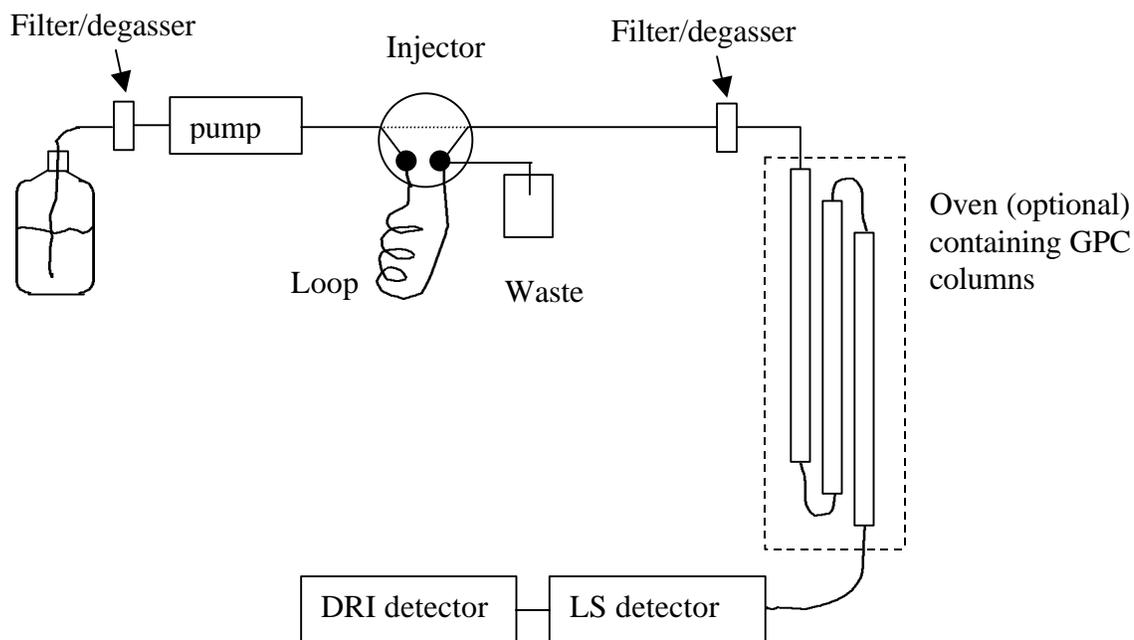


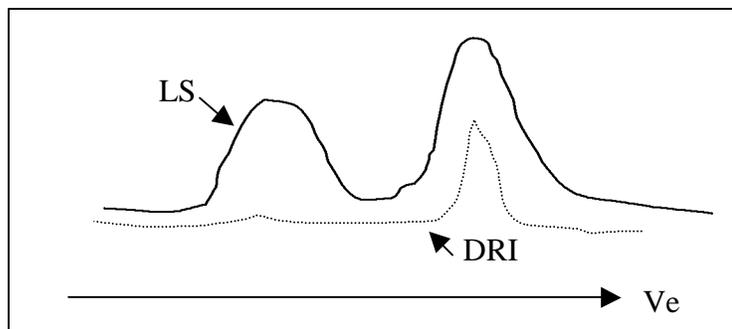
## Gel Permeation Chromatography/Light Scattering

GPC/LS is among the most powerful methods in the polymer characterization arsenal. When coupled with in-line viscosity detection (GPC/LS/Vis) it becomes possibly *the* most powerful weapon available for rapid, routine analysis. It remains to be seen whether new mass spectroscopy methods might ultimately prove better for molecular weight, but since GPC/LS/Vis can also provide shape information, it may last quite some time into the future. A schematic of a GPC/LS device appears below.



A GPC column set is used to separate the macromolecules according to size, in the usual “big first” order of elution. After leaving the column, the molecules flow past a light scattering detector (using one or more angles) and then to a concentration detector. The usual choice for the concentration detector is a differential refractive index instrument. As it is easily damaged by back pressure, the DRI is placed last. If the time lag between the two detectors is well known, the scattering intensity at a time  $t$  can be compared well to the concentration of scatterers in the cell. The intensities may be converted to the Rayleigh factors in the usual way, as for a conventional Zimm plot (although some compromises are usually required). If the intensities are measured at sufficiently low angles, or if multi-angle detectors are used so that low-angle intensities may be obtained by extrapolation, then absolute molecular weights can be obtained “on the fly.” If the column separates the polymers well, then not only all the moments of the distribution, but also the distribution itself, can be recreated. All this in the same time, about 20-60 minutes, as a simple GPC run. Additionally, GPC/LS can see certain components that GPC misses. This is because the differential refractive index signal in normal GPC is proportional to concentration,  $c$ , while the light scattering signal is proportional to  $cM$ ,

where  $M$  is the molecular weight. This is very handy in identifying the existence of high-mass components present at low concentrations, as in some preparations of polyethylene. It is also useful in identifying aggregates. This type of result is shown schematically below.



The DRI barely shows a blip for the early peak. Since this is a large polymer, the LS detector sees a large peak. Serious problems in industry (like how to copy your competitor!) are solved by such observations. Another cool thing to do with GPC/LS (with a multi-angle detector or one of the systems that fake multi-angle operation using only 2 or 3 angles) is to obtain the distribution of radius. Given a sufficiently broad sample, one can obtain radius vs. molecular weight in a wide range--and thus infer the shape!

That is the promise of GPC/LS. The reality is not very far different, but the experiment does require extraordinary care and patience. One should not expect to walk into an idle GPC/LS lab and have an answer in one day. Even if the pump is working already! To the usual demands of LS (elimination of stray light, removal of “dust”, and careful alignment) GPC/LS adds all the paraphernalia of GPC (pulsating pumps, leaking seals, check valves, gallons of solvent to de-gas and dispose of, easily damaged columns). The calibration steps are somewhat tricky compared to normal LS. It’s a wonder it works at all, but then chromatography equipment is designed...how to put it gracefully?... ”with the intelligence of the end user in mind.”

### **Complications and Simplifications to the Light Scattering**

The alignment in time between the LS and concentration detectors is essential, and will be particularly difficult when analyzing narrowly distributed material with sharp elution peaks. These are the kinds of samples desired in the best physical chemistry research, so if the GPC/LS is supporting this type of effort, it is especially important to do the time alignment very well. The instrument should be set up with narrow bore tubing between the detectors, and the lengths kept to the absolute minimum. Also, for materials of this type, traditional light scattering (e.g., Zimm plots on a serious instrument) might be preferable. The main functions of the GPC/LS should be analyzing polydisperse samples and guiding synthetic chemists in their quest to make better samples. GPC/LS can also be

especially useful for studies of branching and shape, since the radius vs. molecular weight behavior can be obtained.

Like normal LS, GPC/LS requires that the Rayleigh factor be measured. There are two approaches: absolute Rayleigh factor detection (KMX-6 type machines) and *calibration* with a known solvent (all other machines). Our Wyatt DAWN machines use calibration, and it is actually somewhat simpler than in normal LS. The toluene sample is simply flowed through the cell. Unfortunately, the instrument may not totally exclude stray light, and this can corrupt the calibration.

Multi angle experiments with a rotating arm detector are impractical for GPC/LS: the detector would have to move very fast. In multi-detector experiments (e.g., our DAWN instruments) all the detectors must be aligned not only with the concentration detector, but with each other. Additionally, in order to prevent line broadening, the volume of the detector must be kept small, and its shape must not induce turbulence. A complication is that the electrical sensitivities of the various detectors are not identical, even when each is presented with the same light intensity. Also, most of the time, the detectors will *not* be presented with the same intensity, even for a Rayleigh scatterer, since the detected volume usually varies with scattering angle. These problems are solved through the process of *normalization*.

These are all serious constraints on light scattering, but there is one very important advantage which applies even if one is not doing a chromatographic separation: *if the flow cell is fixed, then so is its stray light*. The stray light in a conventional instrument, such as the one used to make a Zimm plot, varies with cell position, scratches on the cell, etc. That is why we are so careful to position each new sample to reduce the stray light when making Zimm plots. Poorly designed systems, specially polished and expensive glass cuvettes may even be required, or the beam may have to be tightly focused to hit just a tiny region of the cell. Such strong focusing leads to beam divergence and poorly defined scattering angles. The stray light in a flow cell may be a little bit higher than normal, due to its small dimensions. In the standard-issue Wyatt DAWN that we use, the weak laser beam necessitates rather poor volume definition, which adds again to the stray light problem. But as long as the stray light from a flow detector does not overwhelm the detector, it should be possible to subtract it off, since it is constant. In fact, the Wyatt software enables you to perform "micro-batch" experiments: Zimm plots using the flow cell. Unfortunately, the sensitivity of the Wyatt cell's stray light pattern to pressure changes requires a good syringe pump for this to work effectively.

## Operating a GPC/LS from the Very Beginning (“*Aller anfang ist schwer,*” *sagdt der dieb--und stahl einen amboss.*)<sup>1</sup>

About 20 years ago, most computers were equipped with several rows of toggle switches. You didn't just turn them on and start up your word processor. Rather, you turned the computer on and went through a large list of toggle switch settings from a book to "boot" the computer (the term comes from the saying, lifting oneself up by one's bootstraps, to indicate a difficult beginning). Starting a GPC/LS involves a fair amount of bootstrapping, too. The following instructions apply mostly to our DAWN type apparatus. You must:

- Take all the precautions of normal GPC, paying special attention to good pump operation and column performance.
- This means starting with a determination of the number of theoretical plates!
- Bite the bullet and read the cryptic instrument manuals.
- Align the LS apparatus (only if necessary; consult an expert user)
- Calibrate the DRI detector (unlike normal GPC, we will actually try to get *real* concentrations from it).
- Ensure the software is correctly configured.
- Rayleigh calibrate the LS detector at one angle.
- Normalize the other detectors (if doing a multi-angle measurement).
- Align the DRI and LS detectors.
- Check with one or more standard materials.
- Measure dn/dc for your material (the specific refractive index increment).
- Measure your material.
- Repeat measure your material at least two more times.
- Fiddle with analysis; there are usually many options and user choices to optimize.

Normal GPC Precautions. See GPC HowTo.

### Bite Bullet...

GPC/LS is a complex business, and I could never prepare a document as detailed as the instrument manuals. You should at least *scan* through the instrument manuals (especially the Astra section and theory sections) to familiarize yourself with the calibration, normalization and other steps. Then read the rest of this HowTo document, but keep the manual around as you actually begin your work.

The following mostly applies to our Wyatt DAWN instruments. First of all, realize that there are *two* of them: an ambient temperature instrument currently dedicated to aqueous work and a variable temperature instrument for non-aqueous work. The two use slightly

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<sup>1</sup> “All beginnings are difficult,” said the thief and he stole an anvil.

different cells, with important optical consequences. A computer is devoted to each instrument. You should always use the software as configured on the appropriate computer because it reflects the right optical settings.

Align the LS Apparatus. Ask us first if this is necessary! Probably not, because commercial GPC/LS instruments are designed to stay aligned for long periods of time. If you feel that you absolutely must align the machine, consult me first!

