Model 302 TDA Detectors

Instrument Manual

1002
Revision 2.00

15600 West Hardy Road
Houston, Texas 77060
Telephone: (281) 445-5966
Facsimile: (281) 931-4336

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS AND DRAWINGS

LIST OF TABLES

NOTICES AND WARNINGS

The information contained in this manual is subject to change without notice. Viscotek Corporation assumes no responsibility for errors that may appear in this document. The manual is believed to be accurate at the time of printing. Viscotek Corporation shall not be liable for damages resulting from the use of this document.

⚠ **ATTENTION:** This detector is a highly sensitive instrument. Read the manual before using, and follow appropriate safety procedures.

To avoid the risk of electrical shock, disconnect the power cord before removing top cover.

To avoid possible damage to electrical components, power off the TDA before removing detector module.

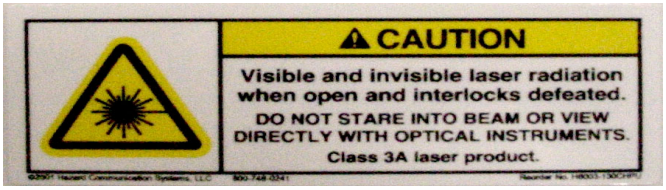
WARNING: Set fuse module and switch (sw-6) for correct line voltage (110/240 VAC 50/60 Hz) to avoid damage and possible fire hazard. When fuse replacement is necessary, use only the fuse specified on the chassis rear cover (5.0 Amperes 250V type T).

WARNING: HOT surfaces inside. Wear appropriate protection when handling detector module.

When connecting power cord to detector, use a properly grounded receptacle.

⚠ **Attention:** The detector has two flow cells that are pressure rated at 150 psi. Do not connect any tubing or device that might cause the back pressure to exceed the above pressure rating.

⚠ Note: The operating temperature of the instrument is from 5 to 30 degrees centigrade. The maximum operating humidity rating is 80% non-condensing. The current draw for the unit is 3.5 amperes.



⚠ The laser diode used in this product has been classified as a Class 1 Laser Product that complies with 21 CFR 1040.10 and 21 CFR 1040.11

⚠ The Light Scattering Detector assembly contains no user serviceable parts. Contact the Viscotek Corporation if service is needed.

This product has been tested for and is in compliance with low voltage safety, EMC, and laser safety according the directives of

CE

If further information is needed about these or any other notices or warnings, please contact Viscotek Corporation Technical Service and Support Department.

A. DESCRIPTION OF INSTRUMENT

A.1 GENERAL DESCRIPTION

A.1.a Purpose and Applications

The Model 302 TDA Differential Refractometer is the basic detector component of a system for polymer analysis by gel permeation chromatography (GPC), also called size exclusion chromatography (SEC). SEC involves separation through the hydrodynamic volume of the molecular distribution. The refractometer can also be used to determine the concentration of solutions after calibration.

SEC SEPARATION

In SEC, columns are packed with porous packing material. Molecules which are smaller than the pores are trapped for short periods of time within the pores with which they come in contact. A common term for this is that "the molecules are totally included." Molecules larger than the size of the pores are not trapped at all, and will elute earliest. These molecules are said to be "totally excluded." In these two cases, the column has little or no separation as a function of molecular weight and the values fall on a non-linear section of the calibration curve (log Hydrodynamic Volume (V_h) vs. Retention Volume). Within a given column, there is a distribution of pore sizes which allows most molecules to stay resident in many of the pores while being excluded from many others. Molecules in this size range, generally will fall on a fairly linear calibration range of the calibration curve.

MEASURING THE INTRINSIC VISCOSITY DISTRIBUTION

The Model 302 TDA is specifically designed for polymer analysis by SEC^3 or FIPA. In an SEC^3 system, the Deflection-type refractometers are the most common type used in SEC separation; they operate by measuring the deflection of a light source caused by a difference in the refractive index of a sample-solvent mixture and pure solvent. Because different polymer types have different differential refractive index responses with concentration (dn/dc), each polymer type must have a unique RI response factor. Therefore, the area of the RI is proportional to the product of the concentration, injection volume and inverse flowrate of the sample only across a single sample type. The RI area is exactly proportional to the quantity of the mass injected of the sample and its dn/dc value.

Thus, the refractometer provides a signal which is generally proportional to concentration of sample as it elutes from the column.

[A.1-1] $$Y_i = k_{ri} \frac{dn}{dc} C_i$$

where for species i,

k_{ri} = Refractometer response constant

$\frac{dn}{dc}$ = Polymer refractive index increment

C_i = Concentration

If the usual assumption is made that all of the sample injected into the column elutes from the column, the sum can be taken over all data points in the concentration chromatogram. Then Equation [A.1-1] can be normalized, yielding a cancellation of constants.

[A.1-2] $$\frac{Y_i}{\sum Y_i} = \frac{C_i}{\sum C_i}$$

But,

[A.1-2a] $$\sum C_i = \frac{C_s V_s}{\Delta V}$$

where,

C_s = Concentration of sample injected
V_s = Volume of sample injected
ΔV = Elution volume increment

Therefore it can be written that,

[A.1-2b] $$C_i = \frac{Y_i}{\sum Y_i} \frac{C_s V_s}{\Delta V}$$

The viscometer provides a signal likewise proportional to the specific viscosity of the sample.

[A.1-3] $$\eta_{sp} = \frac{4DP}{IP - 2DP}$$

where,

η_{sp} = Specific viscosity
DP = Differential pressure
IP = Inlet pressure

Equation [A.1-3] can easily be solved for the differential pressure value, DP:

$$\eta_{sp}(IP - 2DP) = 4DP$$

$$\eta_{sp} IP = 4DP + 2DP\eta_{sp}$$

$$\eta_{sp} IP = 2DP(2 + \eta_{sp})$$

[A.1-3a] $$DP_i = \frac{1}{2}\left(\frac{\eta_{sp\,i}}{2 + \eta_{sp\,i}}\right) IP_i$$

These are the two primary pieces of information provided by the refractometer and viscometer detectors. Taken together, the specific viscosity and concentration permit the calculation of intrinsic viscosity of the sample at every elution point.[1]

[1] Equation [A.1-4] is exact only in the limiting case of infinite dilution of the sample. At normal concentrations encountered in SEC, it is usually adequate. However, the Viscotek TriSEC software actually uses an empirical improvement called the Soloman-Gottesman equation to calculate more accurate intrinsic viscosities. See the TriSEC manual, Part 2.e (Further Derivations) for more details.

[A.1-4] $$[\eta]_i \cong \frac{\eta_{sp\,i}}{C_i}$$ where $[\eta]$ = intrinsic viscosity

The set of data points $\{C_i, [\eta]_i, V_i\}$ is thus obtained across the entire chromatogram. The first pair of this set constitutes the *Intrinsic Viscosity Distribution* (IVD) of the polymer sample. Note that it is obtained directly from the detector responses of the detector.

DERIVING MOLECULAR WEIGHT DISTRIBUTION

The *Molecular Weight Distribution* (MWD) can be obtained at least 3 ways:

(1) Conventional Calibration

Conventional calibration generally calibrates the molecular weight or intrinsic viscosity of a given substance to the retention volume. The only case where this can be rigorously held true is when there is no variation of structure (including branching, conformation, etc.) between the standards and the unknowns. Therefore, for each different structure or substance, a new calibration curve must be made to obtain a true molecular weight or intrinsic viscosity distribution. "Apparent molecular weights" referenced to standards such as polystyrene are commonly used; however, this leads to disproportionate information if there are major structural differences within the sample such as branching. This type of error can cause gross underestimation of sample polydispersity.

In conventional calibration techniques, the absolute concentration is not required for the sample measurement. The overriding concern is the ability to measure relative concentrations along the elution profile. Therefore, the RI detector is often used to determine relative mass.

(2) Universal Calibration

By calibrating the column with primary molecular weight standards, one can convert the intrinsic viscosity distribution into the molecular weight distribution via the Universal Calibration Curve. This is usually accomplished by measuring the retention volumes V_i and intrinsic viscosities $[\eta]_i$ for a set of narrow distribution polymer standards. The product of intrinsic viscosity and molecular weight for each of the standards is then plotted against the retention volumes, obtaining a smooth curve.

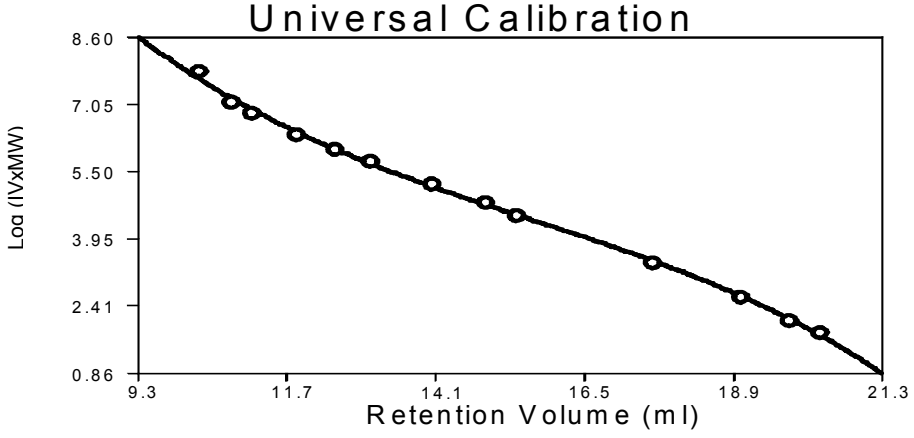


Figure 1 Typical Narrow Standard Universal Calibration Curve

Then for an unknown the data set $\{[\eta]_i, V_i\}$ is also measured.

