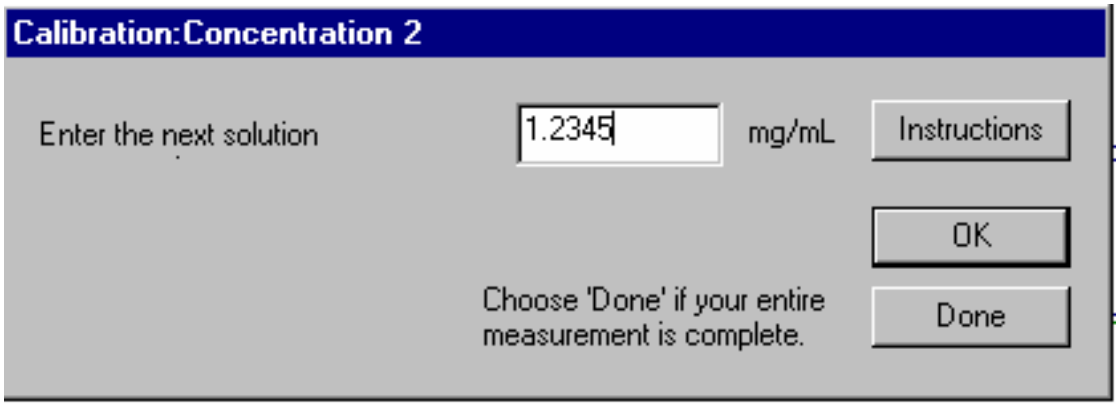
Type the first concentration in mg/mL. Then click on **OK**.



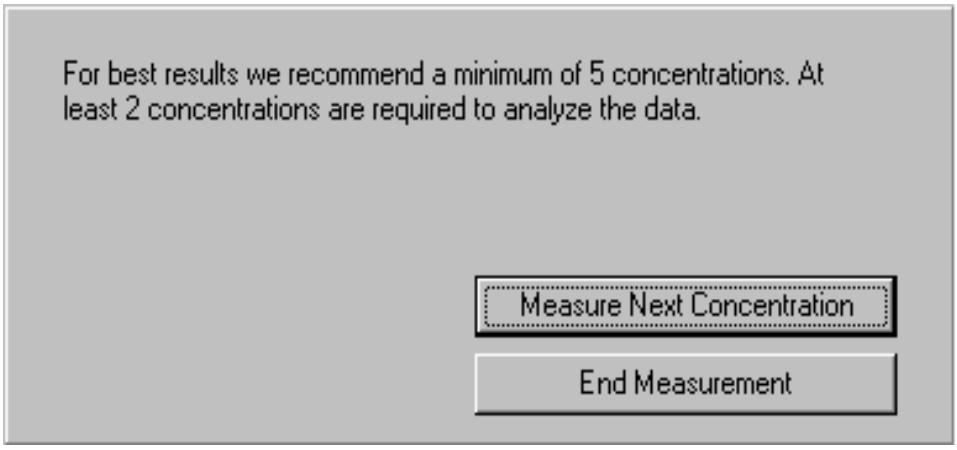
Draw about 0.5 mL of the first solution into the syringe and rinse the syringe by drawing the plunger back with the needle pointed up. Then, expel the air, remove the needle, and connect the syringe to the inlet tube. Inject all of this liquid into Inlet 1. Repeat. Now the syringe and cell are completely rinsed with the new solution.

For measurement, draw about 1 mL of the first solution into the syringe. Then, expel the air, remove the needle, and connect the syringe to the inlet tube. Inject most of this liquid into Inlet 1 and press **OK**.

You will see bumps in the signal vs. time line for each injection. These bumps are normal. However, the signal should level off. When the signal is sufficiently level, the red points will turn black to show the data points used to obtain the voltage level for your sample. Next, the following window will appear:



Type the second concentration in mg/mL. Click **OK**. Repeat the rinsing and injection sequence described above for the second solution. If you click the **Done** command button, you will see the following message:

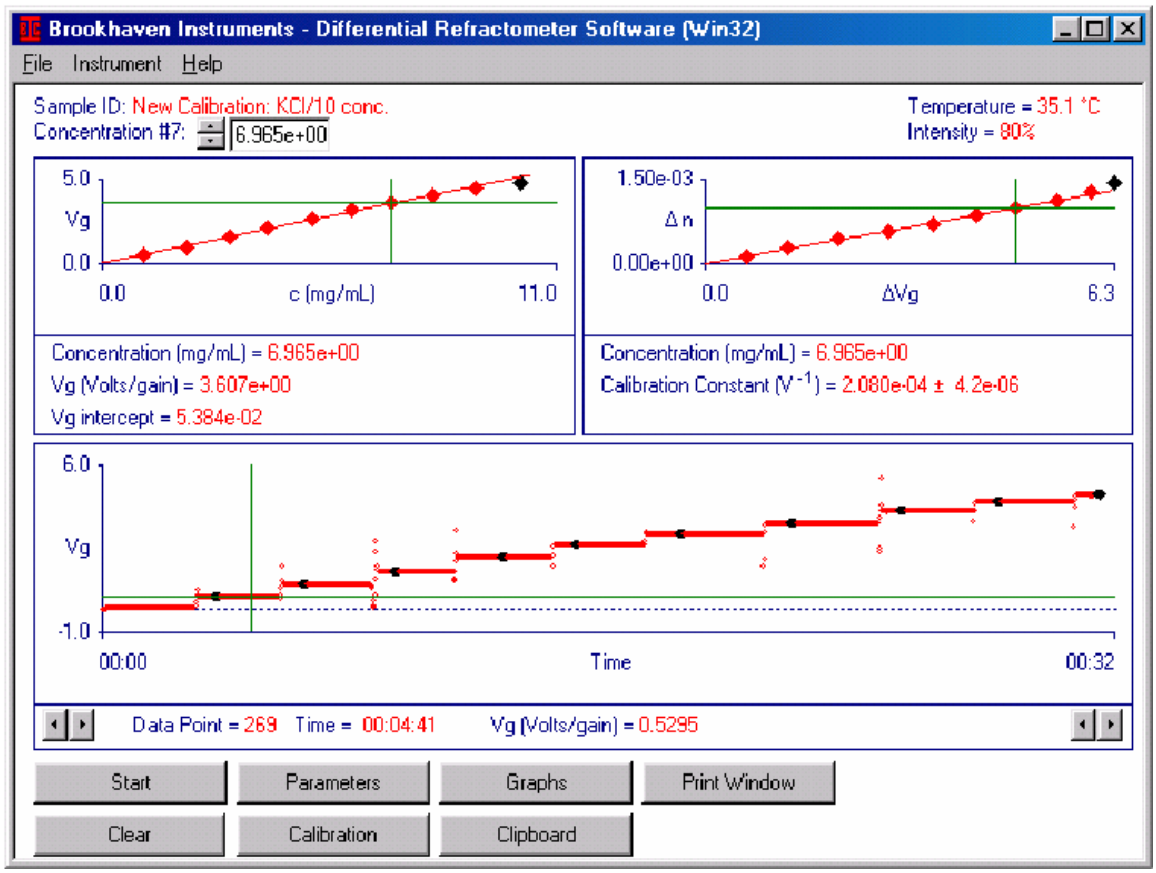


Continue measuring each concentration until you have measured all of your solutions. Then, click **Done**. You will be asked if you wish to measure the baseline for a drift correction. Click yes. Rinse the syringe and cell thoroughly with solvent and inject pure solvent. Then, click **OK**. The baseline will be measured again and a drift correction will be applied. The baseline correction can be removed by clicking on **Parameters** and by clearing the *Use drift* checkbox.