Calibrate the DRI detector. In normal GPC, you don't really need to know the exact concentration--you just need a signal proportional to it. But the guiding principle of GPC/LS is that, to first approximation,  $R = KcM$  where  $R$  is the Rayleigh factor and  $K$  is a known constant (involving  $dn/dc$ ). Thus, to get  $M$ , you must actually know  $c$ ... *accurately*.

The purpose of DRI calibration is to measure  $dn/dV$ , which is the inverse of how much voltage change the device produces for a certain difference in refractive index between the sample and reference sides of the two-chamber DRI detector. Once  $dn/dV$  is known, you can convert above-baseline signals ( $dv$ ) to concentration by applying the  $dn/dc$  value, *which must be known*. More specifically, you can get the difference,  $dc$ , in concentration between the reference side of the DRI detector and the sample side. However, since the reference side is usually just pure solvent,  $c = dc$  and we have:

$$c = dv \cdot (dn/dv) / (dn/dc)$$

The Wyatt manual says, effectively, "calibrate your concentration detector." Only very sketchy instructions are provided. In this section, we fill that gap. It will also be shown that the DRI detector can be used to measure  $dn/dc$  if it is not already known. The accuracy is less than ideal, but the convenience compared to the usual very slow way of measuring  $dn/dc$  makes it tempting. This is especially useful for our "polymere du jour" organic friends, who can make things faster than anyone can measure them.

There are two ways to calibrate the DRI detector. In the first, a series of samples of known concentration and RI is injected. The samples are large enough to produce a steady signal for at least a few seconds, which is measured....and that's it. The second way is to inject a single solution of well known concentration and integrate under the peak to find the calibration constant. The second method is faster, requires less sample and no messy retooling of the instrument. However, it fails if any material adsorbs to the column, and it relies on a very good baseline estimation. (A small baseline error in the first method is just an intercept term that can be ignored during this calibration--but not during measurement of course). Both methods should be made to agree for careful work. The Waters 590 is very stable because it uses a light emitting diode (red) for its light source and stable amplification and detection schemes. Still, DRI calibrations should be repeated "often." A sensible strategy would be to check for instrument drift using Method 2. Worsening agreement between the injected mass of sample and the calculated mass from the Astra program also are a sign of a problem.

### *Method 1. Series of samples*

*Calibration Solutions.* You will need a pure polymer whose  $dn/dc$  is well known in some solvent. Ideally, this would be the solvent currently running in the GPC, but since the columns and LS detector will be disconnected (see next step) it is not too bad if you need to change solvent. Do it slowly, though, to give the pump plenty of time (i.e., volume) to re-wet all vital parts. A logical choice to calibrate the system would be polystyrene in THF. It is *not* important to use narrow-distribution polystyrene standards in this step--the polystyrene just needs to be pure and relatively free of very low-M materials (which may have slightly different  $dn/dc$ ). We assume PS/toluene in what THF.

1. Use vacuum-dried Dow 1683 PS. It is easy to make a nice series of solutions by placing 1,2,3,4,5 pellets into 50-mL volumetric flasks. Weigh on 5-place balance! As each pellet is approx 15 mg, this will give you a range of about 0.3 to 1.5 mg/mL after solvent is added. You could use lower concentrations, but then you might have to chop the PS pellets or use larger volumetrics.
2. Be sure the solvent being pumped is pure: free of water and other impurities.
3. After stirring overnight, remove the stirring bar, if any, and rinse it thoroughly with solvent to carry any polymer from the surface into the volumetric. Top off the volumetric *after* the polymer has dissolved, again using the same solvent from the pump's source. Be careful not to overfill it!
4. Cap each volumetric and invert *at least 25 times* to homogenize the solutions.

### *Plumbing*

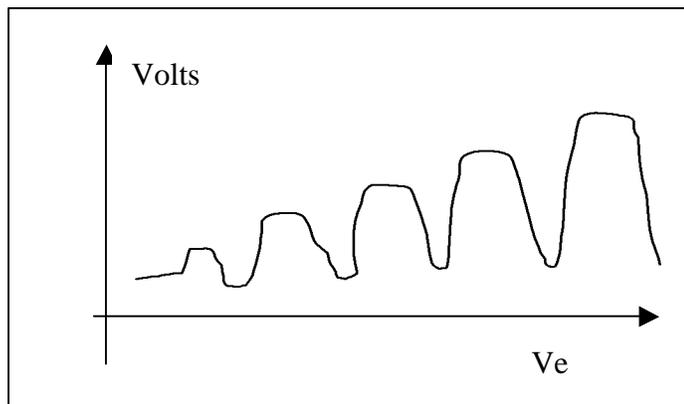
1. Turn the pump off (as usual, reduce the flow rate in small increments separated by about 1 minute. For example, if the pump is running at 1 mL/min, set it down to 0.75 mL/min, wait about 1 minute, then set to 0.5 mL/min, wait 1 minute, go to 0.25 mL/min, wait, then finally set the pump to 0 mL/min. These steps are designed to spare the columns from sudden and damaging pressure pulses).
2. Disconnect the column(s) from the injector, but leave them connected to the LS instrument for convenience.
3. Screw a plastic plug into the column end to prevent it from drying out.
4. Disconnect the LS instrument from the RI detector.
5. Lightly screw a "dead end" plug onto the lead from the LS detector to keep it from drying out.
6. Connect the output of the injector directly to the RI detector.
7. Disconnect the injector loop, which is probably 0.02 mL to 0.5 mL in total volume.
8. Connect a new loop, about 2 mL total volume (we have a nice Teflon loop for this purpose). This is a huge injection volume, but we do want flat peaks. (Note, this step doesn't apply if you are using the U6K injector, which has variable injection volume).

9. Following the guidelines above, turn the pump back on slowly (even though the columns are out, you must make only gradual pump flow rate changes: the crystals in the RI detector are delicate--and the columns are not present to soak up any pressure jumps). Do not exceed 1 mL/min.
10. After you reach the final pump rate, flush the reference cell for (Purge command on the Waters 590 Solvent Delivery System).
11. Re-zero the RI detector (Autozero command on the Waters 590). Consult the manual for the older Waters DRI.
12. Repeat steps 9 and 10 until the system is utterly stable: drifts of about  $\pm 5$  on the RI detector's digital output for hours at a time.
13. When all is ready, set the Wyatt Astra software for a single long run. Inject all samples, waiting a minute or so to establish a clean baseline between each.
14. Save the data and run it through MakeAsc.Exe program (Old DAWN) or use the export facility (New DAWN). This lets you write out what was measured as a text file. Choose only the RI output.

*Instead of putting a new loop onto the injector, one can use the U6K injector, placing it in-between the pump and the DRI. The U6K allows for variable sample volumes, up to several mL, controllable by syringe. It is a convenient way to direct-inject a concentration series of samples. Good conditions for the U6K: 3-5 mL of injected sample @ 0.8 mL/min.*

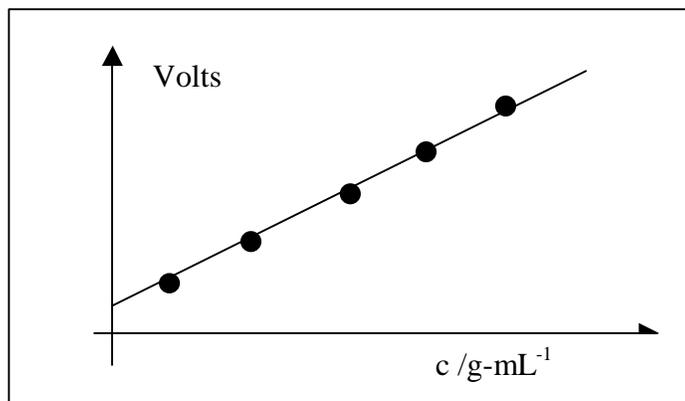
#### Computation

The output text file can be imported into any graphics routine--e.g., Origin, Excel, etc. It should look something like this:



Each concentration should be characterized by a peak with a *flat top*. If the tops are not flat, then a larger sample must be run. You could also try running at a higher flow rate (to reduce diffusion at the interfaces)—but do not exceed 1 mL/min to avoid damaging the DRI. Do get the peaks flat before proceeding. Plot the peak height  $dV$  (voltage output of RI) against concentration,  $c$ . Get the slope  $dV/dc$  from a linear fit. In the example below, the intercept is huge because the THF used to prepare the samples was not the same as was being pumped

through the machine. This is annoying but not fatal because it is the slope that matters.



You know the value of  $dn/dc$  (e.g., 0.185 mL/g for PS/THF for red light of the type the Waters 590 uses or 0.19 mL/g for aqueous bovine serum albumen or 0.1346 mL/g for M~114,000 PEO). Obtain  $dn/dV$  as:

$$\frac{dn}{dV} = \frac{dn/dc}{dV/dc}$$

*Measuring the  $dn/dc$  of an unknown a'la Method 1.* After you know the instrument constant ( $dn/dV$ ), you can just measure  $dV/dc$  for the unknown by again injecting a series of carefully prepared solutions. Then get  $dn/dc = (dn/dV)(dV/dc)$  for the unknown. If a solvent change was required, be sure to rinse the pump thoroughly and, especially to flush the reference chamber of the RI detector.

The appendix shows what *real data* look like!

#### *Method 2. Single sample calibration of RI detector*

*Sample(s).* Prepare a single sample (or a series, if you like!) following generally the guidelines of Method 1. Specifically, pay close attention to knowing the concentration very well. It is even more important to use the same THF as the pump does. You probably do not need such large samples. Enough for triplicate injections should be OK.

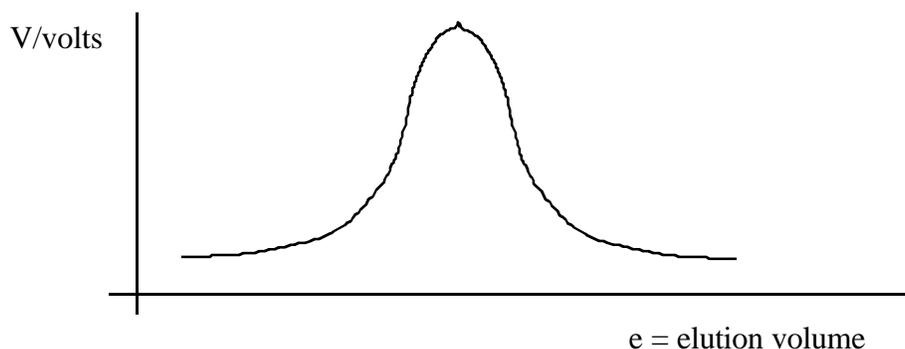
*Instrument Preparation.* Essentially, none! It would be wise to test that the pump is delivering its rated output so that volume is strictly proportional to time. This can be done, as usual, just by timing the output into a graduated cylinder or

weighed bottle. For volatile solvents, it's better to pump into a syringe, so you don't have the odor.