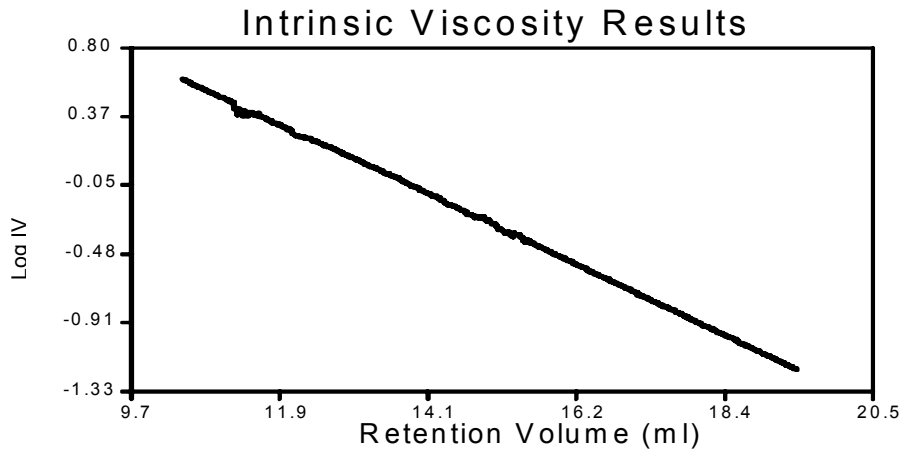


Figure 2 Typical Plot of Intrinsic Viscosity versus Retention Volume for Broad Sample

It is clear that the data set $\{M_i, V_i\}$ for the unknown sample can be mathematically extracted by dividing the data of **Figure 2** into that of **Figure 1**. The full data set $\{C_i, [\eta]_i, M_i, V_i\}$ for the unknown sample could then be constructed, the first and third terms of which constitute the desired MWD.

Universal Calibration is the best means of column calibration in SEC. It is applicable to a wide range of polymers, the polymer standards do not have to be the same type as the unknown, and it applies to copolymers as well as to both linear and branched samples. However, it necessarily assumes that the mechanism of retention on the columns for both sample and standards is pure size exclusion.

(3) SEC^3

The molecular weight distribution can be obtained directly from an on-line light scattering detector connected in series before the refractometer and viscometer detectors. This is the SEC^3 system. The data set $\{C_i, [\eta]_i, M_i, V_i\}$ is obtained just as with Universal Calibration. However, the M_i values are calculated directly from the LS detector signal, not derived through the column calibration. In addition to the convenience of not having to run calibration standards, SEC^3 offers the real advantage of not being constrained by the size exclusion separation mechanism.

MOLECULAR SIZE AND BRANCHING

Regardless of how one determines MWD, whether by Universal Calibration or Triple Detection, the viscometer detector is useful for determining the size of molecules in solution. The molecular size can then be related to the secondary structure of the polymer, either in terms of chain stiffness, conformation, or branching.

Molecular size is commonly computed as the radius of gyration, R_g, which can be determined rigorously only from the initial slope of multi-angle light scattering measurements. Unfortunately, the multi-angle slope approach has only a limited range of applicability, as the

precision of measurement suffers greatly below R_g of 20 nm (MW ≈ 250K for flexible coils) and becomes impossible below 10 nm (MW ≈ 80K).

The radius of gyration can be computed from the intrinsic viscosity and molecular weight via the Ptitsyn-Eizner modification of the Flory-Fox equation.

[A.1-5] $$[\eta]M = \Theta_0\left(1 - 2.63\varepsilon + 2.86\varepsilon^2\right)\left(\sqrt{6}R_g\right)^3$$

where $\varepsilon = \dfrac{2a-1}{3}$ and with a = Mark-Houwink exponent.

Although this viscometry approach is certainly non-rigorous and would likely be inaccurate for polymer molecules deviating significantly from the flexible coil model, it has the necessary virtue of excellent precision over all ranges of molecular size and weight. Precision and range of applicability are most important because it turns out that any subsequent calculations of secondary structural effects, *e.g.*, branching, are only suitable for relative measurements. This is because those theoretical calculations involve the same type of assumptions that affect Equation [A.1-5].

A.1.b General Layout

LIGHT SCATTERING PLUMBING AND FLOW PATH

Refer to the Detector Plumbing Diagram in **Figure 3** for the flow through the system. Flow enters the light scattering cell from the in-line RALLS filter and then exits to the refractometer detector of the 302TDA. A laser beam is focused on the end of the cell and scattered light is measured at 90 degrees and at 7 degrees.

REFRACTOMETER PLUMBING AND FLOW PATH

Refer to the Detector Plumbing Diagram **Figure 3** for a schematic of the flow through the refractometer. Note that the diameter of the tubing is not the same throughout the refractometer. The tubing from the inlet through to the sample side of the cell is 0.01”id; all other tubing is 0.04”id, from the bottom of the reference cell and out to the RI Purge solenoid. Although the pressure drop also depends on length, the strong dependence on bore radius or diameter means that the wide bore tubes shown have small resistance compared to the narrow bore tubes. Only with this relationship clearly in mind can one understand the flow patterns described below.

The reference cell of the refractometer is filled with mobile phase solvent by opening the RI purge valve. Solvent flow is then diverted from the Viscometer to the Reference side of the RI flow cell. Solvent then passes through a 20 micron filter, the positive Inlet Pressure transducer cavity and Cross 3. It then flows through the negative Inlet Pressure transducer cavity, Cross 2 and finally exits to waste via the Outlet bulkhead port fitting.

VAPOR SENSOR DETECTOR

Refer to **Figure 9** for the location of the vapor sensor in the Model 302. The sensor is mounted in the rear of the TDA module. The 4-lead sensor plugs into a socket for easy replacement. Note that the socket has a notch cut for the sensor key alignment. Hence, the

socket's notch should be aligned with the sensor's orientation tab. Should a solvent leak occur in the TDA module, the chemical sensor's output signal will increase and exceed a set threshold level. This will cause the vapor bar LED on the front panel to flash and it turn activate a solid-state relay. This relay will provide a contact closure to shut down the external solvent pump.

VISCOMETER PLUMBING AND FLOW PATH

Refer to the Viscometer Plumbing Diagram in **Figure 3** for the flow through the system. Poiseulle's Law of flow through tubes requires that the pressure drop across any tube is inversely proportional to the diameter raised to the fourth power.

[A.1-6] $$P \propto \frac{8LQ}{\pi R^4}$$ where L = length, Q = flowrate, and R = radius

Note that some of the tubing shown in **Figure 3** is small bore (shown with fine line width) and other tubing is wide bore (shown with wide line width). The wide bore tubing is 0.040" diameter and the fine bore tubing is 0.010" diameter. Although the pressure drop also depends on length, the strong dependence on bore radius or diameter means that the wide bore tubes shown have negligible resistance compared to the narrow bore tubes. Only with this relationship clearly in mind can one understand the flow patterns described below.

The viscometer differential pressure transducers, having positive and negative cavities, are purged using electronic solenoids from the front panel of the instrument. These purge procedures must be carried out independently to properly redirect the flow through the transducer cavities and remove air bubbles and/or purge the cavities with new mobile phase solvent. Normal flow is directed into the viscometer and split at a tee connecting capillaries R_1 and R_2. After closing the DP purge ports, the flow is directed only through the four capillary bridge R_1 - R_4. The IP^+ cavity is in series before the bridge and is purged by the Refractometer Purge valve. The IP^- cavity is in series after the bridge and is continuously purged by the viscometer waste flow.