In general, the drift correction should be small. If it is not, then the condition of the cell is changing (due to dried salt or polymer), or, more likely the sample cell still contains some solute.

Interpreting the Results

Here is an example of a calibration with 10 concentrations of KCl in water.

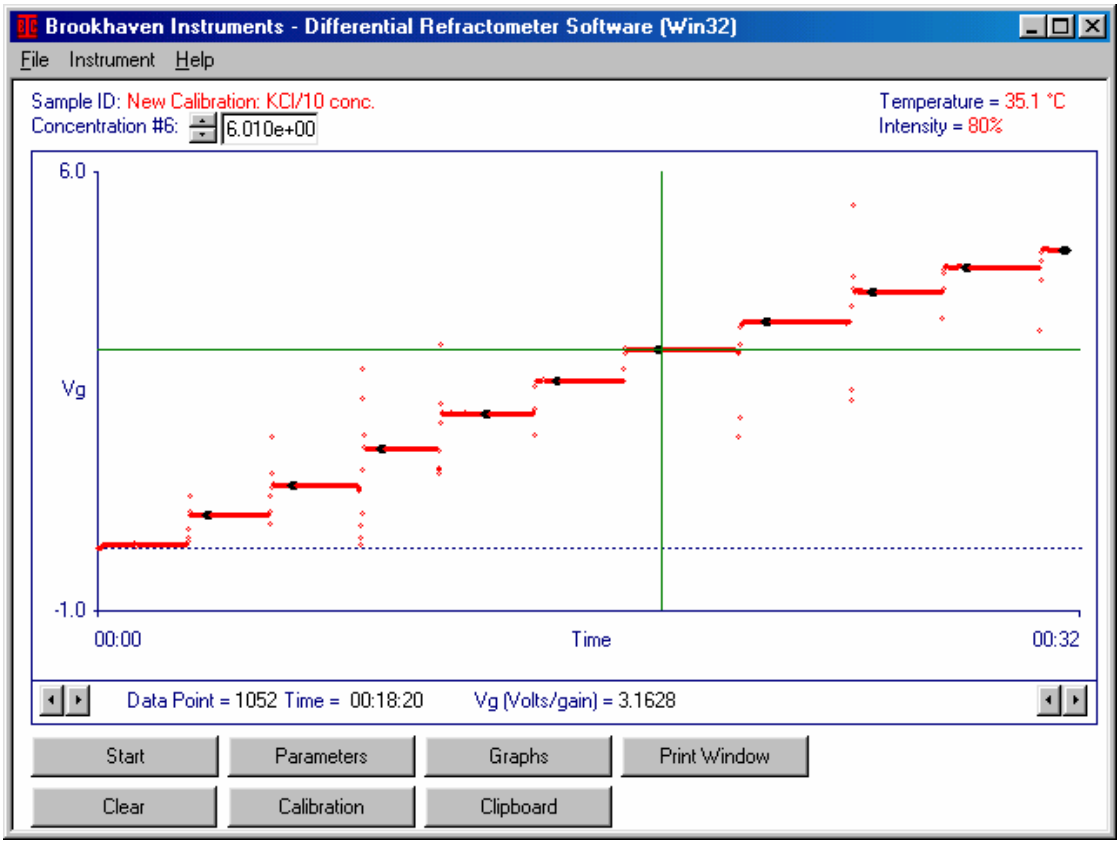


The bottom graph is a plot of Vg (signal) vs. Time. Each step in the plot corresponds to a concentration. The duration per step depends on how long you take to type in the next concentration, remove the syringe, rinse it and the needle, fill it with the next concentration, remove air bubbles, and slowly and gently inject the next concentration. The duration per step here averaged around three minutes, though after the average value is determined (black group of data points in V_g vs. Time graph), there is no reason to wait longer.

A running average of 10 data points is fit to a straight line. Two criteria are used to accept or reject the points for final averaging. First, the slope must be < 2e-4 V/s or be statistically insignificant. Second, no single point in the group can differ from the average by more than 0.005 volts. If either criterion is violated, another data point is taken.

The average value of the signal for each concentration is plotted as a function of concentration in the upper left. The refractive index difference, calculated from the concentration of calibrant, as a function of signal is plotted in the upper right.

By clicking on the Graphs command button, you can enlarge and rotate through the three graphs. Here is an example of V_g vs. time enlarged.