*Loop Volume.* This section assumes you are using a Rheodyne or other injector that meters out the injected volume using an injection loop. Another type of injector, the U6K, is sometimes used; it offers some advantages, one of which is that you can use calibrated syringes to inject. Anyway, if you are using a standard "loop" injector, then you *must* know the volume injected to use Method 2. Even the nice commercially prepared loops are only accurate to about 20%. To determine the loop volume, remove the injector loop and clean its outside from fingerprints, grease, etc. *Temporarily* remove plastic labels that manufacturers sometimes attach. Select a pure, inviscid liquid that is volatile (but not too volatile). Its density should be large, well known and not very sensitive to temperature. Reasonable choices might be carbon tetrachloride or ethylene dichloride. Connect the injection loop to a syringe holding the liquid and place them in an oven (or just a box) at known temperature. Force liquid through the injector, dry any excess liquid from the outside, and weigh carefully to 5 places. Now, place the injector into a vacuum oven, remove the liquid, and reweigh. Again place the injector into the vacuum oven and reweigh to test for complete removal of the fluid. Compute the volume from the known density. The volume from the inner diameter and length of the injector should be computed for comparison.

#### *Measurement*

Just inject as you might for any normal sample to be analyzed. Three to five runs should be made for decent statistics. Save the files and convert them to ASCII using the MakeAsc program (Old DAWN) or export facility (New DAWN). They will look like this:



The voltage (above baseline) from the RI detector can be written:

$$V = k_1 \Delta c = k_2 \Delta n$$

where  $k_1$  and  $k_2$  are constants connecting the output signal (above baseline) to either the concentration difference  $\Delta c$  or refractive index  $\Delta n$  between the reference and sample sides of the split cell RI detector. The area  $A$  under the curve (and above the baseline) is easily measured with a program like Origin. It is given by:

$$A = \int V de = k_1 \int \Delta c de$$

Here,  $de$  is the elution volume increment. The area  $A$  would typically have units of Volt-mL. The integral on the right-hand side of the last equation is just the total mass injected,  $g$ . This is *known* because the concentration and injection volume are known. Thus,  $k_1$  is obtained:

$$k_1 = A/g$$

The first equation of this section, defining  $k_1$  and  $k_2$  can be solved for  $k_2$ :

$$k_2 = k_1 \Delta c / \Delta n \equiv k_1 / (dn/dc)$$

Since  $dn/dc$  is known (i.e., 0.185 mL/g for PS in THF at or near the wavelengths used by the Waters 590 RI detector) and  $k_1$  has just been measured from the integral and the known injection volume, you can get  $k_2$ . The Wyatt software appears to want RI increment per volt--i.e., the Wyatt wants  $dn/dV = 1/k_2$ . The inverse of  $k_2$  should be determined several times to avoid problems with setting the baseline, doing the integral, etc. Ultimately,  $dn/dV$  should agree with the value from Method 1.

Caveats about Method 2. This method is convenient, but.....what if some of the polymer sticks to the column, is lost on a pre-column filter, etc.? Admittedly, you can get around this problem by removing the columns and just injecting into some widebore tubing. Perhaps a more serious weakness to Method 2 is that the injector loop volume cannot be determined very well. The weights to be measured are small and, even ignoring this, any dead volume between the injection loop and the injection is not included. If the  $1/k_2$  value disagrees with the Method 1 measurement, you should be very suspicious.

Note: if you have performed method 1, then you can inject a sample (skip the columns) and re-arrange the equations above to compute the volume of the loop *as it is actually connected*. Thereafter, Method 2 would be convenient.

### *Measuring an unknown dn/dc a'la Method 2*

Once  $k_2$  is known, the  $dn/dc$  for some other polymer/solvent pair could be measured:

$$dn/dc = k_1/k_2$$

where  $k_1 = A/g$  is obtained from injection of a known mass of the polymer whose  $dn/dc$  is not known. The measurement should be repeated because there is always some uncertainty in estimating the integral. Values of  $dn/dc$  determined this way should probably be regarded as provisional because of the errors in integrals and how they get magnified by taking the quotient above. For the best work, a conventional differential refractometer (like the old Brice Phoenix device) should be used.

### Ensure Correct Software Configuration

The new Astra software is pretty straightforward--follow the manual. Start by creating a new file (process menu/select directory followed by sample run info and establish the name). You must enter cell type (it's taped to the DAWN) and solvent/Rayleigh standard refractive indices in BOTH the SETUP screen and in the CALIBRATION screen. Save the correct values as Defaults. Load them again! Get out of the program and start it again to see that the settings are right! In both screens! It matters!

### Rayleigh Factor Calibration.

Just as with conventional static light scattering (see the Zimm Plot HowTo for the GuiDe program) it is necessary to convert electrical signals into the Rayleigh factor. On KMX-6 type instruments, this is done absolutely. DAWN and most other instruments require a calibration. The procedure is conceptually similar to that used when collecting data for a standard Zimm plot on our conventional instruments. Extra problems arise because the DAWN is not as good at excluding stray light.

Step 1. Prepare the DAWN cell to receive toluene (*not necessary if you are using toluene as the solvent already!!!*)

- Detach the low-dead volume (narrow bore) tubing from the DAWN flow cell and replace it with wider-bore tubing (this will decrease the pressure you need to apply to make samples flow).
- Be sure the new tubing does not leak, but don't overtighten!
- Attach a 0.1  $\mu\text{m}$  or smaller pre-fabricated filter (solvent compatible!) to the inlet.
- Using a syringe, flush 20-50 mL of a good solvent for the last polymers run through the DAWN to remove all traces of it.
- If the previous solvent was an aqueous buffer, flush 20-50 mL of clean water.
- Inject 20-50 mL of appropriate solvents to prepare the system for toluene (e.g., after water, you might go to acetone first and then toluene).
- Remove the little cap so you can view the solvents flowing through the DAWN cell, preferably with the little surface magnifier supplied by the manufacturer.

- Place the syringe at least one foot above the DAWN and remove the plunger. Allow a slow gravity feed. This avoids pressure pulses that can adversely affect the DAWN's stray light characteristics.

Step 2. Run the DAWN calibration program. The instrument will collect data and it should be steady to within  $\pm 1$  mV. The program will also ask you to turn off the laser to obtain the dark count, and it will instruct you to turn it back on again.

Step 3. Pray there is no stray light. The DAWN gives you no independent method for testing for the absence of stray light. You will simply get the wrong answers if some of the light that was supposed to emanate from within your sample comes, in fact, from stray light and if the stray light is not constant. If it subtracts out cleanly, and if the detectors have sufficient dynamic range, then even stray light isn't *too* bad. Our experience, however, suggests that stray light leads to noise. For example, the DAWN cell is not pressure-perfect: its dimensions fluctuate some with pressure pulses. When there is stray light of any kind, this also fluctuates. See the instructions on "fiddling" below.

### Normalization

- As mentioned previously, the multiple detectors do not respond equally to light. Given the same signal, they do not produce the same output. Also, the detectors do not see the same volume, may lose different amounts of light to reflections, etc. For a general description of this kind of problem, check the *LS Intensity Measurements* HowTo. The Wyatt manual deals with the problems in detail, but here are the main steps.
- Method 1. If you are getting very nice quality signals, you can simply inject a solution as in normal operation. The new DAWN has built-in normalization routine in Astra (use the Help menu and search 'normalization'). So does the old system, but the commands are located in a completely illogical place (the Renormalize menu is hidden under the Debye analysis section; seek and ye shall find). This may be the best way to normalize the system, because the DAWN cell would be operating at normal pressure (therefore, be distorted to the same degree as during measurements). However, there is one disadvantage: the detectors do not look at precisely the same spot in the cell. If you are normalizing on a very sharp peak, this might produce some skew. A compromise solution might be to insert a long length of wide-bore tubing between the GPC column and the DAWN to enable diffusion to broaden the peak. Larger injection volumes may also help.

Method 2. Another way to perform the normalization is by introducing a large quantity of an appropriately small, nearly monodisperse Rayleigh scatterer in batch mode using a syringe, as in Rayleigh calibration. This will produce a level signal at all detectors and prevent skew. The Astra software can be used normally (set baseline on the solvent before injection and use Peak ID to select a steady signal). One has as an

option the ability to use the DAWN software (instead of Astra). The numbers can be copied or typed into the normalization file. A problem with this is getting the desired flow rate so that the pressure-induced distortions to the DAWN cell are the same as they will be during measurement. Probably the best approach is to try Method 1 first.

- The Rayleigh factor of the small polymer used for normalization need not be known. It is only important that the polymer is small enough to scatter equally in all directions, and it must be in the same solvent that you will use for your actual measurements. If you have any doubts, try measuring the radius on the conventional SLS machine (see the Guinier HowTo). If you *fail* to get a decent radius, the polymer is small enough! This normalization polymer solution is used to establish the differences among the detectors. In principle, you could have done this with the toluene, which is a Rayleigh standard--but there would have to be no stray light (and, as already mentioned, that's not guaranteed in the Wyatt, especially at small angles). The purpose of the normalization solution is to give a strong, angle-independent scattering signal that will drown out any stray light. The need to get these instrument response factors with a polymer solution (a microemulsion would also work) rather than from toluene or some other pure solvent is an admission, really, that the DAWN does a mediocre poor job of excluding stray light compared to a conventional instrument. As discussed in the *LS Intensity Measurements* HowTo, there are both additive and multiplicative factors affecting the light intensity at a detector. In the Wyatt, it is hard to separate these effects, and you basically wind up hoping that the detector-to-detector normalization is based totally on multiplicative effects. In short, you put in such a strong scatterer that any additive signals (stray light) are negligible. There is no way to test whether or not this is really so. In a conventional instrument, the geometry is well known (the scattering volume follows a simple  $(\sin\theta)^{-1}$  relationship) and you can TEST for the absence of stray light by seeing how well the solvent adheres to this expectation (typically, to within several percent). The geometry of the DAWN is weird; in the manual, the builders claim to have combined analytical optics with ray tracing and empirical testing to arrive at the optical geometry.
- It's a good idea to print out the normalization constants and store them in a file. Try to remember some of the numbers, in case the DAWN software (which can be somewhat tricky) serves up the wrong file sometime (in the old Astra software it is very easy to make mistakes).

## Run Some Standards

- If necessary, hook the columns back into the system and allow it to run *to waste (not recycle)* overnight before attempting to do serious work. It takes awhile for the DAWN cell to rid itself of all the dust, and for the columns to purge themselves of any crud that formed while they were disconnected.
- Look at the log-book. It has a place for you to put the  $dn/dV$  value, the calibration constant (DAWN constant; see the manual--it's modified by the software after you determine it) and also the normalization file in use.
- Narrow polystyrenes are good choices for non-aqueous work, while bovine serum albumen is a good choice for aqueous.
- For BSA and medium polystyrenes, the total load (product of loop volume and concentration) should be around 1 mg or less.
- Lower concentrations are always better, IF you have enough signal in *both* detectors. For very high molecular weights, it's easy to get good LS signals and have almost no DRI signal. For very small molecular weights, the opposite is true.
- Change loop volumes or concentrations to get decent results.
- Concentrations should remain below 1% to avoid loop plugging and fouling the injector. If you need more polymer, put on a bigger loop.
- Once you have good signals, run the sample *several times*.
- Inject a narrow-distribution standard of modest molecular weight. Consult the manual to *align the detectors!* The alignment step is important, because it's how you compare concentrations of polymers in the DRI at one time to their LS signals at some previous time. Be sure to save the alignment value.
- Refer to the DAWN manual for analysis. Some tips:
  - Avoid using noisy data. You can get really wild answers when either detector has only a small signal. For example, a weak DRI response means the total signal is very uncertain. This can lead to large errors (huge!) in molecular weight.
  - Don't just trust the Auto Baseline feature. Look at each angle.
  - Exclude bad angles! Sometimes, a bit of dust or bubble occludes an angle, and it will be weird.
  - Consider carefully whether or not you NEED to use the low angles. Small polymers don't need low-angle data, and it's invariably noisier.
  - Repeat the analyses with different baseline and peak selections; you'll get some idea of the true uncertainty of the measurement by doing this! (Hint: several percent is good).