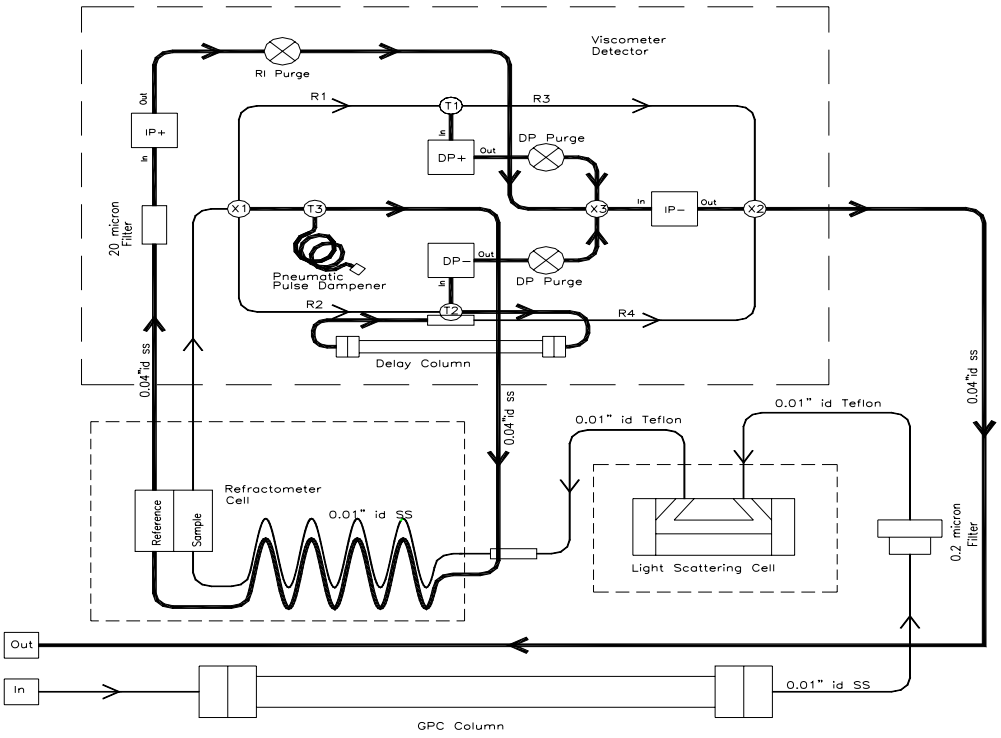


Figure 3 Viscometer, RI and LS Plumbing Diagram

FRONT PANEL

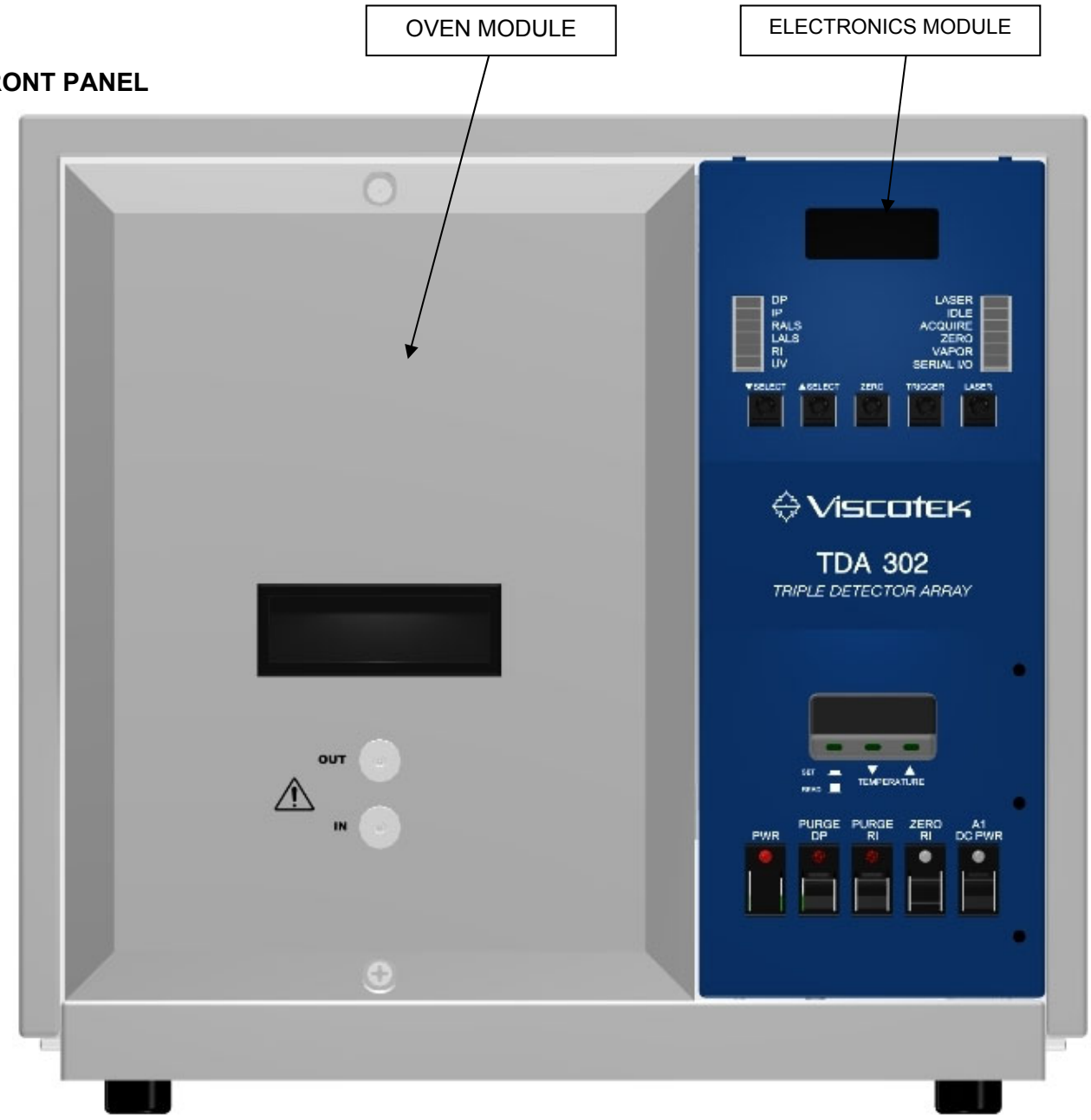


Figure 4 Front Panel of the Model 301/302 TDA

Located in the center at the top of the electronics module is the LED voltage display of the signal (**FIGURE 4**), which is indicated as DP, IP, RALS, LALS, RI or UV by the appropriate light on the left of the electronics module. Readout shown on the meter is in units of millivolts, which matches the analog output voltages available on the back panel. For the DP signal, 1.0 mV corresponds to a pressure of 1 Pa. For the IP signal, 10 mV corresponds to 1 kPa. The LS signal corresponds to mV from the photodiode amplifier of the light scattering detector and the refractometer signal corresponds to the mV output of the refractometer. Full scale data ranges are established in the TriSEC software package for each channel between 100mV, 500mV, and 2500mV FS. Default ranges are 2500mV FS for IP, 500mV FS for DP, 2500mV FS for LS and 500mV FS for RI.

In the center of the module is a set of five push-buttons: two for SELECT, ZERO, TRIGGER, and LASER:

- The SELECT button is a selection switch to choose the signal displayed on the LED display meter. You can cycle up or down through the display using either the up button or the down button.

- The ZERO button is an electronic zeroing adjustment control for offsetting the IP and the LS signals only. DP, RI and LS are strictly baseline signals, that is, measurements are made in the computer after subtracting the baseline. Zeroing these signals is primarily a matter of convenience or visual reference. On the other hand, the IP signal is read by the computer as an absolute pressure, so *it is critical that the IP signal be zeroed only when the flow is off*.

- The TRIGGER button can be used to start an acquisition with a manual injector. Remember to connect a contact closure signal cable output to the VAC terminal strip if using an auto-sampler.

- The LASER button turns the power on to the laser diode. When this button is depressed for the first time, it illuminates, indicating that the power is on. The power to the laser diode is turned off by depressing this button a second time. The light will go off indicating that the power is off to the laser diode.

On the right is another bank of lights, which indicates the status of operation: LASER, IDLE, ACQUIRE, ZERO, VAPOR and SERIAL I/O:

- The LASER light indicates whether or not the power to the laser diode is on. It is recommended that this switch be left ON at all times since a warm-up period is required for the LASER source to become stable. NOTE: The laser operates in a constant optical power output mode. This is not a pulsed laser unit.

- When the IDLE indicator light is illuminated, the instrument is ready to communicate to the host computer. This light also serves as an indicator light for the self diagnostic routine during the power up routine. At unit power up, this LED is off until self diagnosis is completed. Should the unit fail self diagnosis, the LED will not be properly set.

Note: An improper set may be indicated by either none or several of the LEDS being lit.

- When the ACQUIRE indicator light is illuminated, the instrument is in the process of acquiring data from the analog inputs. This diode will turn off after each runtime cycle.

- The ZERO indicator light is only illuminated when either the RALS, LALS, or IP signal is selected and the ZERO switch is pushed.

- The VAPOR light will only illuminate when the vapor sensor is activated by a leak in the detector module. This will also shut off the solvent delivery system if a shut off cable is attached to the terminal strip on the back of the detector. **The connector is polarity sensitive! Pin #1 must be connected to the pump stop and pin #2 connects to pump ground.**

- When the SERIAL I/O light is on serial data is being received from the computer.

Below the Viscotek Corporation Logo On the lower portion of the electronics module is a smoked plastic cover that encloses several switches and a push-button used for operating the Model 300TDA. They include: MAIN POWER button, REFRACTOMETER and VISCOMETER PURGE, REFRACTOMETER ZERO, as well as a DC POWER RESET switch.

- The MAIN POWER push-button turns the AC power On/Off. This switch will disable all electronic components including the heater circuit. This switch is always left ON during data acquisition.