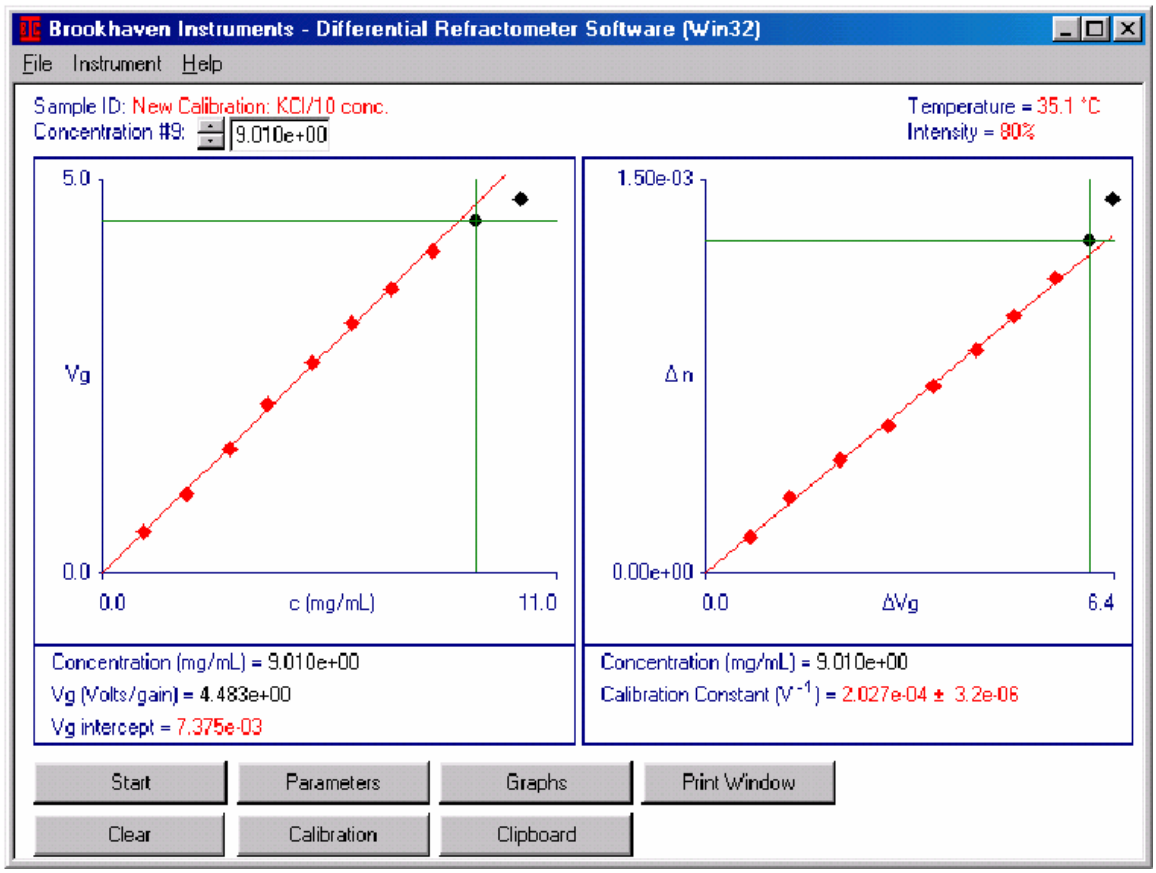


Notice that some of the steps have unusually high and low values at the interface between them. This is normal when using a syringe without a syringe pump. Unless you inject very slowly and smoothly, you will get a little turbulent mixing in the cell when the new concentration pushes the old one out. This turbulence will cause a greater signal, thus accounting for the sudden and sharp rise. Once the turbulence passes (since flow has ceased), a smooth, flat portion of each step is obtained. After at least 10 seconds, the average of 10 data points will satisfy the criteria and an average voltage (V_g) will be established corresponding to the concentration injected.

By clicking on the scroll box arrows above the graph, you can review the concentrations. If you made a mistake in entering the concentrations, you can also edit them. By clicking on any point in the graph, you can see its concentration and corresponding V_g value below the graph. By double clicking on any point *in the graph* it turns black, and it is removed from the calculation to determine the best straight-line fit.

From the fit, the intercept, labeled V_g intercept, is determined. This value accounts for the inevitable deviations from a perfect zero voltage reading with both sides of the cell filled with the same liquid. The difference in voltage $V_g - V_{g,intercept} = \Delta V_g$ is formed in order to calculate the instrument's calibration constant.

Below is an example showing the same data with the two highest concentration points removed.



The right-hand graph is a plot of Δn vs. ΔV_g, where Δn is the literature value for the difference in refractive index between the calibration solution and the solvent at the concentration where ΔV_g has been determined. The function should be a straight line through zero with a slope equal to the calibration constant, here $(2.080 \pm 0.042) \times 10^{-4}$ V^{-1}. Thus, in this example, the calibration constant has been determined to ± 2%, after deleting the highest concentration, 10.01 mg/mL. Note: When concentrations are too high for a particular machine's optics to handle properly, the effect is a low V_g value as shown in the left hand graph.

V_g intercept is seven times smaller than before. The calibration constant is reduced to ± 1.6%. The calibration factor has changed by – 2.5%. It is now $(2.027 \pm 0.032) \times 10^{-4}$ V^{-1}. This is a significant shift, because an error of 2.5% in the calibration constant will contribute an error of 2 x 2.5% = 5% to the molecular weight and second virial coefficient that are calculated in a SLS experiment. Note: In the classic, static, measurement of $\langle M_w \rangle$, where the polymer concentration is determined by direct gravimetric/volumetric analysis, this statement is true.

Deleting any of the other points one at a time does not materially change the calibration constant, the gain-adjusted intercept, or the random error of the fit. If you measure with several concentrations, this is a good method to try. If you measure with only a few concentrations, you will not have this luxury.

The lesson is clear: It is difficult to determine dn/dc to better than 2% unless extraordinary care is taken with sample preparation, instrument warm up and stability, and calibration. In fact, a very good procedure for improving such determinations is to practice calibration until you get 2% or better repeatability in the calibration constant over the short term. Longer term, the value may drift. For example, on this same machine, five days earlier, the calibration constant was found to be $(1.972 \pm 0.031) \times 10^{-4}\ V^{-1}$. The shift is 2.75%/5 days or ~ 0.5%/day. For this reason, we recommend calibration before any significant, new measurements of dn/dc will be made.

Suppose, because of serial dilution from a concentrate, all the concentrations in the above measurement ($2.027 \times 10^{-4}\ V^{-1}$) were 1% too high. What would be the effect? The result, after deleting the two highest concentrations again, is $(2.047 \pm 0.032) \times 10^{-4}\ V^{-1}$, a shift of 1%.

After the calibration data is correct, that is outliers removed, then click on **File**->**Save** followed by **clear**. You will be asked if you want to use the new calibration constant. Click **Yes**.

After calibrating with KCl/ H_2O, it is a good idea to then measure the same solutions and see if you can obtain straight lines and dn/dc for KCl. This is an excellent way to sharpen your sample preparation skills as well as learning how to operate the machine. We urge you to make such measurements before you launch into a difficult sequence using either a volatile solvent or the remaining 10 mg of protein that will cure all forms of cancer. Practice, practice, practice. Do it with patience and acquire the skill necessary to get better than 2% repeatability.