## Fiddle

Recent experience suggests that the machine really can nail the correct answers for standard materials after a complete calibration/normalization procedure. Unfortunately, it has sometimes been our experience that, when the Rayleigh factor calibration comes from toluene, the measured molecular weights are “off” by perhaps 10% compared to  $M$ 's from a machine known to be reliable (e.g., the KMX-6 at Dow or our own instrument). This is

because the Wyatt cannot eliminate all the stray light and so cannot really get an accurate reading on the intensity from toluene, even at  $90^\circ$ . A somewhat unsatisfying solution is to fine tune Rayleigh calibration coefficient until the  $M$ 's agree with known standards over a wide range of molecular weights. It must be emphasized that these are *small* changes; the toluene calibration gets you pretty close to the right answers. The very best solution to this problem is to re-align the laser until the data are not even 10% off.

Reference: [http://www.waters.com/waters\\_website/applications/polymer/gpc\\_chem.htm](http://www.waters.com/waters_website/applications/polymer/gpc_chem.htm)

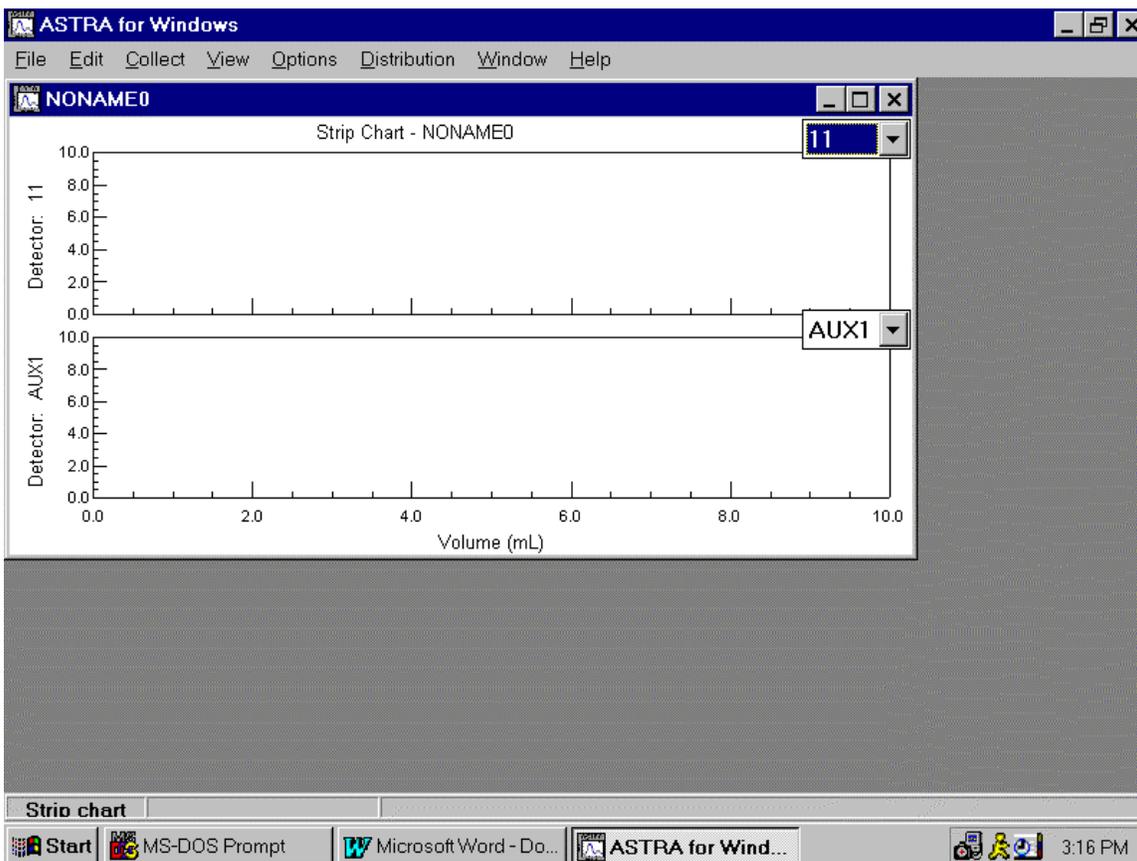
# A Sample GPC/MALLS Experiment

## COLLECTION PHASE

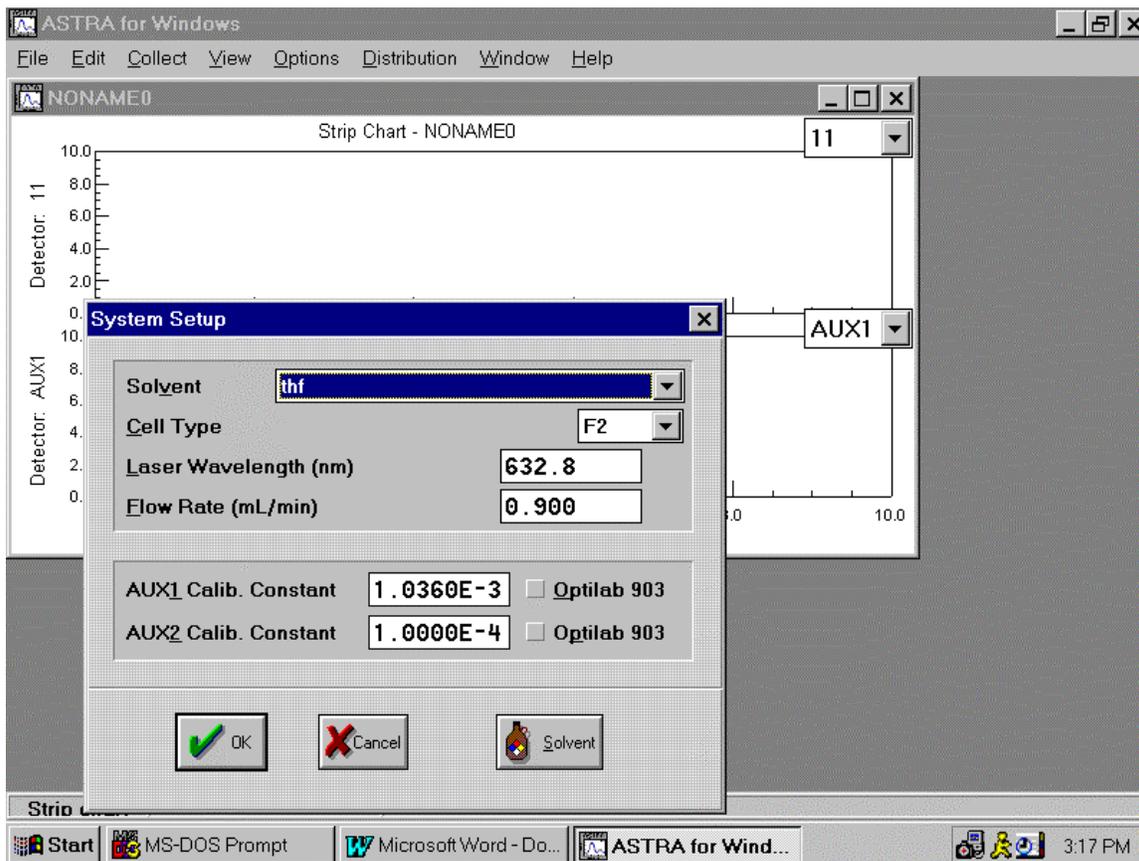
### Prepare Sample

- Put 1 pellet of Dow PS-1683 in a tared vial.
- Note mass (typically 15 mg)
- Add 10 ml of THF from the well-sparged bottle atop the GPC
- Allow time to dissolve (sonication helps)
- A Resulting solution is about 1.5 mg/ml.
- Load into loop (usually 100  $\mu$ l) so that total injected mass is about 0.15 mg or  $1.5 \times 10^{-4}$  g.

Start the Astra software and go to File:New. Screen looks like this:

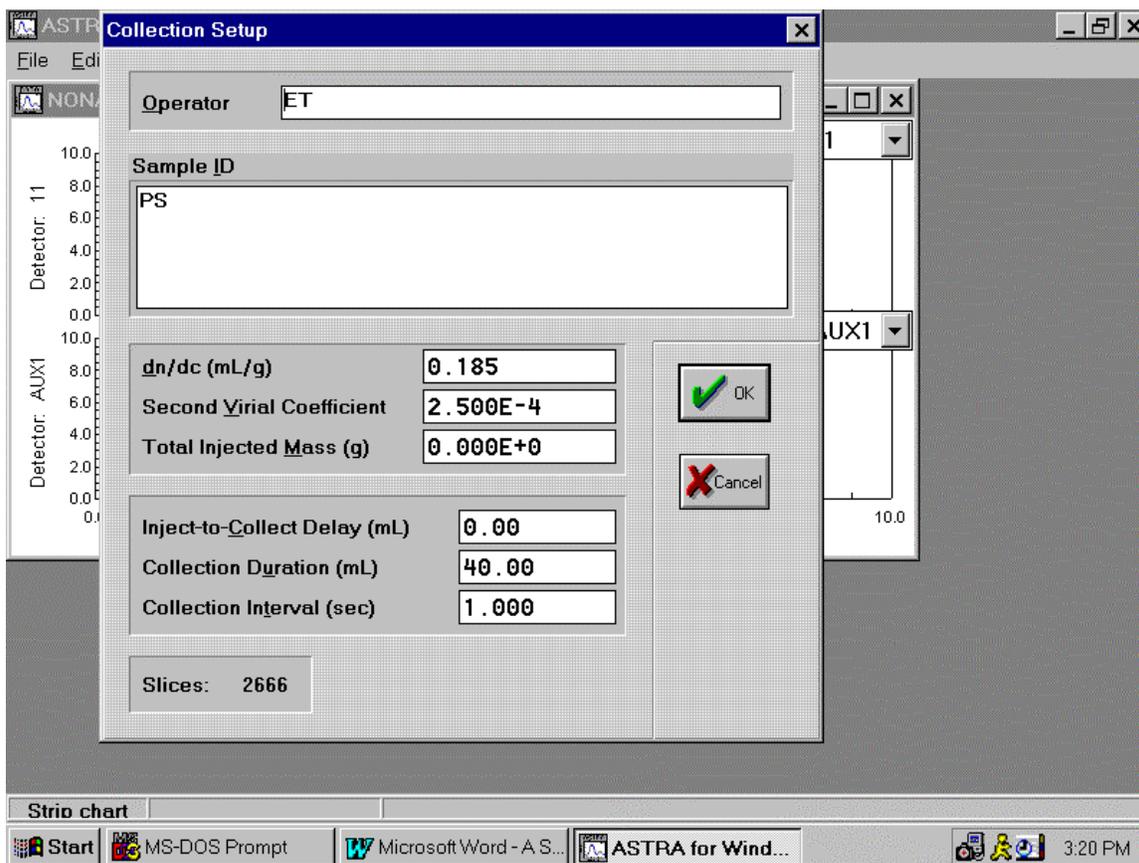


## Select Collect: System Setup (see figure below)



- Make sure the dn/dV value is correct in AUX1 (inverse of the slope determined when you calibrated the DRI).
- Also check wavelength, cell type and solvent (but these should not change often).
- Be very sure to input the correct flow rate (usually 0.9 ml/min in our instrument. Ask me why!)
- Close the window (by selecting OK or Cancel, as appropriate)