- The REFRACTOMETER PURGE switch is used to control an electronic valve that is used to purge the reference side of the cell with fresh solvent. Normal HPLC pump flow-rate is required for a period of approximately 5 minutes (or until a stable Display Meter reading is achieved) to adequately purge the Refractometer. Additionally, the positive signal side of the Inlet Pressure transducer is purged simultaneously. This switch must be in the OFF position for data acquisition.

- The VISCOMETER PURGE switch is used to control a pair of electronic valves that are used to purge the two halves of the Differential Pressure transducer. Again, normal HPLC flow is required for approximately 5 minutes (or until a stable Display Meter reading is achieved) to adequately purge the viscometer. The negative signal side of the Inlet Pressure transducer is continuously purged during normal operation of the Model 300TDA.

- The REFRACTOMETER ZERO switch is used to adjust the optical alignment of the Refractometer signal during the initial setup and upon solvent change-over. To make adjustments hold the switch either up or push down depending upon the algebraic sign of the signal displayed. *NOTE: Once the zero has been obtained, do not change or re-zero the signal while running samples. If it is necessary to re-zero the signal, you may need to re-calibrate the RI detector. See the TriSEC 3.0 Software manual for this procedure.*

- The DC POWER RESET switch is used to reestablish serial communication between Model 302TDA and its data station. If either power surges or signal saturation are experienced during normal data acquisition result in an apparent “lock-up” of the data stream it will be necessary to STOP data acquisition and use this switch to reset the internal CPU for proper operation. Use of this switch does not effect proper temperature control.

Located to the left hand side of the electronics module is the Oven Compartment that contains space for up to 3 separation columns and the Model 302TDA detectors. This compartment may be removed from its housing by loosening the two panel screws located at the center top and bottom of the compartment front panel. A handle is provided in the front and rear of the drawer as an aid in removal.

Note: No locking mechanism is provided for sliding the detector drawer out of the oven chamber. ***Also, HOT surfaces will be encountered upon removal****.*

On the outside and to the bottom of the oven compartment are the Sample In/Out Flow Ports. These are used to connect the Model 302TDA to the HPLC system.

NOTE: An optional manual sample injection valve may also be located on the front panel of the Oven Module. This is often used in the FIPA application. This option may also be selected for GPC applications not requiring auto-sampler capabilities.

BACK PANEL

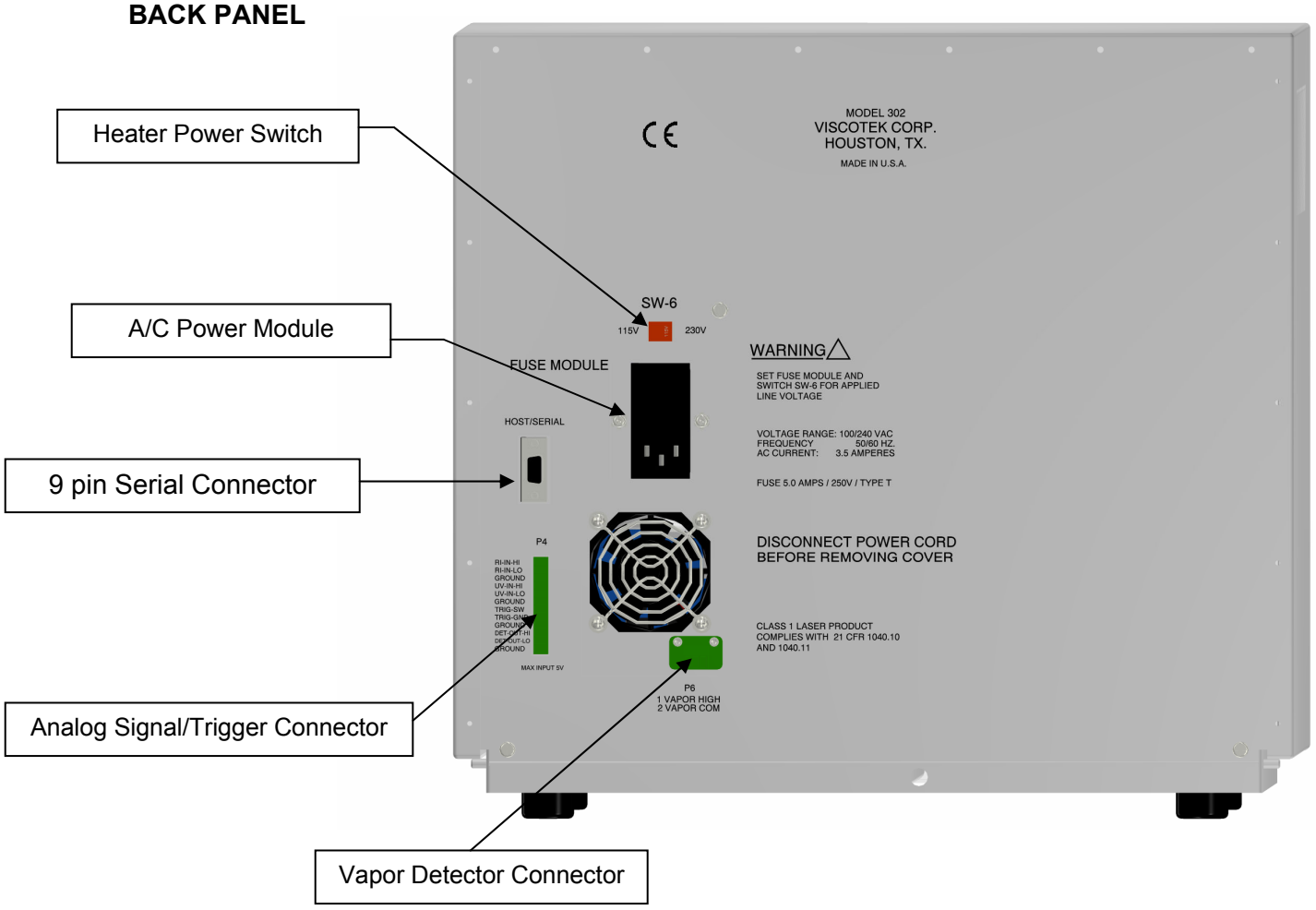


Figure 5 Back Panel of the Model 301/302 TDA

The back panel, **Figure 5,** contains the fused A/C power module. This module contains the receptacle, a fuse holder and a voltage selector switch. The voltage is selectable for either 100, 120, 220 or 240 VAC. An additional HEATER POWER switch is located above the A/C power module. It may either be selected for 110 or 220 VAC. The main power on/off push-button is wired directly to the AC power supply.

The back panel also contains the analog signal input/output connector and the auto-sampler trigger input. This includes inputs for an external UV detector, and a RI detector as well as an output for one detector signal. Connections to the barrier strip may be made by using a small flat-head screwdriver supplied with the instrument. The maximum level of signal inputs is 2.5 VDC. The device may be damaged by voltages exceeding 12VDC

A nine-pin serial connection port is located on the rear of the instrument. This communication socket allows the instrument to be connected directly to a PC through the standard RS232 serial port. The nine-pin connector cable may be oriented in one direction only.

The back panel also contains the vapor detector connector. A two wire connector can be connected here and to the back of the solvent delivery unit to the pump stop or shut down terminal. When the vapor sensor is activated by a leak, the detector will send out a contact closure signal to the pump to shut off flow to the system. **The connector is polarity sensitive! Pin #1 must connect to the pump stop and pin #2 must connect to pump ground.**

DC POWER SUPPLY

The DC power supply is located toward the back of the detector. It provides ±15 volts and ±5 volts to the electronic components of the detector. The primary side of its transformer is wired directly to the AC power module on the back panel.

DATA ACQUISITION

A separate data acquisition device is not necessary for either of the Model 302TDA. The data acquisition device (DAD) has been integrated into each T model detector, available through the serial interface located on the back panel.

The DAD system is more than a simple chromatography board, which only converts analog voltage signals from GPC detectors into digital values for processing by a computer. It is designed for unattended data acquisition, leaving the computer free to run other applications. The DAD collects and stores analog data and controls linked accessories. On request, data can be transferred to the host PC for further evaluation with the TriSEC GPC Windows Modules.

A.2 LIGHT SCATTERING DETECTOR

A.2.a Theory

LIGHT SCATTERING THEORY

Light scattering is a well established technique for determining weight-average molecular weights (MW) of polymers in solution. When light interacts with a molecule, it induces a temporary dipole moment which oscillates in phase with the incident beam. In fact, the molecule acts as an antenna and re-radiates light in all directions. This scattered light is referred to as Rayleigh scattering and is of the same wavelength as the incident beam.

Due to thermal fluctuation, pure solvent also scatters light, although to a lesser degree than the polymer solution. The information about the size and molecular weight of polymer is experimentally derived from the excess light scattering intensity above the solvent background.