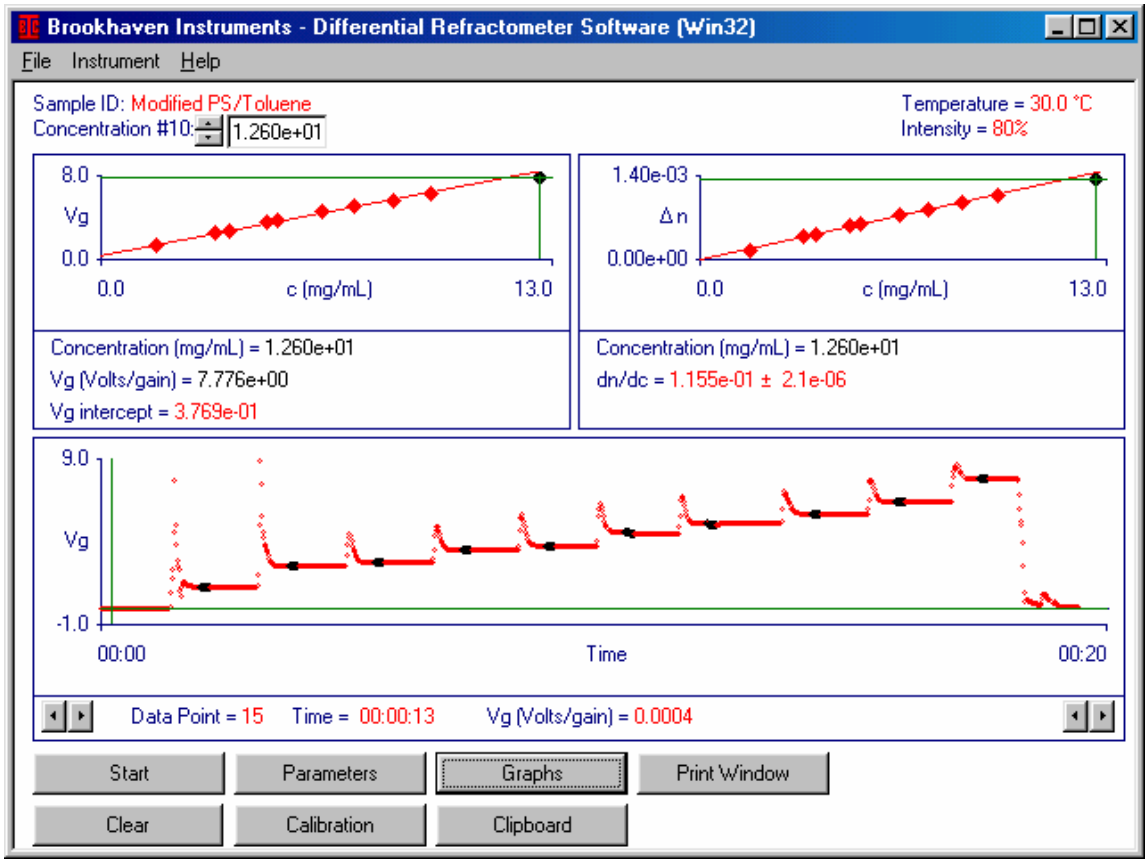
Section V: Making a Measurement: Polymer/Solvent

Preliminary Comments

Read again Section IV on calibration of the instrument. Almost every statement there applies equally to this section. All the steps are the same, except, instead of clicking on the **Calibration** command button after filling out the **Parameters** window information, you click on **Start** to initiate a measurement.

CCFor example, you have to warm up the instrument until solvent in both cell compartments yields a flat signal for many minutes to ± 5 mV. You must prepare polymer/solvent solutions with concentrations known to better than 0.5% and keep them covered to prevent evaporation (increase in concentration). The instrument should be calibrated if it has been more than a day or two since it was. Click **Calibration** to see the date and time of the last calibration as well as the constant itself. Click **Parameters** and fill in the Sample ID, Operator ID, and any relevant Notes. The wavelength is automatically determined for you. And, remember, if you change the temperature it may take a long time to re-establish thermal equilibrium at the cell.

Here is an example using a modified polystyrene in toluene.



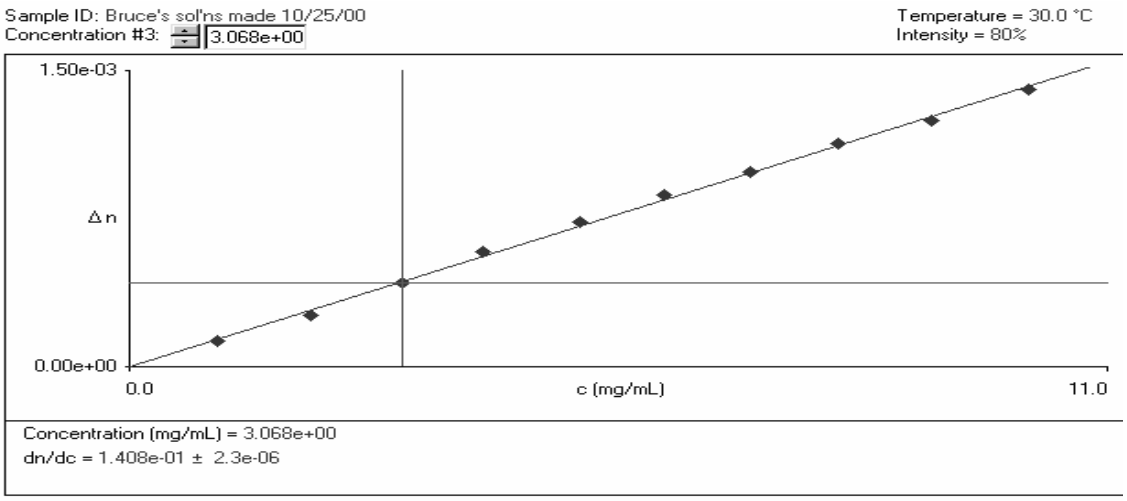
Two of the three plots are the same as the ones formed during calibration. The voltage, V_g, is plotted against time, where the thick, black points represent the average of 10 previous points. Read the section on calibration for the acceptance criteria of these points. Sudden, upward spikes indicate an apparent sharp increase in Δn, probably due

to mixing. If the sample is injected extremely slowly, these spikes do not appear. The final results are not affected by the appearance of such spikes. In this particular plot, the user decided to check the solvent baseline again by injecting solvent after the last concentration. This procedure is not required, but is recommended.

The plot in the upper left-hand corner is also the same as the one formed during calibration, V_g vs. c, polymer concentration. A straight line is fit to the data to determine the intercept. At each polymer concentration the difference in voltage $V_g - V_{g,intercept} = \Delta V_g$ is determined. Given the calibration constant, Δn is calculated from ΔV_g.

The plot in the upper right-hand corner is different. Here it is Δn vs. c, the polymer concentration. The slope of this line, $\Delta n/c$, is dn/dc, because it is assumed that the best fit is a linear one. See the section on the theory of dn/dc.

In this example, the last data point at 12.60 mg/mL did not fit as well as the other points to a straight line. By double clicking on it, it is removed from the fit. The final result is dn/dc = 0.1155 mL/g. The random error in the fit (±2.1 x 10^{-6} mL/g) is unusually small. Therefore, a few repeats of the measurement, perhaps with new concentrations, perhaps with a fresh calibration, are needed to determine the error on dn/dc itself.



Here is another example of dn/dc. The plot above shows Δn vs. c for KCl/H_2O. First, the machine was calibrated with one set of KCl solutions. Then a second set, prepared two months earlier, was used to determine dn/dc for KCl. The value, 0.1408 mL/g, is within 0.1% of the expected value for this 470 nm instrument. This shows that carefully prepared samples can be preserved for extended periods if they are not allowed to evaporate. It also shows that the instrument and technique are reproducible. However, do not expect agreement with known values to 0.1%. This is an extreme case.