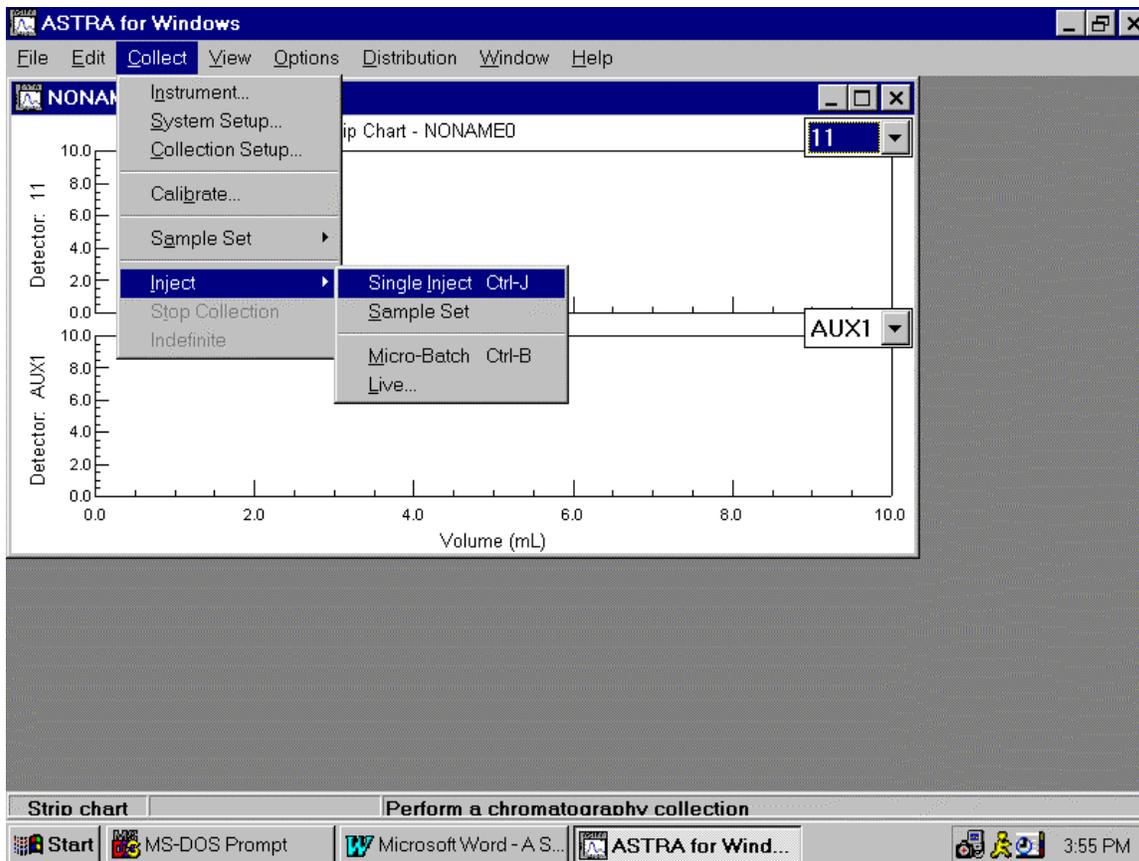
Now go to Collect:Setup; screen looks like this:



- Input the total injected mass! It matters! Do it!
- Input the right dn/dc value
- 2<sup>nd</sup> virial coefficient is not very important if you operate at sufficiently low concentrations; you have to determine it from Zimm plot (another machine, or “trick” this one into doing it—ask how). You can change A2 just to see if it matters.
- Flow rate: even though this is MALLS, an absolute method, the flow rate does matter because you specify the interdetector distance at some particular flow rate. You must use the same flow rate when actually making the measurement as you do when setting up the interdetector distance.

## Prepare to Inject

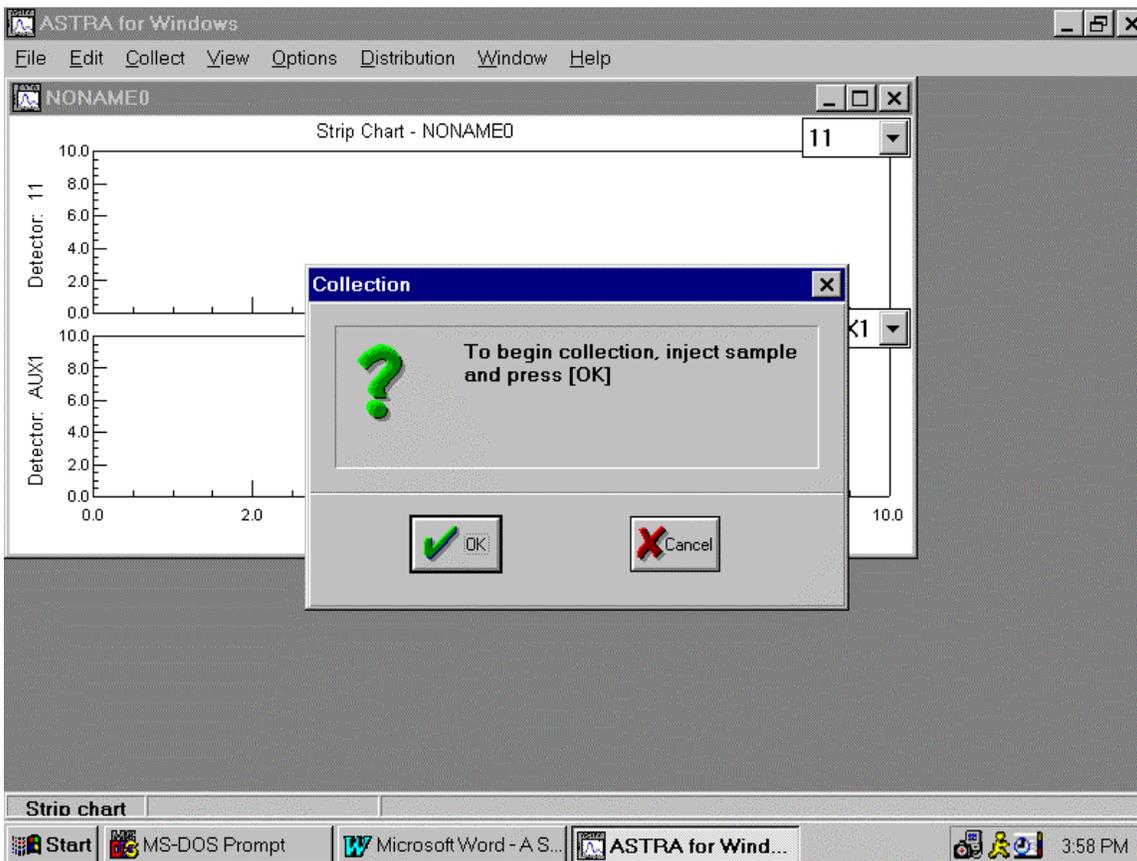
Go to Collect:Inject→Single Inject (this screen)



You will see a progress bar while DAWN initializes communications with the computer. If laser is not on yet, you may see other messages.

INJECT!!!

Now you should have this screen.



Make sure there are no bubbles flowing out of the injector. Throw the injector and push “Return” simultaneously (doesn’t really matter if it is *exactly* simultaneous in MALLS, but do try).

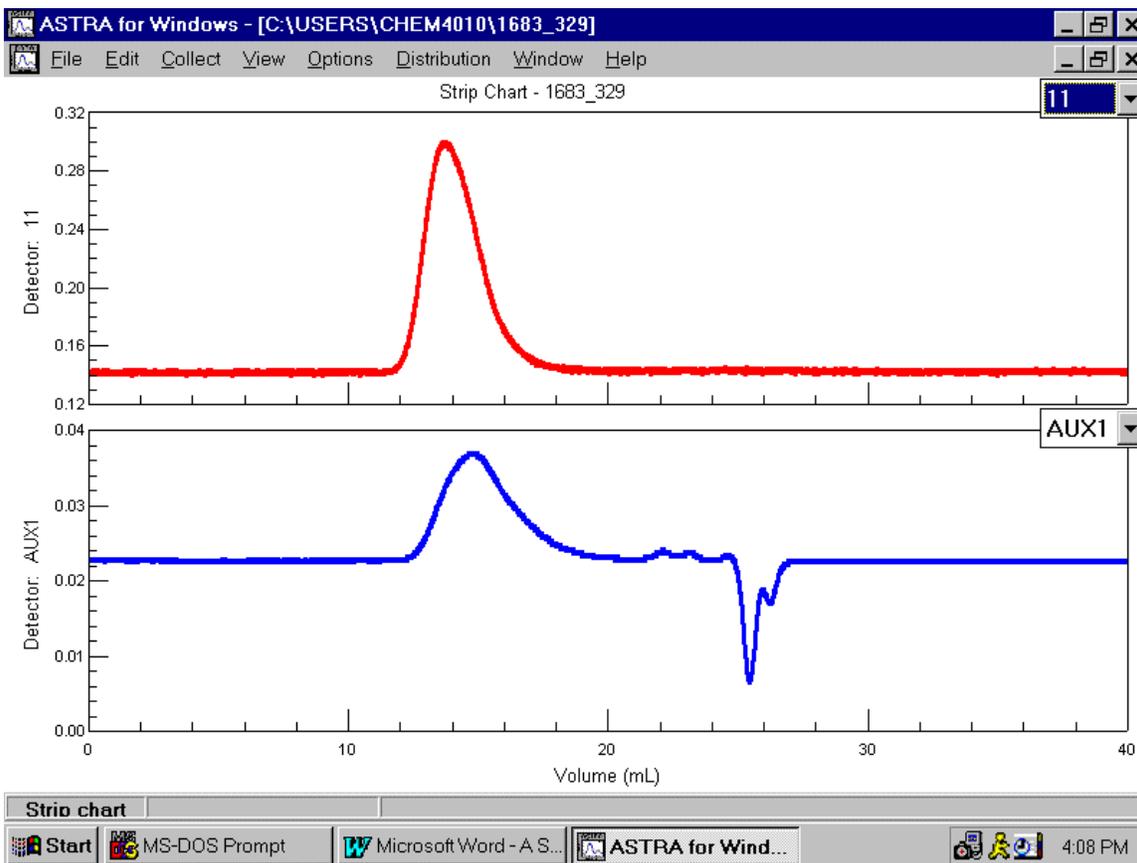
Now there is nothing to do but wait. When you are satisfied that everything worth measuring has come out (usually, it’s best to wait until the solvent blips at the very end of the run) you can stop the run by going to Collect:Stop Collection.