The excess light scattering intensity caused by the presence of polymer molecules in the sample solution is directly proportional to polymer MW and sample concentration:

[A.2-1] $$\frac{KC}{R_\theta} = \frac{1}{M_w P(\theta)} + 2A_2 C$$

where C is the sample concentration, M_w is the weight average MW, and A_2 is the second virial coefficient. The term $P(\theta)$ is the particle scattering function[2,3] which describes the angular dependence of light scattering intensity. The term $P(\theta)$ is a function of the geometry and size of the polymer molecules with respect to wavelength of the incident light. The K term in Equation [A.2-1] is an optical constant:

[A.2-2] $$K = \left(\frac{2\pi^2 n_0^{\,2}}{\lambda_0^{\,4} N_A}\right)\left(\frac{dn}{dc}\right)^2$$

where n is the refractive index of the medium, λ_0 is the wavelength of the incident beam, N_A is Avagadro's number (6.023 x 10^{23}), and dn/dc is the refractive index increment. The excess Rayleigh ration R_θ in Equation [A.3-1] gives the normalized scattering intensity with respect to the scattered volume v, distance r, and incident intensity I_0:

[A.2-3] $$R_\theta = \left(I_{\theta\,\text{solution}} - I_{\theta\,\text{solvent}}\right)\left(\frac{r^2}{I_0 v}\right)$$

where $I_{\theta\,\text{solution}}$ and $I_{\theta\,\text{solvent}}$ represent the scattering intensity observed at the scattered angle θ, for the polymer solution respectively.

Experimentally, the $P(\theta)$ value can be determined as the ratio of scattering intensity at the scattering angle θ, versus the scattering intensity at $\theta = 0°$:

[A.2-4] $$P(\theta) = \frac{I_{\theta\,\text{solution}}}{I_{\theta = 0^\circ\,\text{solution}}}$$

Due to the dissymmetry and depending on the size of the polymer molecule, the $P(\theta)$ function can take on values that are equal to, or less than unity.

The angular dependence of light scattering intensity is the consequence of the destructive interference of the scattered radiations.[4] When a scattering particle has its sizes comparable to the wavelength of the incident beam, the scattering radiation from different parts of the particle may get out of phase when they reach the detector. The extent of this phase mismatch varies with the scattering angle. For example, due to the difference in the optical path distances, the radiations from scattering point A and B would be more out of phase as they reach detector 2 than detector 1. Because of this destructive radiation field interference, a lower scattering intensity would be observed at the larger scattering angles, such as the case for

[2] P. Kratochvil, "Classical Light Scattering from Polymer Solutions", Elsevier, Amsterdam, 1987.
[3] P. Kratochvil, in "Light Scattering of Polymer Solutions", M. B. Huglin (Ed.), Academic Press, New York, 1967, Ch. 7.
[4] B. H. Zimm, R. S. Stein, and P. Doty, Polymer Bulletin, *1*, 90, (1945).

detector 2. This angular dependency of light scattering intensity is represented by the particle scattering function $P(\theta)$ in light scattering theory, see Equation [A.2-1].

Quantitative theories of $P(\theta)$ for the solute shape models of hard-sphere, random-coil, and rigid-rod types have long been worked out and published.[4] For example, the $P(\theta)$ function according to Debye's theory for random coil polymers[5] gives:

[A.2-5] $$P(\theta) = \frac{2}{X^2}\left[e^{-X} - (1 - X)\right]$$

or,

[A.2-6] $$P(\theta) = 1 - \frac{X}{3} + \frac{X^2}{12} - \ \ldots$$

where,

[A.2-7] $$X = \frac{8}{3}\left[R_g \frac{\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right)\right]^2$$

Equation [A.2-6] shows that it is the initial slope of the $P(\theta)$ function which is proportional to the square of the polymer radius of gyration, R_g, value. In this manner a light scattering instrument can be used to determine the R_g of the polymer samples.

Since there are two variables, θ and C in Equation [A.2-1], a double-extrapolation procedure is used to obtain M_w as well as R_g and A_2. This is done by plotting K_c/R_g versus $\sin^2(\theta/2)$ and C, known as the Zimm plot method.[6]

SEC LIGHT SCATTERING DETECTOR

Potentially, the coupling of an on-line light scattering detector can give a very powerful polymer characterization capability. Considerable efforts have been invested in the past two decades or more in the attempt to develop an on-line light scattering detector for SEC to achieve absolute molecular weight distribution determination of polymer samples. Earlier commercial light scattering detectors for SEC were far less than satisfactory because of the lack of detection precision.[7,8] The concentration of the polymer solution eluting from an SEC column is extremely dilute. The polymer concentration level in an SEC effluent is typically an order of magnitude less than the sample concentration commonly used in classical light scattering experiments. The signal-to-noise of a light scattering instrument is the most important consideration in the SEC detector application.

Viscotek's choice of the 90° right angle in the light scattering instrument is for the reason of optimum signal-to-noise performance in the SEC-LS detection. Compared to the other scattering angles, the 90° scattering intensity is the least affected by the problems of stray-light,

[5] P. Debye, Technical Report CR-637, Private Communication to Reconstruction Finance Corporation, Office of Rubber Reserve (1945). Reprinted in "Light Scattering from Dilute Polymer Solutions", (D. McIntyre, and F. Fornick, eds.) pp. 139-147, Cordon and Breach, New York (1964).
[6] B. H. Zimm, J. Chem. Phys., *16*, 1093 (1948).
[7] Grinshpun, O'Driscoll, and Rudin, ACS Symposium SER., *245*, 273 (1984).
[8] S. Kim, P. M. Cotts, and W. Volksen, J. Polymer Science, Part B: Polymer Physics, *30*, 177 (1992).

cell window reflections, air bubbles, and particle contaminations. An example of this is where light scattering data at four different scattering angles is presented: the 90° signal is clearly the best among all the scattering angles, while the baseline noise is the least.

There is another advantage of choosing the right-angle light scattering detection. In the current 2-angle or multi-angle LS instrument, a correction factor is needed to convert the detector angle θ_{DET} to the actual scattering angle θ_{LS}. The difference between these two angles varies, depending on the scattering angle and the bending of the scattered beam through the liquid-glass interface. The angular correction factor is needed to compensate for this liquid-glass interface refraction effect. Unfortunately, this angular correction of θ_{DET} to θ_{LS} is a source of error in the molecular weight and size calculations. At the 90° angle the scattering beam exits at a normal angle to the liquid-glass interface. This means that no detector angle correction is needed for θ_{LS} at the 90° scattering angle.

Since the actual polymer concentration of the samples eluting from the SEC column is highly dilute, the second term in Equation [A.2-1] is often negligible in the SEC application:

[A.2-8] $$\frac{KC}{R_\theta} = \frac{1}{M_w P(\theta)}$$

In theory, the polymer size parameter can be derived from the $P(\theta)$ function. Again, in theory, the $P(\theta)$ function and therefore the polymer R_g value can be determined by a multi-angle approach to light scattering detection. However, the molecular size measurement by SEC and multi-angle LS has may severe practical limitations.

The $P(\theta)$ function approaches unity for molecules having sizes less than 1/20 of the wavelength of the incident light. When that happens, the scattering intensity alone can no longer be used as a tool to measure molecular sizes. Under the circumstances, the LS intensity becomes a function of only the molecular weight. Light scattering loses its angular dependency and its discriminating power on molecular sizes. As $P(\theta)$ approaches 1.0, LS becomes strictly a MW measurement, no longer sensitive to molecular size:

[A.2-9] $$\frac{KC}{R_\theta} = \frac{1}{M_w}$$

or,

[A.2-10] $$R_\theta = KCM_w$$

Signal-to-noise limitation presents the other problem in the multi-angle light scattering detection of the SEC effluent. Reliable $P(\theta)$ determination for molecular size measurement is not yet possible by the SEC-multi-angle LS combination, especially for the determination of the initial $P(\theta)$ slope at the low scattering angle near θ_{LS} approaches zero. Meaningful R_g values are obtainable only for MW > 200,000 or more.[9,9]

SEC-VISCOMETRY-LS

[9] W. W. Yau, Chemtracts-Macromolecular Chemistry, *1*, 1 (1990).

With SEC-LS coupled with an on-line viscometer in the triple-detector configuration, additional precision can be gained in determining molecular sizes, and thus providing meaningful information of polymer branching and conformational differences.[10,11]

The coupling of a viscometer and a light scattering detector in one SEC instrument greatly improves the precision and dynamic range of SEC for polymer conformation studies. The SEC-Viscometry-LS results are insensitive to typical adverse variations of SEC experimental conditions, such as flowrate inconsistency, band broadening, column deterioration, non-SEC retention effect, moderate sample overloading, etc.