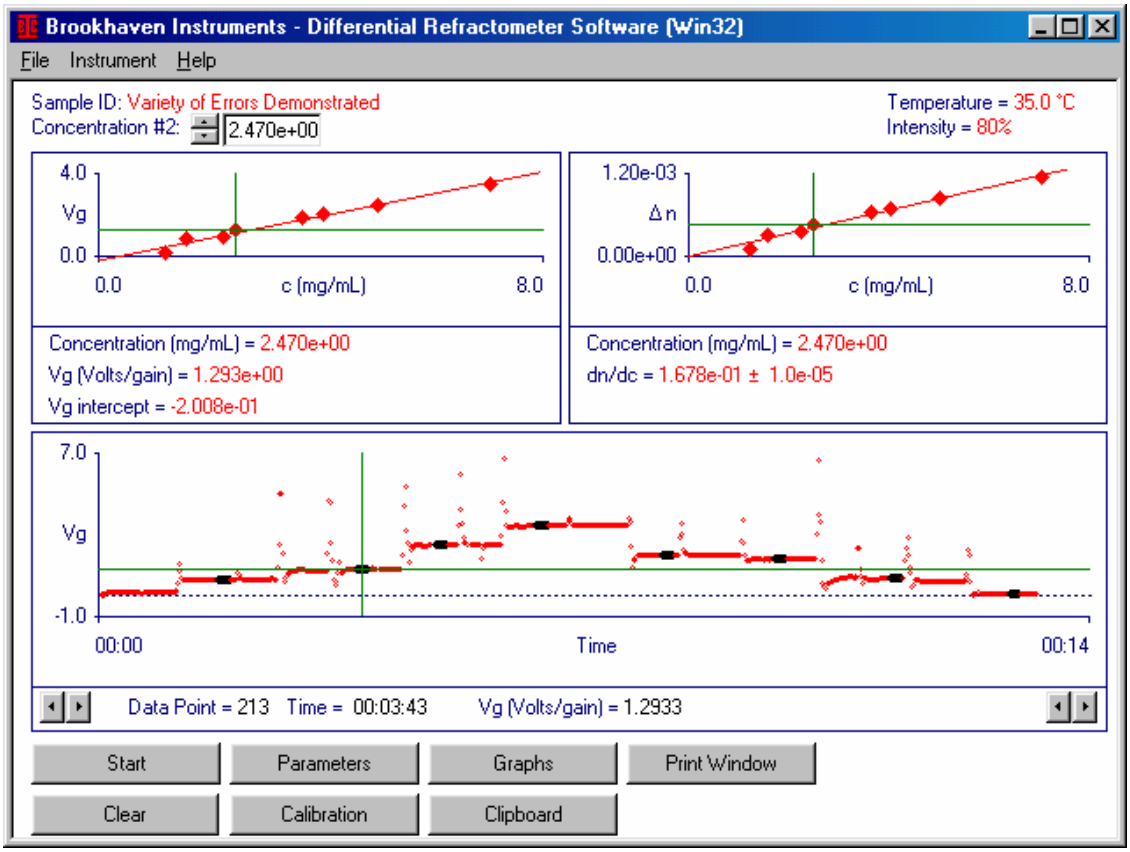
For future reference, here are the expected values for dn/dc at the three possible wavelengths of a BI-DNDC:

dn/dc vs λ , KCl/H_2O

Wavelength	dn/dc @ 25°C
470 nm	0.1408 ± 0.0005 mL/g
535	0.1383 ± 0.0005
620	0.1360 ± 0.0005

Here is an example that demonstrates a variety of errors.

The plot of V_g vs. Time is not monotonic: it rises and then falls. This is because

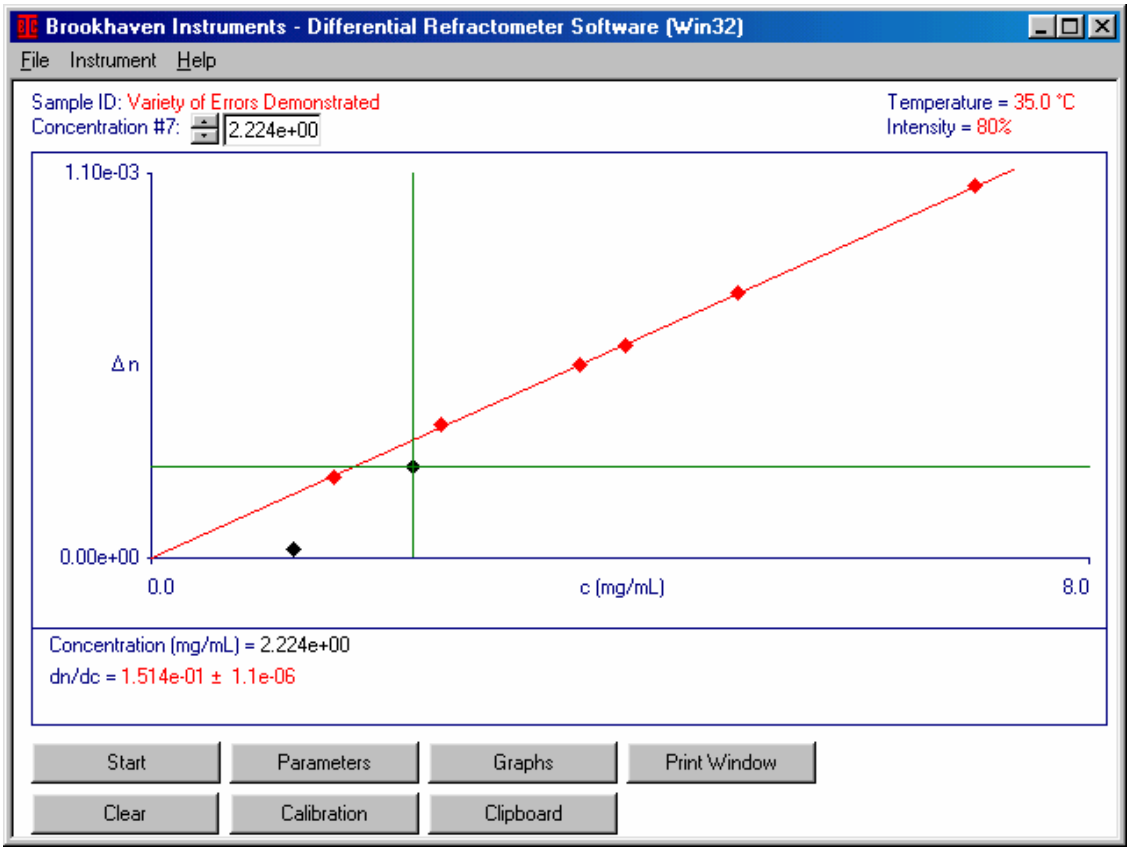


the concentrations were not run in order. Yet, as will be seen at the end of this discussion, it is possible to retrieve apparently acceptable data. If you must run the concentrations out of sequence, then it is best to rinse the syringe and cell with pure solvent between concentrations, then rinse the syringe and cell twice with the new solution before measurement.