**THAT CONCLUDES THE COLLECTION PHASE**

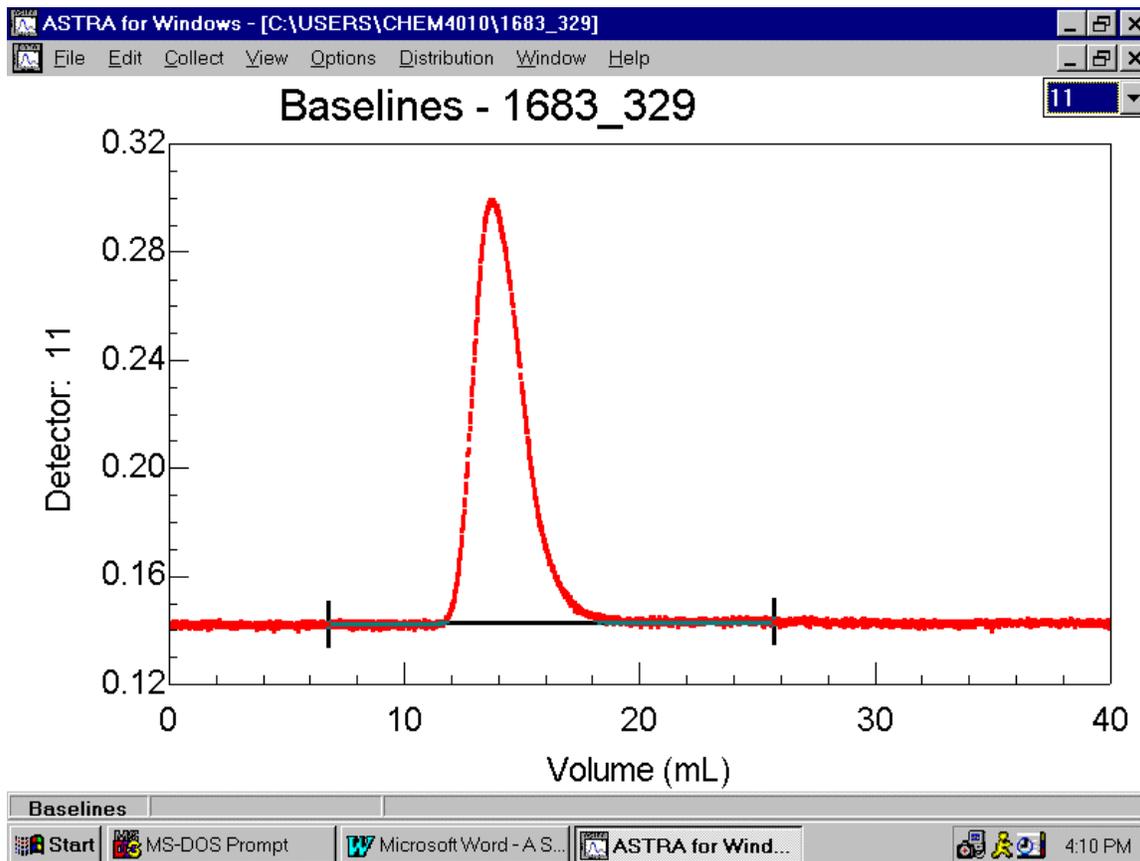
## ANALYSIS PHASE

Go to File:Save and give the file the NEXT NAME IN THE LOGBOOK!  
Be sure to write everything else about the sample into the logbook. Don't be a piker about putting details here—takes time, but worth it later!

File might look like this, typically:

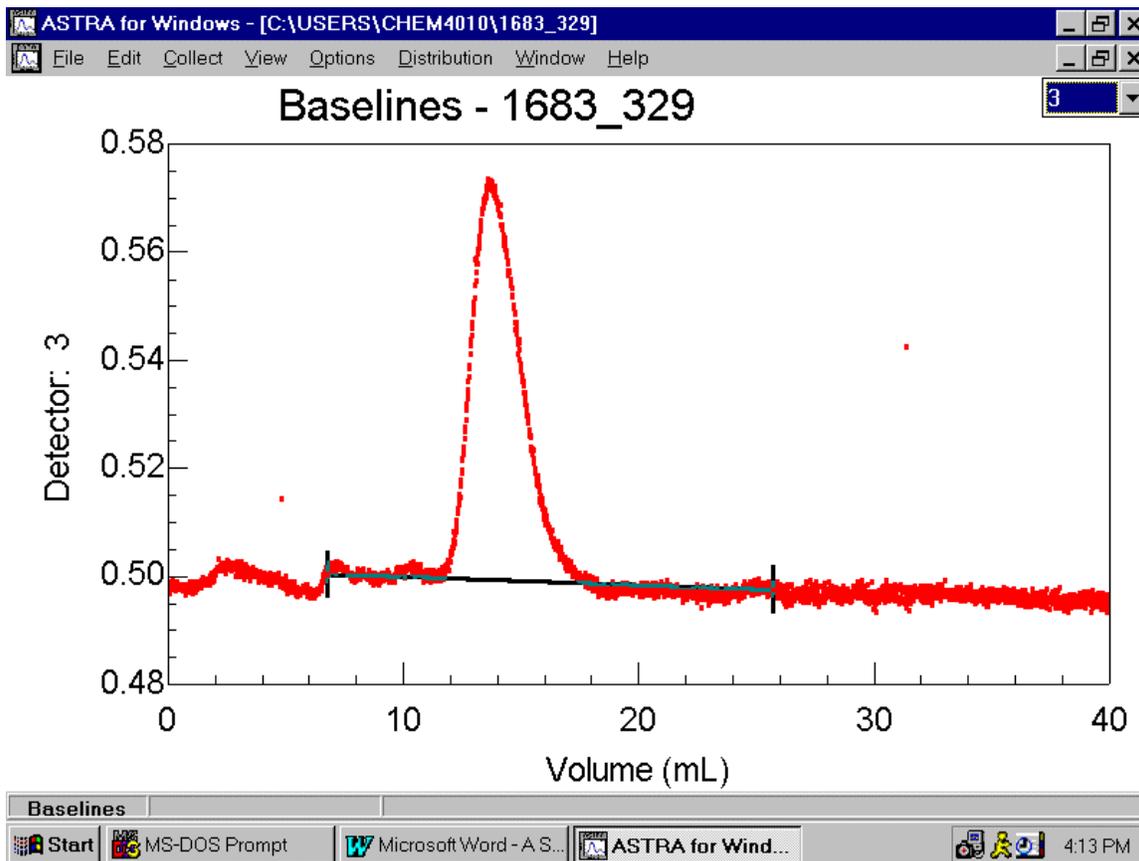


## Go to View:Baselines



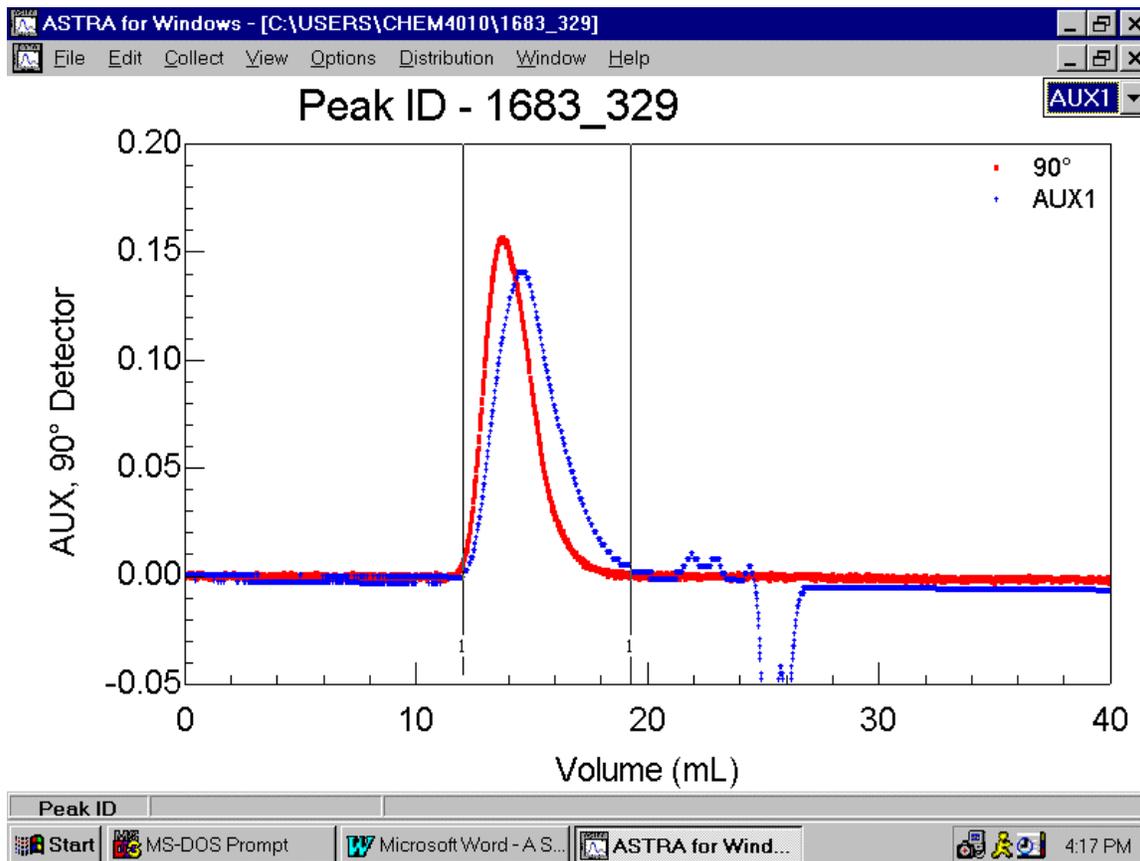
- Using the mouse, set baselines kind of as shown.
- In the box at upper right, select AUX1 instead of Channel 11. Then set the baseline for the DRI detector.
- Now go to Options:AutoBaseline.