In the SEC-Viscometry-LS experiment, the molecular size at every elution volume can be calculated in addition to the molecular weight value. For the hydrodynamic radius of the molecule (R_h):

[A.2-11] $$R_h = \left[\frac{3}{4\pi} \left(\frac{[\eta]M}{0.025} \right) \right]^{1/3}$$

For linear flexible chain polymers, this can be related to the radius of gyration, using the Flory-Fox and Ptitsyn-Eizner equations,[12,13] where,

[A.2-12] $$R_g = \left(\frac{1}{6} \right)^{1/2} \left(\frac{[\eta]M}{\Phi} \right)^{1/3}$$

[A.2-13] $$\Phi = 2.55 \times 10^{21} \left(1 - 2.63\varepsilon + 2.86\varepsilon^2 \right)$$

[A.2-14] $$\varepsilon = \frac{2a - 1}{3}$$

where a is the exponent of the Mark-Houwink equation:

[A.2-15] $$[\eta] = KM^a$$

SEC-VISCOMETRY-RALLS/LALLS

In this triple detector method, the angular dissymmetry in the scattered intensity is accounted for by using the combined measurements of intrinsic viscosity at 90° light scattering intensity (RALLS: Right Angle Laser Light Scattering- **Figure 6**) to determine both molecular size and the molecular weight. Size determination of small polymer molecules is possible with this new method, even when there is no observable multi-angle LS dissymmetry.

[10] M. A. Haney, C. Jackson, and W. W. Yau, Water's International GPC Symposium Proceedings, 1991.
[11] C. Jackson, H. G. Barth, and W. W. Yau, Water's International GPC Symposium Proceedings, 1991.
[12] T. G. Fox and P. J. Flory, J. American Chemical Society, *73*, 1904 (1951).
[13] O. B. Ptitsyn, and Y. E. Eizner, Sov. Phys., Tech. Phys. 4, 1020 (1960).

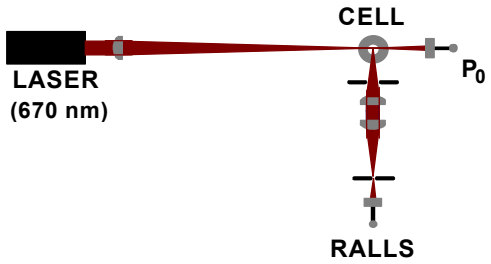


Figure 6: RALLS Diagram

Angular light scattering dissymmetry is not a factor for many important commercial random-coil type polymers having molecular weight values in the order of 100,000 or less, like most polycondensation, Nylon, or Dacron type polymers. For higher MW samples, a proper account of LS dissymmetry is highly desirable to assure a better than 5% accuracy in the molecular weight determination. In Viscotek's software, the following algorithm is used to account for LS dissymmetry automatically to guarantee accuracy:

1) Initially, the particle scattering function at 90°, $P(90°)$, is assumed a value of 1.0 and a molecular weight estimate M_{est} is calculated directly from the RALLS intensity R_{90} from Equation [A.2-10], that is:

[A.2-16] $$M_{est} = \frac{R_{\theta=90^\circ}}{KC}$$

where K is the optical constant defined earlier.

2) A Flory-Fox radius R_{FF} is then calculated from the above M_{est} and the experimental intrinsic viscosity value $[\eta]$:

[A.2-17] $$R_{FF,est} = \left(\frac{1}{6}\right)^{1/2}\left(\frac{[\eta]M_{est}}{\Phi}\right)^{1/3}$$

where Φ is the Flory viscosity constant defined earlier in Equations [A.2-12] and [A.2-13] that take into account the different degrees of solvent expansion factor for the polymer molecules.

3) An improved 90° dissymmetry value of $P(90°)_{est}$ is then calculated from a specific particle scattering function that fits either the rigid-rod, hard-sphere, or random coil type; the following Debye $P(\theta)$ function is chosen to update the $P(90°)_{est}$ value.

[A.2-18] $$P\left(90^\circ\right)_{est} = \frac{2}{X^2}\left(e^{-X} - \left(1 - X\right)\right)$$

where,

[A.2-19] $$X = \frac{4\pi n_0}{\lambda_0} R_{FF,est}$$

where n_0 is the solvent refractive index, and λ_0 is the wavelength of the incident light.

4) A new estimate of the molecular weight is calculated from:

[A.2-20] $$\text{New } M_{est} = \frac{M_{est}}{P(90°)_{est}}$$

5) Steps of 2 and 4 are repeated using the new estimate of molecular weight until the molecular weight and R_{FF} values no longer change. This usually takes three iterations or less.

A similar algorithm, but with the different $P(\theta)$ functions is provided in the software as an option for treating polymer samples of other conformational architectures. Either a rigid-rod or a hard-sphere particle scattering function can be used besides the random coil model used as an example to explain the basic features of the SEC-Viscometry-RALLS software package.

The accuracy of the MW determination by the SEC-Viscometry-RALLS triple detector technique is illustrated by the results obtained on polybutadiene standards shown in **Table A.2-1** shown below.

Nominal MW of Supplier	[η] (dl/g)	Rg (nm)	P(90°)	MW by SEC-Visc-RALLS	MW by UNICAL
1K	0.053	1.29	1.00	1.03K	1.36K
3	0.108	2.36	1.00	3.09	3.45
5	0.18	3.71	1.00	7.07	7.33
22	0.439	7.7	1.00	26.3	26.3
43	0.725	11.3	0.99	51.9	50.6
170	1.82	23.8	0.94	188	161
272	2.5	30.7	0.89	295	273

Table A.2-1 Accurate MW and Rg Determination by Triple Detector SEC of Polybutadiene Standards in THF

The P(90°) values in the fourth column are the angular dissymmetry factor of the particle scattering function measured at the 90° scattering angle. Remember that the P(90°) value equals the ration of the scattering intensity at the 0° scattering angle. The P(90°) factor can be 1.00 or less. A P(90°) value of 1.00 is indicative of the fat that there is no angular dependence of the scattering intensity, reflecting the presence of an isotropic scattering of small molecular size. When P(90°) is close to 1.00, no angular dissymmetry correction of the 90° scattering intensity is needed for the accurate MW determination.

The MW values from the triple detector measurement are shown in the fifth column of **Table A.2-1**. These values have been corrected for the angular dissymmetry factor of the P(90°) value. It is apparent from the P(90°) data that no correction is needed for the low MW samples, and there is up to an 11% correction made for the 272K MW standard. It has been shown that accurate MW determination is possible for samples up to several million MW by the RALLS measurement with the dissymmetry correction. The RALLS dissymmetry correction is automatically done in the triple detector software using the R_g values calculated from the combined IV and MW information derived from the viscometer and LS detector signals.

The MW values in the last column of **Table A.2-1** are calculated by a different approach that incorporates the universal calibration method and only the SEC viscometer data, but not the RALLS data. The close agreement of the MW values of the last two columns, which are determined by two totally different methods, gives the strong evidence for the accuracy of the triple detector approach in determining polymer MW and size. The most amazing feature of the triple detector technique is its capability of measuring polymer R_g values down to a value as low as one nanometer, which is an equivalent of 10 angstroms, a value approaching the measurement of chemical bond dimensions! (See the third column of **Table A.2-1**.)

In certain situations, however, the dissymmetry correction becomes important. Rod-like materials of high molecular weight can exhibit a large angular dissymmetry that can alter the correction algorithm used by the RALLS detector. The low angle (LALLS) detector can be used for these materials, or can be used on any other sample with molecular shapes having undefined size where very accurate values of molecular weight are desired.

In the case of a light scattering detector that can measure responses below 10 degrees, the particle scattering function P(θ) approaches a value of unity. Therefore, no assumptions are needed, and the calculated molecular weight is highly accurate. The Viscotek LALLS detector measures signal response at an angle of 7 degrees having virtually no background noise and a high signal-to-noise ratio compared to earlier low angle instruments.

The remarkable ability of triple detector SEC to detect polymer branching has been illustrated for an experimental bimodal polymer. The RALLS detected a very high MW species in the prepeak while the viscometer showed a weak signal for a relatively small molecular size. This is indicative of a highly branched structure. The presence of this branched structure would not have been detected by the conventional SEC using a single RI or UV detector.

The quantitative determination of polymer branching and chain conformation relies on the measurement of the Mark-Houwink exponent a in the viscosity-to-MW relationship shown earlier in Equation [A.2-15]. Because [η] and MW values in SEC-Viscometry-RALLS/LALLS are measured directly from detector signals, the precision of determining this Mark Houwink a value by triple detector SEC is significantly better than any other technique that exists today. As shown in **Table A.2-2** below, with triple detector SEC, the precision of the a value determination is now quite good, reliable to two significant digits or better. This degree of precision is necessary for the meaningful use of this a value for the investigation of polymer branching and conformational differences. The standard deviation of the a value determined by the triple detector is almost two orders of magnitude better than that by the multi-angle laser light scattering approach.

a values, where $[\eta] = KM^a$

	SEC-MALLS	SEC-Visc-RALLS
	0.47	0.729
	0.41	0.726
	0.23	0.731
	0.59	0.727
	1.19	0.723
	0.38	0.739
	1.52	0.735
	0.44	0.724
Average	0.62	0.729
Std. Dev.	0.044	0.005

Table A.2-2 Unprecedented Precision of SEC-Viscometry-RALLS in Determining Polymer Conformation, Repeated Runs of Polystyrene Standard (NBS 706) in Toluene

The Mark-Houwink a value is a polymer conformation parameter. The a value decreases for molecules of more compact structure. This a parameter can have a near zero value for globular proteins. For random coil molecules, it can have a value between 0.5 for poor solvent conditions and 0.8 for good solvent conditions. An a value of 1.0 or greater is possible for extended rod-like molecules. For polymers containing long chain branching, the a value can fall significantly below 0.5, depending on the degree of branching. The g' branching index is calculated as the ratio of sample $[\eta]_B$ value against its linear reference $[\eta]_L$ value of same molecular weight:

[A.2-21] $$g' = \frac{[\eta]_B}{[\eta]_L}$$

In conclusion, SEC-Viscometry-RALLS/LALLS is capable of determining accurate molecular weight and molecular distribution of polymer samples over the widest range of molecular weights. The technique provides extra sensitivity at low molecular weights. The triple detector combination also eliminates the need of SEC column calibration and instrumental band broadening correction.[14] In addition, the low angle LALLS detector can confirm the molecular weight calculations of odd shaped species where the angular dissymmetry correction factor is altered. Therefore, the technique can provide the most accurate determinations today for the polymer MW, intrinsic viscosity $[\eta]$, Mark Houwink exponent, and branching factors. The triple detector SEC system represents the most powerful tool existing today to meet the challenges of the polymer and biopolymer characterization needs. Viscotek's SEC-Viscometry-RALLS/LALLS instrument is supported with a smart A/D Data Manager and the fully integrated PC-based Windows TriSEC software.