Besides the sudden, sharp increases/decrease in signal at the edge of each new injection (due to mixing as previously described), there are mini-spikes that sometimes occur in the middle of a concentration plateau. These may be due to air bubbles, especially if they weren't removed before the syringe was attached. Then, at the end of the syringe travel, after all the solution has been injected, the air bubble is injected.

Note also that for several of the steps, no V_g was determined before something else occurred (a step, for example). In this case, the user decided to remove the syringe—perhaps because it was empty; perhaps because air bubbles were about to be injected— load up with more solution, and re-inject. The software allows this, even though, as explained before, injecting higher concentrations after lower ones can lead to trouble.

For example, the 7th and 8th injections in time correspond to the 1st and 3rd lowest concentrations. The measured Δn values from these two injections are clearly below the best fit of a straight line as evidenced by the following blowup:



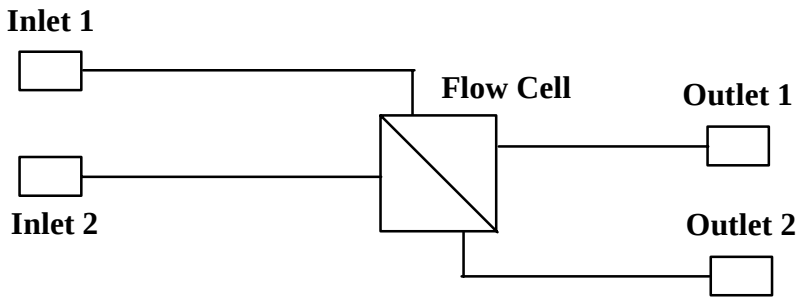
This example demonstrates a few facts. First, always enlarge the plots by clicking on **Graphs**. It is easier to see which points fit best. Second, if you do not inject from lowest to highest concentration as is strongly recommended, make very sure there is no cross-contamination by cleaning scrupulously the needle (inside and out) and rinsing, perhaps more than once, with the next concentration. Third, the lowest concentrations are suspect, especially if injected last, as is the case here. Last, you can still fit a very good straight line provided you have enough points remaining.

The shift in dn/dc is from 0.1678 to 0.1514 mL/g, a nearly 10% decrease. Is 0.1514 mL/g the best answer? If this is all the data available, then it is the best answer at hand. But for truly accurate measurement, it is worth repeating: let the machine warm up sufficiently, and calibrate again with freshly prepared KCl/H_2O of known concentrations.

Then, prepare fresh polymer/solvent solutions taking great care with sample preparation and concentration determination. Some polymers require vacuum drying at low temperature to drive off the residual solvent so that a mass determination corresponds just to the polymer. Still other polymer samples form microgels and require a long time to dissolve.

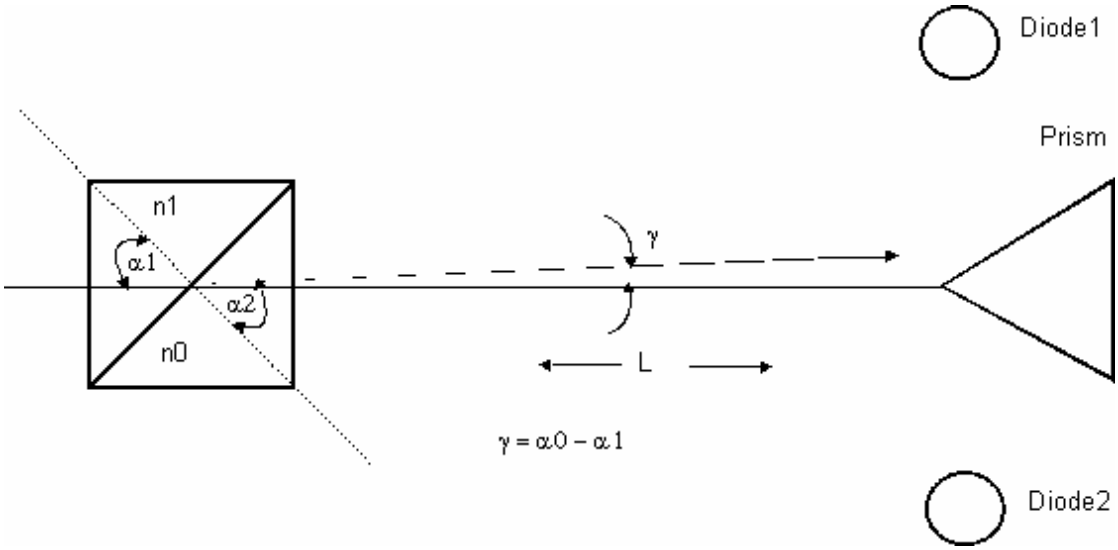
Section VI: Theory: Instrument

A simple flow diagram for the instrument looks something like this:



Other configurations are possible such as a single inlet with solenoids used to route the liquid to either the sample or reference paths in the cell. If you wish to purchase an instrument with another configuration, please contact BIC.

The optical configuration is shown below.



The basic equation that is derived from this configuration is

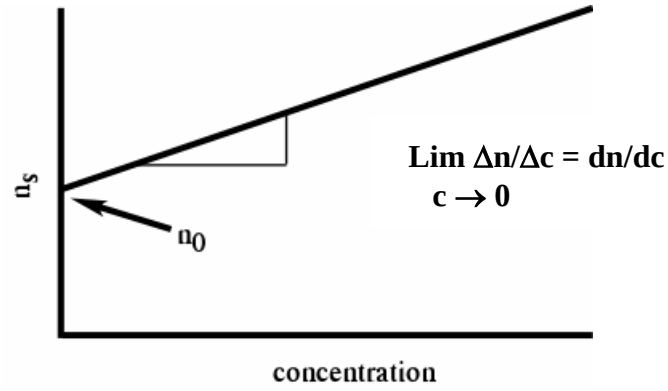
$$\Delta n = k\,\Delta V_g \sim \alpha_1 - \alpha_2$$

Here Δn is the difference in refractive index between the two sides of the split cell; k is the calibration constant and ΔV_g is the gain adjusted voltage that is generated by the difference in refractive index. The diodes and prism serve to convert the angular deviation of the beam into an electrical signal, ΔV_g. The calibration constant converts the electrical signal back to a refractive index difference.

Section VII: Theory: dn/dc

Definition of dn/dc

The differential refractive index increment, sometimes referred to as the specific refractive index increment, or SRII, is the slope of n_s vs. concentration in the limit of zero concentration. Here n_s is the refractive index of a solute/solvent solution. The solute may be a simple salt as in calibration measurements, or it may be a polymer. The SRII is often referred to as just dn/dc. 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.