**Now Select Channel 3** (or 1 or 2, whatever is actually lowest measured channel) in the box at upper right.



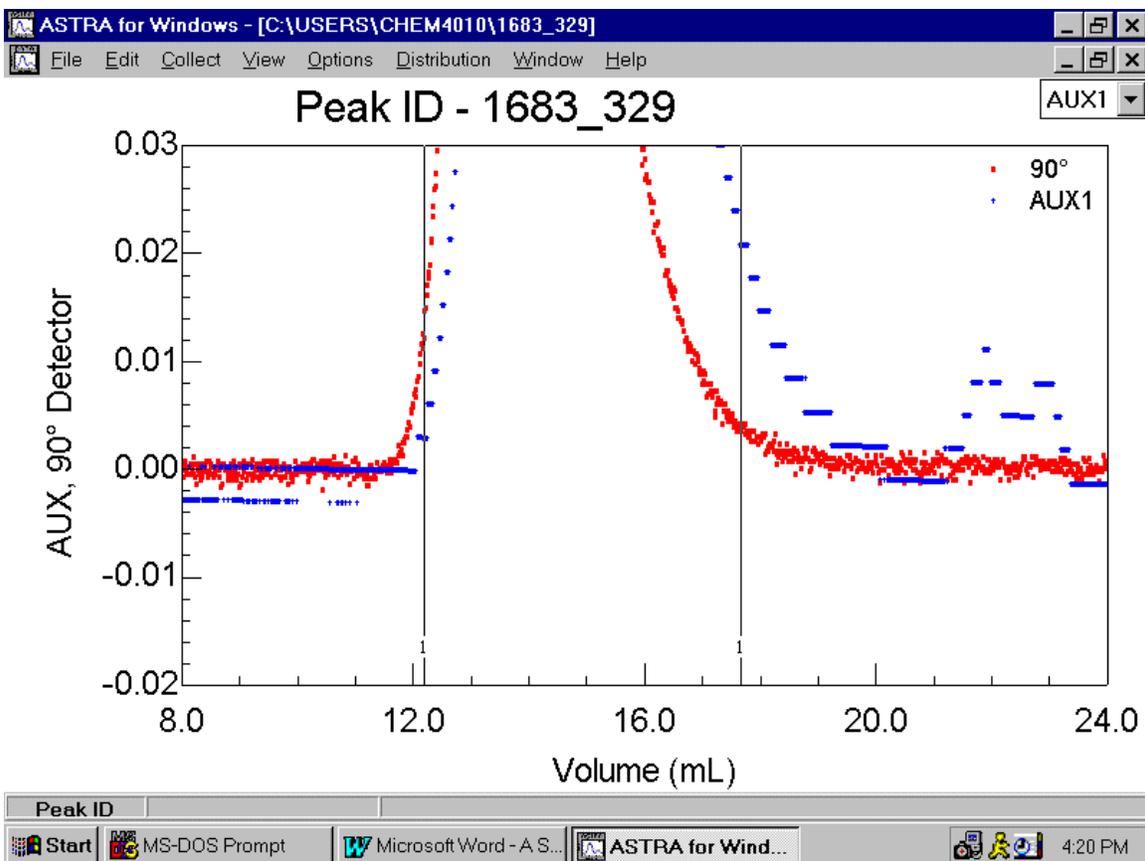
You may wish to reset the baseline for EACH channel!!!

## Now go to View:Peaks



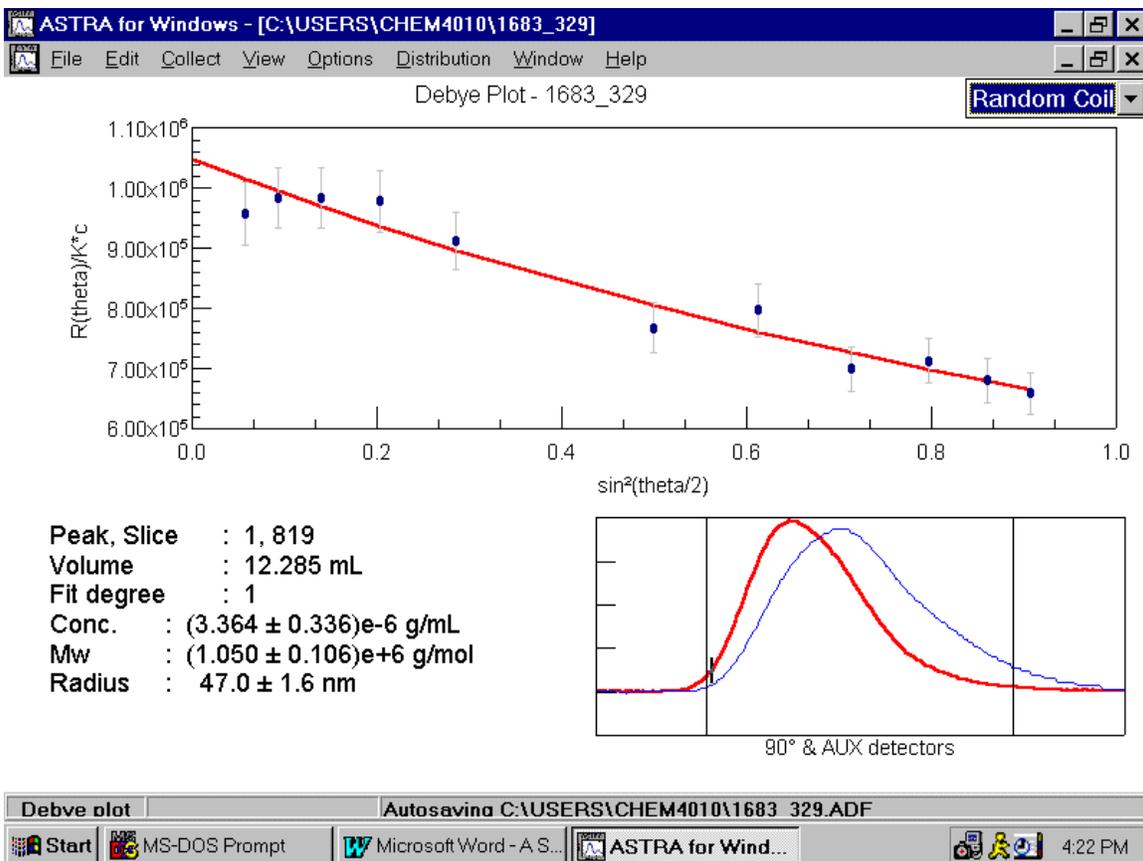
Astra presents you with an overlay of DRI and 90° responses. Hold down the left mouse button and drag until the peaks seem reasonable, using the criterion that there must be significant concentration AND significant LS response.

You can use CTRL-MouseDrag to ZOOM in on a region to see how much noise there really is, like this:



Note the digitization limitations (horizontal lines) for DRI response. This signifies that, near the baseline, there isn't enough digitization resolution. Errors are being made here! If we had to operate out in the peak wings all the time, we might boost the sensitivity or range of the DRI....and recalibrate!

Make a note where your peak begins & ends (e.g., 12.2 ml and 17.7 ml in the above)  
Select View:DebyePlot



Click in between the vertical lines that define the peak limits. Also use your cursor keys to move around and see how the plots vary.

Do the plots look reasonable? Negative Radii?

Try selecting different fit types (option box in upper right, currently saying Random Coil)

Decide if your selected channels really are the right ones (if not, try Options → Processing Parameters). Maybe the lowest angle, or the third, is always weird (normalization problem, stray light?) or maybe there is a regular oscillation from channel to channel (alignment problem?).

Decide if you set your peaks correctly (repeat Peak setting if not)

## Select Options: Processing Parameters

ASTRA for Windows - [C:\USERS\CHEM4010\1683\_329]

File Edit Collect View Options Distribution Window Help

Debye Plot: 1683\_329

Processing Parameters

Concentration Detector:

- AUX1
- AUX2
- Batch mode

Detector Fit Method:

- Zimm ( $K^*c/R$ )
- Random coil
- Debye ( $R/K^*c$ )
- Berry  $\sqrt{K^*c/R}$

Known Parameters (select any two):

- Known  $dn/dc$
- Known AUX calibration constant
- Assume 100% mass recovery

Results Fitting:

Poly.	Exp.	Degree
<input type="checkbox"/>	<input type="checkbox"/> Mass	1
<input type="checkbox"/>	<input type="checkbox"/> Radius	1

Detectors to Use:

<input type="checkbox"/> 1: -----	<input checked="" type="checkbox"/> 7: 44°	<input checked="" type="checkbox"/> 13: 115°
<input type="checkbox"/> 2: -----	<input checked="" type="checkbox"/> 8: 54°	<input checked="" type="checkbox"/> 14: 126°
<input type="checkbox"/> 3: 12°	<input checked="" type="checkbox"/> 9: 65°	<input checked="" type="checkbox"/> 15: 136°
<input type="checkbox"/> 4: 20°	<input type="checkbox"/> 10: 77°	<input checked="" type="checkbox"/> 16: 145°
<input checked="" type="checkbox"/> 5: 28°	<input checked="" type="checkbox"/> 11: 90°	<input type="checkbox"/> 17: 152°
<input checked="" type="checkbox"/> 6: 35°	<input checked="" type="checkbox"/> 12: 103°	<input type="checkbox"/> 18: 160°

Intrinsic Viscosity:

Epsilon: 0.000

Phi: 2.87E+23

OK

Cancel

Random Coil

R(theta)/K\*c

Pea

Vol

Fit

Co

Mw

Rac

Debye

Start MS-DOS Prompt Microsoft Word - A S... ASTRA for Wind... 4:27 PM

There are lots of things to try! Main thing is to select/deselect channels to be used.

## Select View:Report→Detailed

There is TONS of info here. Try to approach it this way.

Do injected and calculated mass agree?

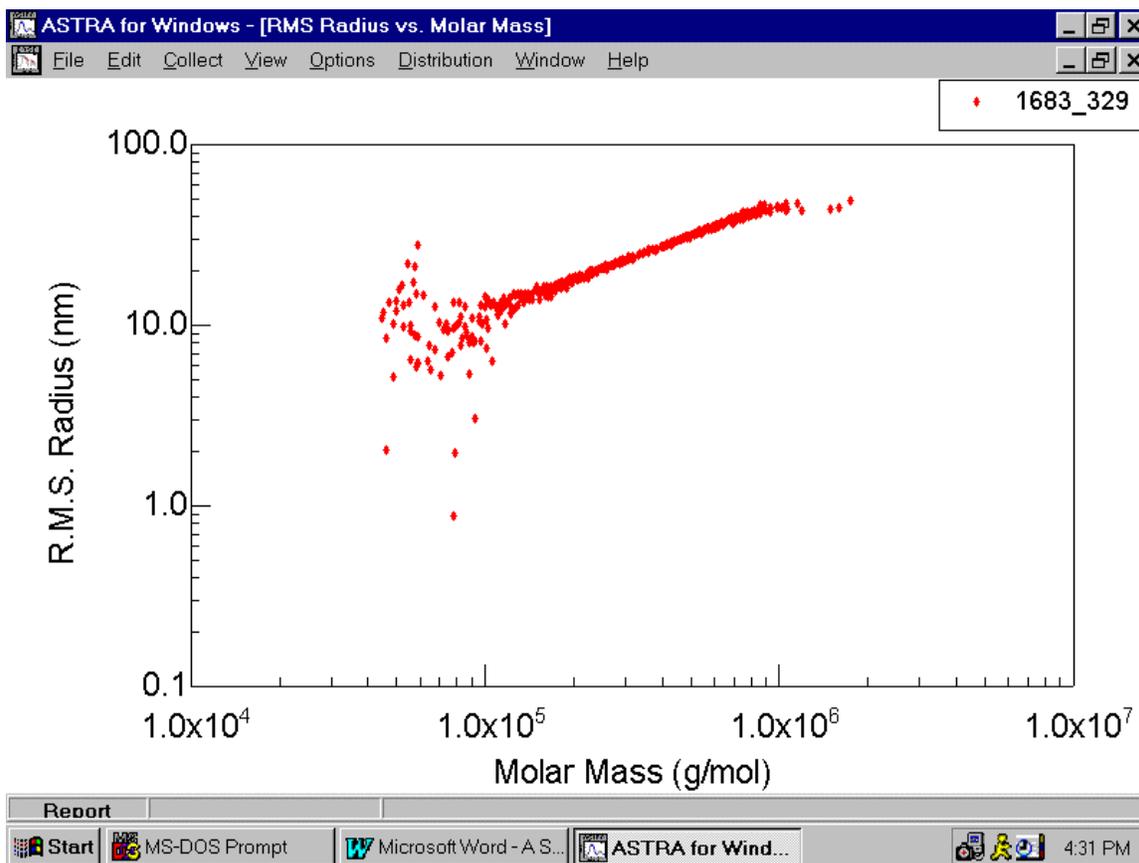
Is there a trend from yesterday's calculated:injected ratio?