A.2.b Components

The Light Scattering detector is composed of a cell block and Laser diode mount. **Figure 7** is an exploded view of the cell block. It is contained inside the heated oven compartment. It contains both the 90° and the 7° detectors housed in separate optical blocks.

[14] W. W. Yau, J. J. Kirkland, and D. D. Bly, "Modern Size Exclusion Liquid Chromatography", Wiley, New York, 1979.

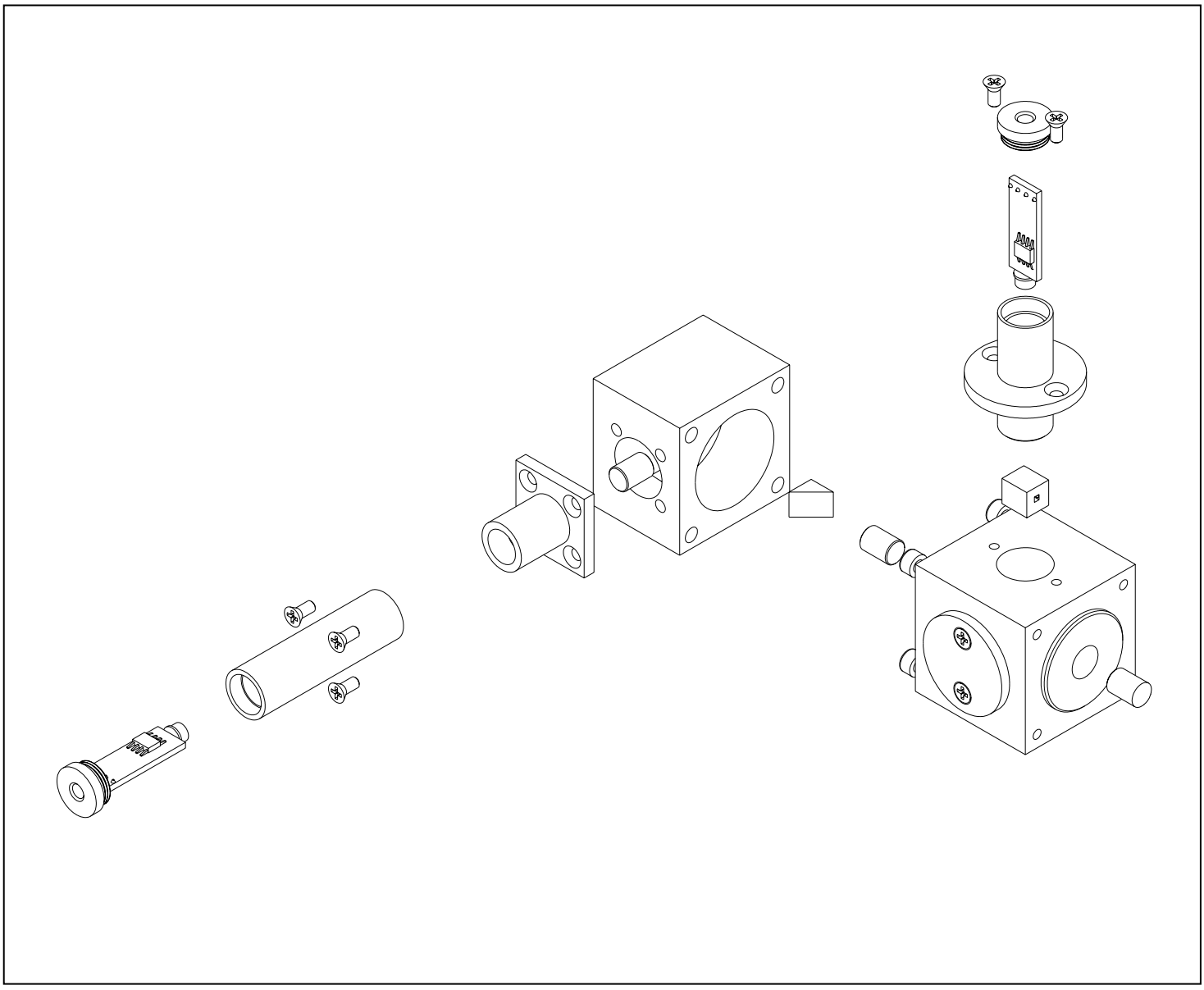


Figure 7: Light Scattering Detector Detail

A.2.c Specifications

Measuring Angle	90° and 7°
Light Source	Constant optical power output laser diode
Wavelength	670 nm
Output Power	3 mW
Detectors	Photodiode/Amplifier
Cell Volume	10 μL

A.3 REFRACTOMETER DETECTOR

A.3.a Theory

The RI detector is a conventional dual cell, deflection design **Figure 8.**. Diffuse light from the source is directed onto the mask behind the dual cell, passed through the mask, cell, slit arrangement, and is then reflected by the adjustable concave mirror. The concave mirror focuses and aims the beam back through the cell onto the mirrored prism. The prism splits the beam into two component beams, each component hitting a separate photodiode detector. The photodiodes generate an electric current proportional to the light received. These electric currents are then subtracted from each other and the amplified difference forms the RI signal output.

When both sides of the cell (sample and reference) contain liquid of identical refractive indices, no deflection of the beam is observed. This would be the expected condition at chromatographic baseline, for example. The concave mirror is then aligned so that near zero signal is output. Now when the sample elutes into the sample side of the dual cell, the light beam is refracted (deflected) according to Snell's Law of Refraction:

$$[A.3-1] \quad \frac{n_s}{n_r} = \frac{\sin\theta_r}{\sin\theta_s}$$

where q is the angle of incidence with respect to the normal of the plane separating the two liquids, and r and s refer to the reference and sample liquids, respectively.

The RI signal output can be shown to be proportional to the difference in refractive index of the sample and reference liquids.

$$[A.3-2] \quad \frac{n_s}{n_r} = \frac{\sin 45}{\sin(45-\theta)} \cong \frac{\sin 45}{\sin 45 - \sin\theta}$$ (For small angular deflections of θ.)

Here it is assumed that the RI cell is a 45° cell, which is the usual case. We then rearrange to:

$$[A.3-3] \quad \frac{n_s - n_r}{n_s} = 1.414 \sin\theta$$

However, $\sin\theta \cong \frac{d}{L}$ and $V_{output} \propto d = L \sin\theta$.

Inserting and rearranging, we find

$$[A.3-4] \quad \frac{n_s - n_r}{n_s} = k' V_{output}$$ where k' is an instrument constant.

In the usual application, the reference liquid is the solvent for the sample, so that we may equally state that the signal is proportional to the sample concentration and the refractive index increment (dn/dc) of the sample.

$$[A.3-5] \quad V_{output} = k C \frac{dn}{dc}$$

The constant k is an instrument sensitivity constant (calibration constant) for the refractometer. Because the refractometer is not designed to be an absolute refractometer, but a detector, the only way to determine the instrument constant is by calibration with a standard solution of known concentration and dn/dc value. This procedure is available in Viscotek's TriSEC software. Once the instrument constant is determined by calibration, it should remain constant for an indefinite period of time unless the solvent is changed, some optical re-alignment is made, or the cell is discolored due to residue accumulating on its surface.