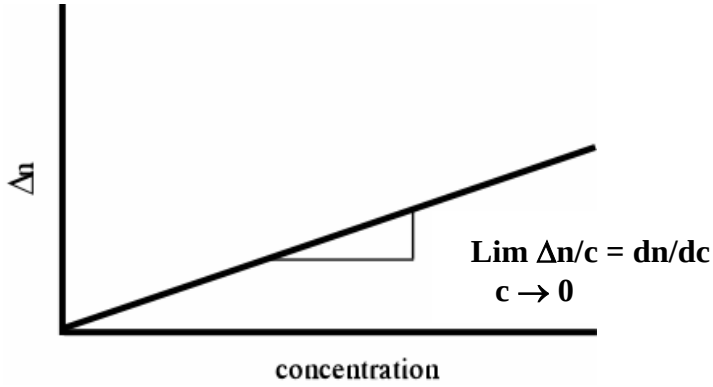
These concepts are illustrated above and serve to define conceptually dn/dc. In practice, the variation in n_s with temperature is larger than its variation with concentration, and the above plot is nearly impossible to obtain with precision unless extreme measures are taken for temperature stability. 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. To improve resolution, the beam is reflected back

through the cell to double the displacement. The displacement is proportional to the difference in refractive index of the solution and solvent-sides of the cell. $\Delta n \equiv n_s - n_o$. The basic plot is illustrated shown below.



The value of dn/dc normally varies from about 0.05 to 0.25 mL/g. Occasionally negative values occur when the refractive index of the solvent is greater than that of the polymer molecule. Since 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 the latter use.

Static light scattering measurements on dilute polymer solutions yield the weight-average molecular weight (molar mass), $\langle M_w \rangle$, the z-average radius of gyration (rms radius), $\langle R_g \rangle_z$, 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

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

where λ_o is the wavelength of the light source in vacuum, n_0 is the refractive index of the solvent, and θ is the scattering angle. 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. Values for proteins in water.

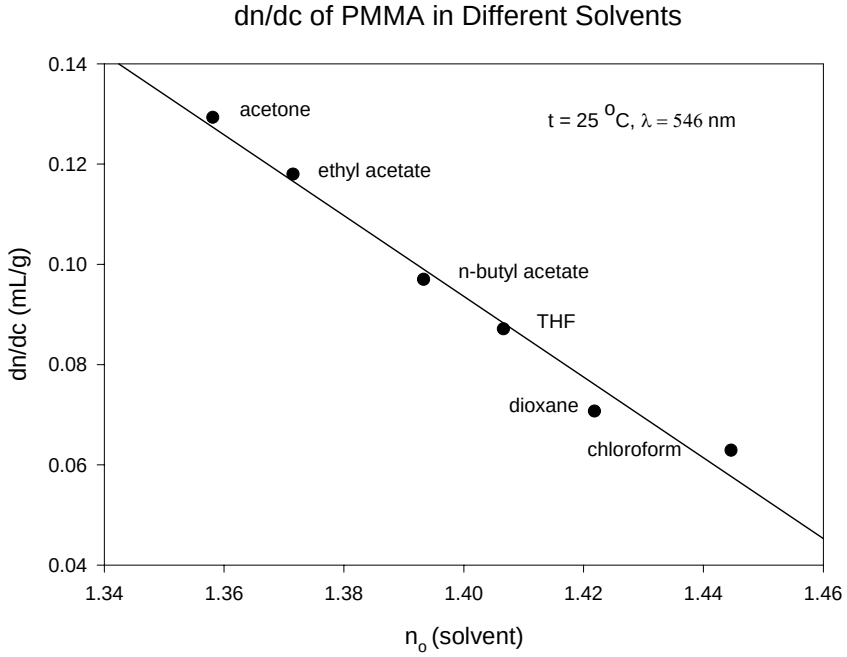
Refractive Increment Data-Book for Polymer and Biomolecular Scientists, compiled by A. Theisen, C. Johann, M.P. Deacon, and S.E. Harding, Nottingham University Press, 2000. Many entries are more recent than the classic compilations above.

The classic values (before the widespread use of lasers, around 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 is from Huglin's book, the first reference 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

dilution is the same in all the solvents. As will be shown in one of the appendices, in this case 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.

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.

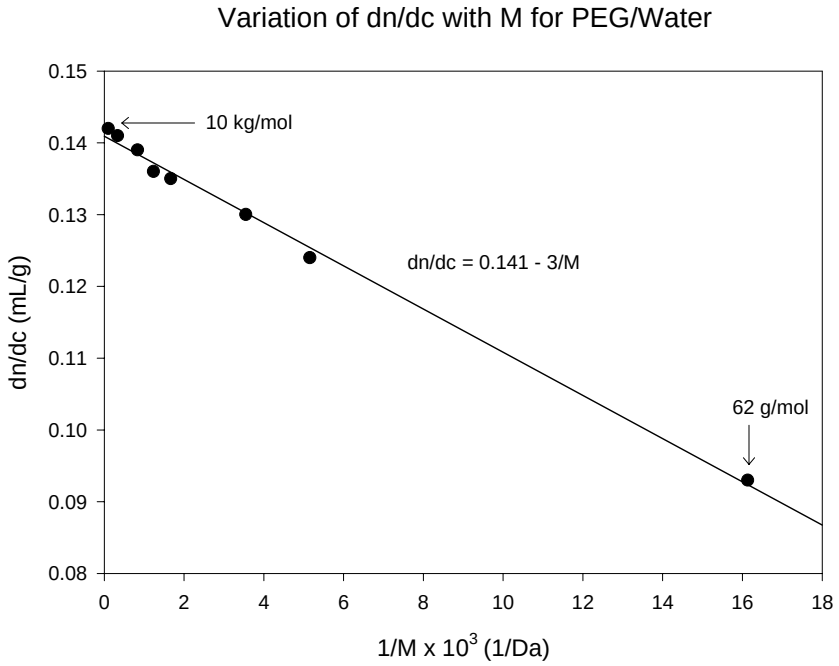
Sample Preparation Skill

As discussed in **Section IV: Making a Measurement: Calibration**, subsection **Preparing KCl Solutions for Calibration**, making solutions of known concentration to better than ± 1% is imperative, better ± 0.5%. Take care not to let solvents evaporate from solutions. This is a serious problem with solvents like acetone, but do not ignore it even with water.

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.