From last month's ratio?

This info tells you about DRI drift—e.g., is it time to recalibrate the DRI?

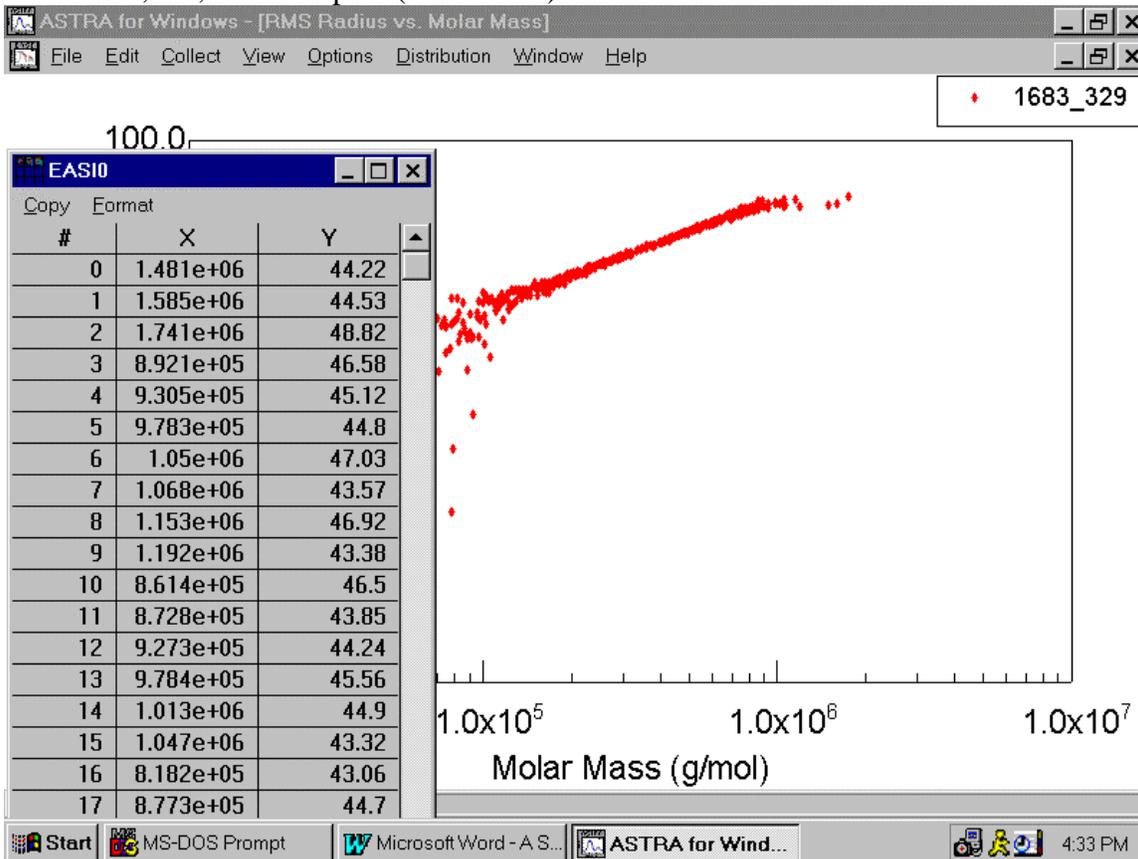
THEN you can look at the Mn/Mw/Mz stuff.

Now, try Distribution:RMSRadius vs. MM



Cool, eh? The slope of that is  $\nu$  as in  $R_g \sim M^\nu$ . That used to take years! On the other hand, people had an easier time spanning wide ranges of  $M$ , so they tended to get better answers.

If you right click on the plot, Astra makes a data table that you can export to Excel or Origin using the copy feature. There, you can easily analyze to figure out what  $v$  is. Astra can do this, too, but it's a pain (see manual).



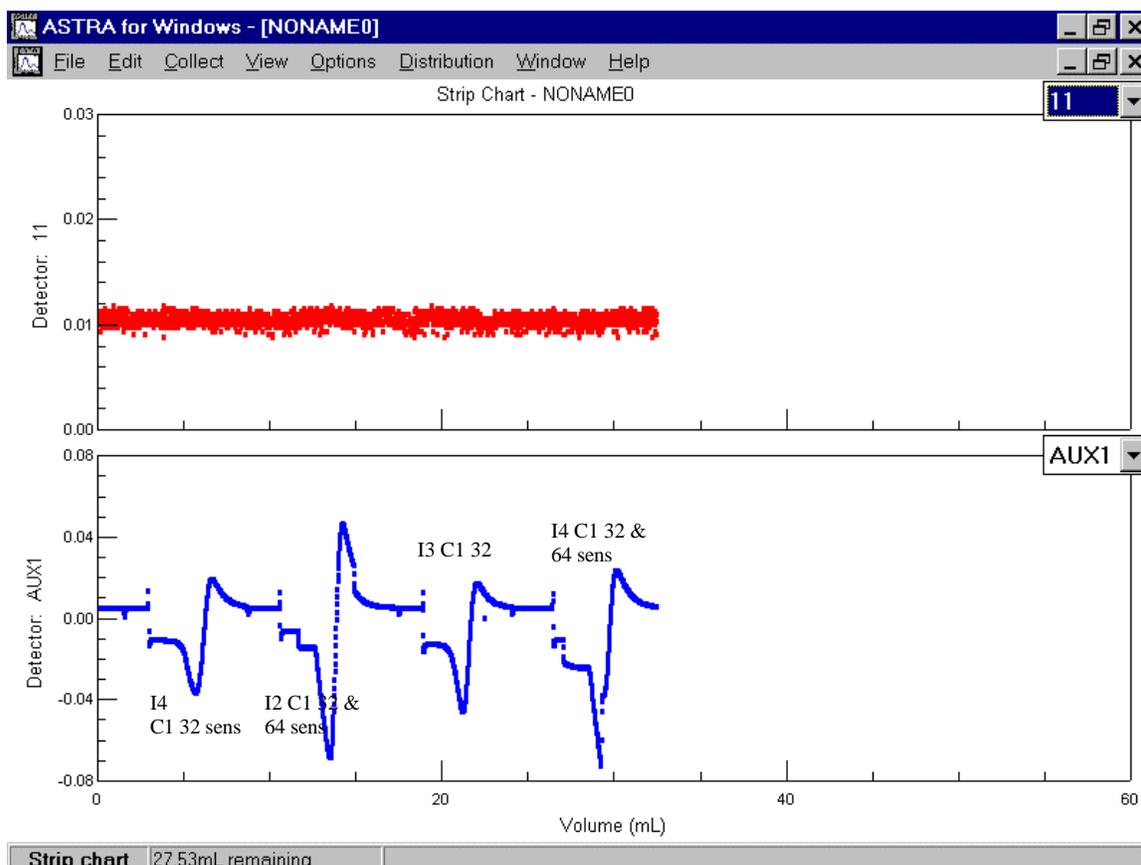
## **SUMMARY**

The MALLS and Astra software is VERY powerful.  
You should really read the manual, which shows many additional features and options.

## APPENDIX

These screen-dump images were placed into a Word file as we were doing the calibration. To do this, hit Print Screen, then Open Word and Paste. To make annotations, we used the Draw menu/text feature. This is a lot of work compared to a strip chart, but one does what one must!

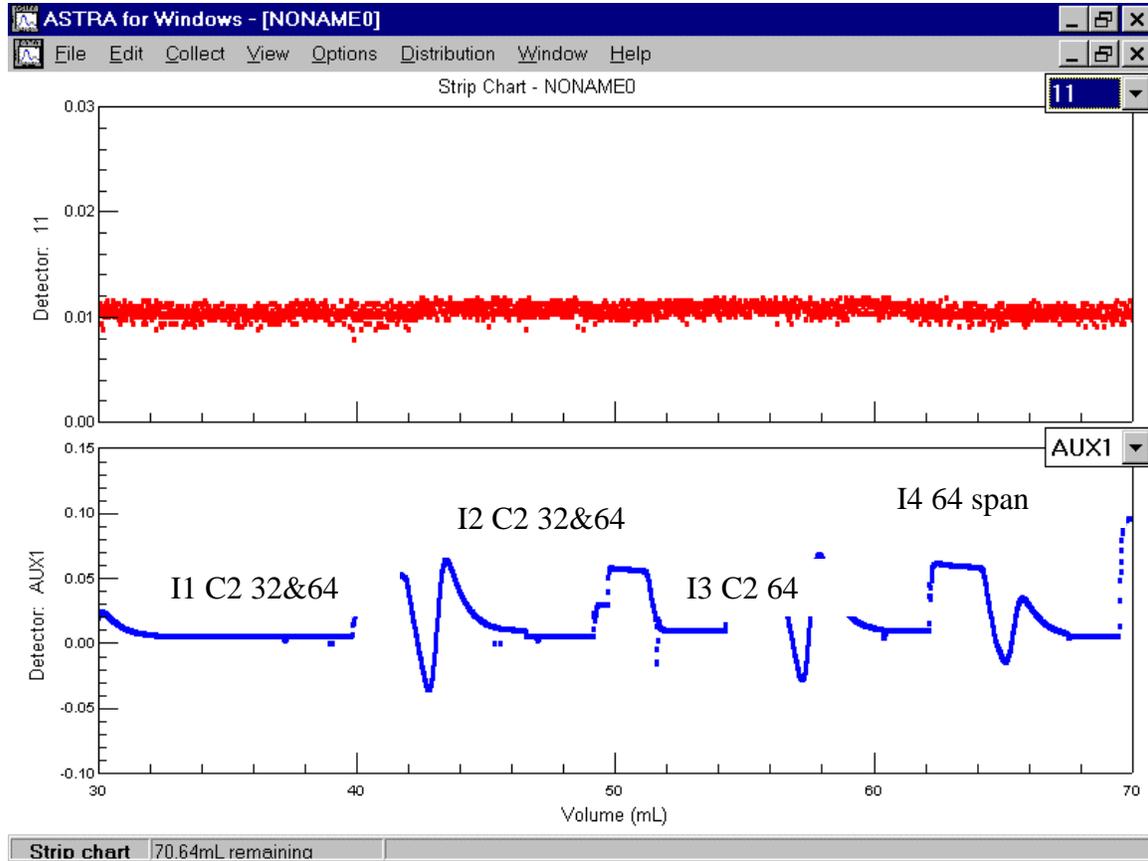
Although the DRI calibration traces themselves are rather ugly, have a look at the resultant plot of (flat) peak height vs. concentration at the end!



Concentration C1

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### Concentration C2



### Concentration C3