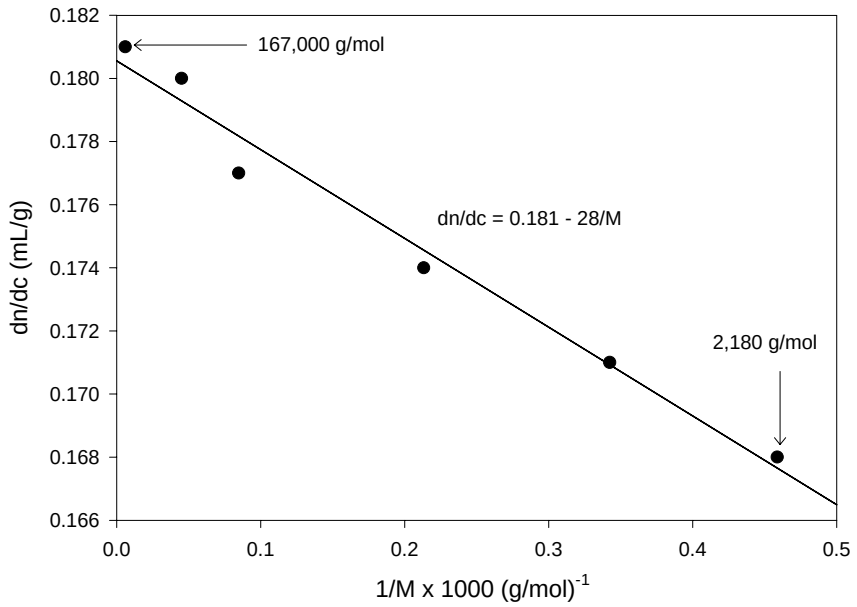
An early example from the literature involved polyethylene glycol in water. Here are the results:



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, $|\, d\nu/dT \,|$ 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 $d\nu/dT$ = 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 1×10^{-4}. If you want to measure that difference to within 2%, you would need to measure Δn with a resolution of $\pm 2 \times 10^{-6}$. 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.

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. Therefore, 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 wavlength 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.

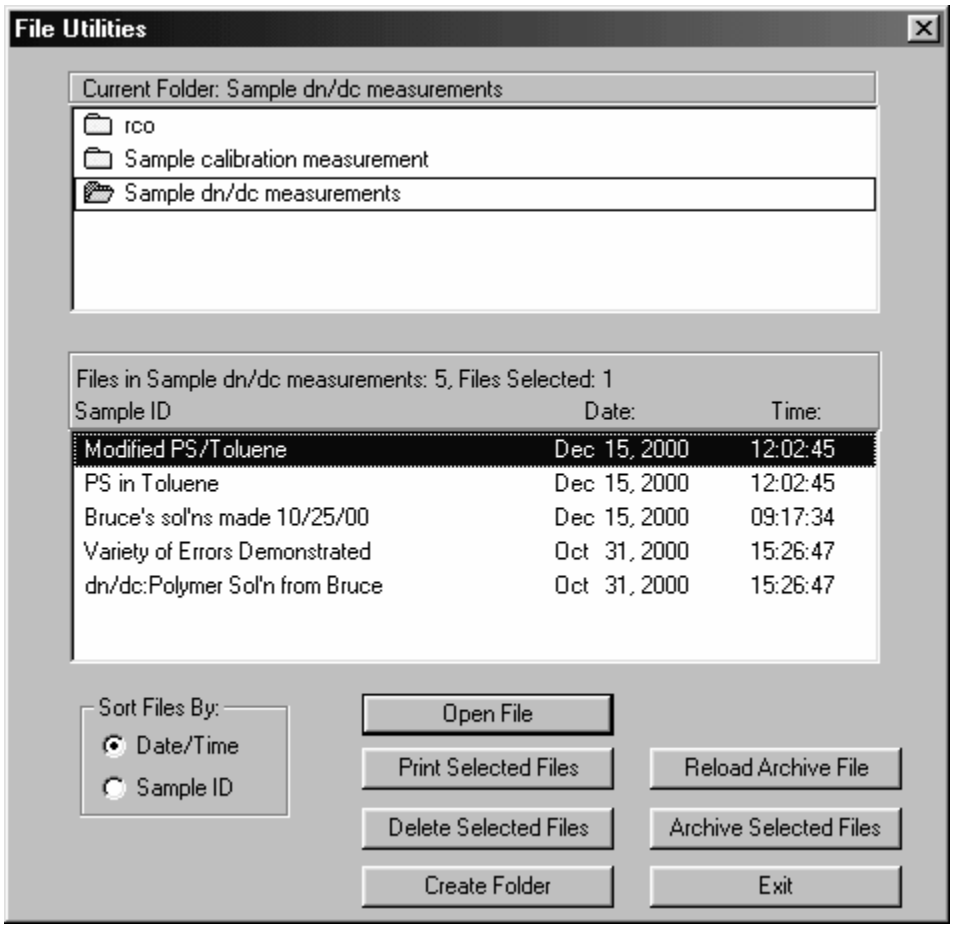
It is up to the user to decide if making a small wavelength correction is useful. If it is smaller than the precision of multiple dn/dc measurements, it is probably not worth the effort.

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.

Section VIII: Database and File Format

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

To create folders, click on **File/Database**. Click on the **Create Folder** command button. Enter a folder name; it can up to 30 characters; it may include spaces and punctuation. Double click on the folder icon to open it. The file folder icon *opens* when the folder is active. Single clicking on a folder selects the entire folder but does not open it. When selected, but not opened, the entire folder and its contents may be deleted, printed, or archived by clicking on **Delete Selected Folder**, **Print Selected Folder**, or **Archive Selected Folder**.



Select a single file in an open folder by clicking on it. Select a string of consecutive files by clicking on the first, then, while holding down the *Shift* key, click on the last. Select a string of nonconsecutive files by clicking on the first, then, while holding down the *Control* key, click on any number of files, consecutive or not, one at a

time. When selected, a single file or multiple files may be deleted, printed, or archived by clicking on **Delete Selected File(s)**, **Print Selected File(s)**, or **Archive Selected File(s)**.

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

Files are **archived** in a binary format in the following path: c:\bicw\dndcw\data\archive.bak using the extension .bak by default. You may change the drive\directory, but you will then have to remember it. If you do not change the filename, the default filename, archive.bak, is used. You can archive an entire folder by selecting it, but not opening it. You can archive single files or groups of files. When archived to an existing .bak file, the selected folder or file(s) are added to it.

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

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

Section IX: Specifications

The specifications of interest when the BI-DNDC is used in the static mode to determine values of dn/dc are as follows:

Split Cell Angle	45°
Total Cell Volume	8 μL
Dead Volume	250 μL, inlet to cell 240 μL, cell to outlet
Solvent RI range, n_o	1.0 to 1.75
Sensitivity	1.5 ng fructose/water detectable
Noise	$<5 \times 10^{-9}$ RI units, 25 °C
Temperature Accuracy	± 0.5 °C, after warmup
Temperature Stability	± 0.01 °C, after warmup
Wavelength	620, 535, or 470 nm
Internal Tubing ID	0.75 mm (0.030”)
Flowrate: Typical/Maximum	1 mL/3 mL/min static mode
Time Constant	0.1 to 2 seconds
Signal Input/Output: Digital	RS-232C, 4800 to 115200 baud
Signal Output: Analog	1 V and 10 V bipolar
Weight	8 kg
Size	160 x 175 x 340 mm

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 μ.

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: $\lambda_m = \lambda_o + 1\text{nm}$. 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). 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 VII, the slope of the line is minus 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

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

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

G-D equation can be written as:

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

Appendix C: Temperature Dependence of dn/dc for KCl/H_2O

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.

Appendix D: Tubing Coding

1/16’’ OD tubing color coding

Upchurch, Alltech (striped or not) and Victrex all 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.