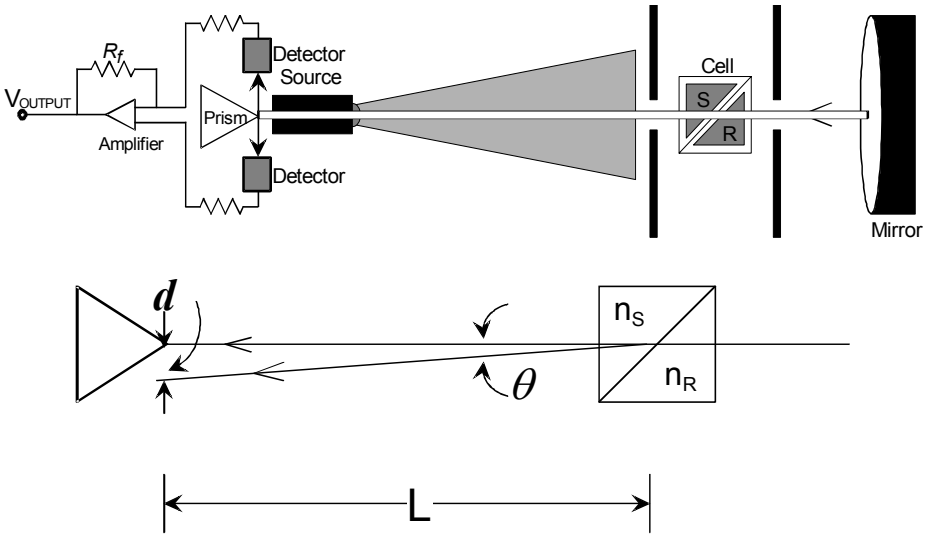


Figure 8 Optical Schematic of RI detector

A.3.b Components

The RI detector is fully contained within the RI block assembly, as noted previously. It consists of the aluminum block and its two endcaps, one covering the source/detector end (not shown in **Figure 9**), and the other cap covering the cell/mirror end (the front end).

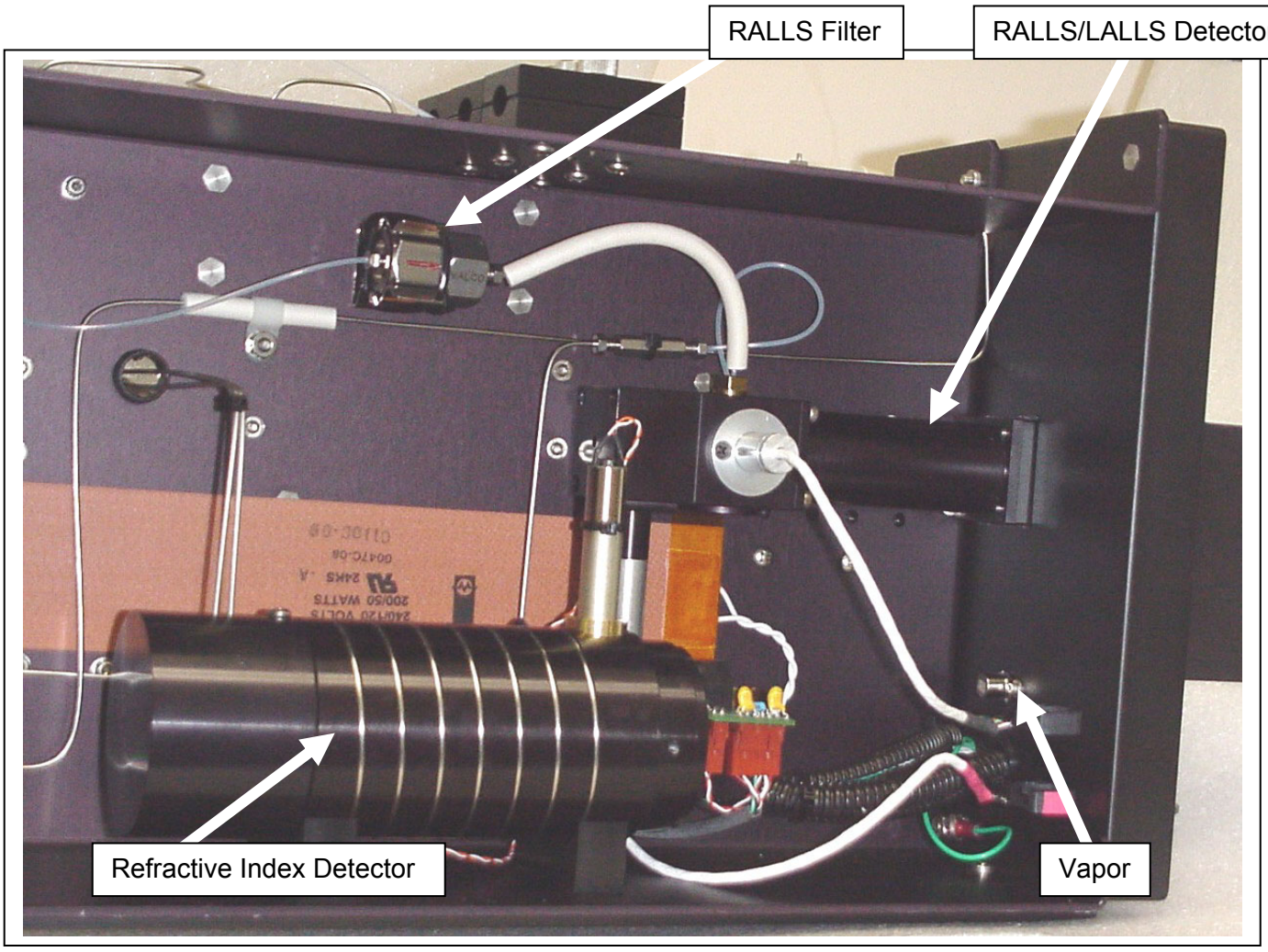


Figure 9: Vapor sensor, Light Scattering and Refrective index detectors

CELL / MIRROR END

The sample equilibration tubing is wrapped in a helical groove on the outside diameter of the RI cylinder block, **Figure 10**. This tubing connects directly into the bottom and top of the stainless steel cell mounts. The cell mounts consists of a small metal blocks with two stainless steel tubes protruding through them to hold the cell seals. An anodized black metal cell block with mounting holes to the RI cylinder block is used to assemble these components. A slit diaphragm is attached to the inside of the cell block. The slit diaphragm defines the reflected beam before it leaves the cell on its path back toward the detector The dual RI cell is dropped into the cell block on top of the lower seal mount containing two TFE seals. Seals are placed on the upper seal mount and it is placed on top of the cell and then secured in place with a brass nut.

The concave mirror is mounted (with permanent epoxy adhesive) on the mirror mount, which in turn is mounted on end of the cell block. A blackened aluminum endcap encases the entire cell/mirror assembly.

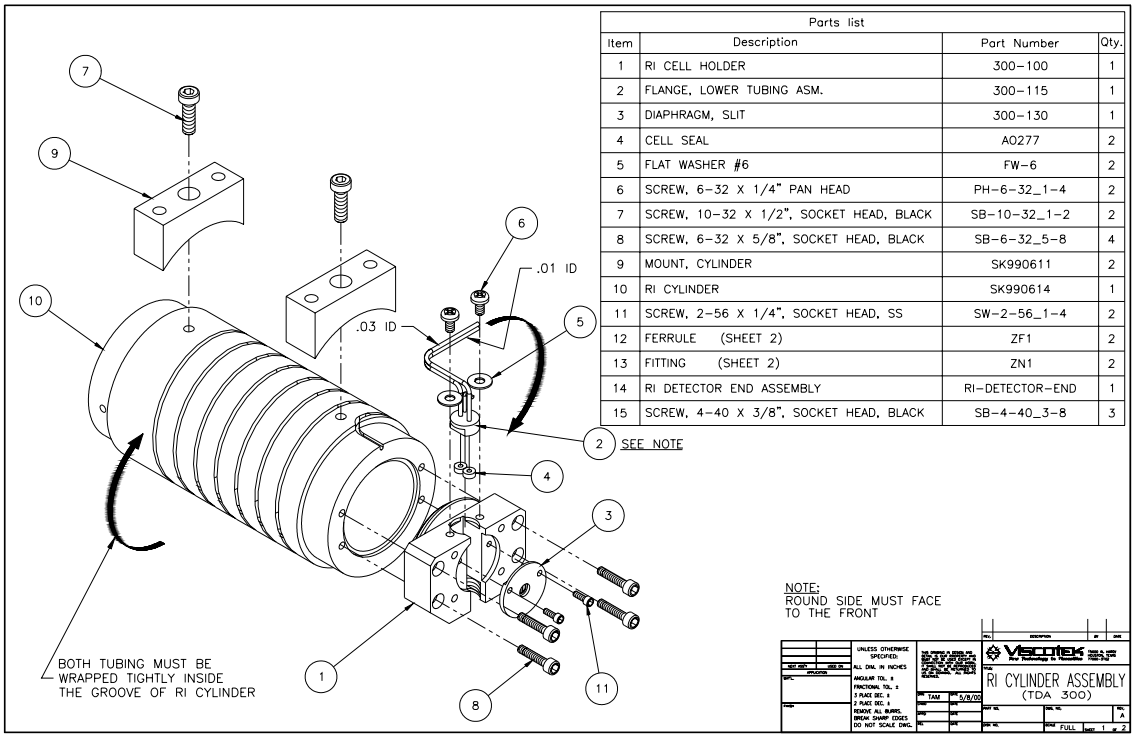


Figure 10 Cell /Mirror End of the RI Block Assembly

SOURCE / DETECTOR END

The light source and detector are mounted on the detector end plate, which is mounted with three screws to the end of the RI cylinder block. The source is mounted in a clamp, which

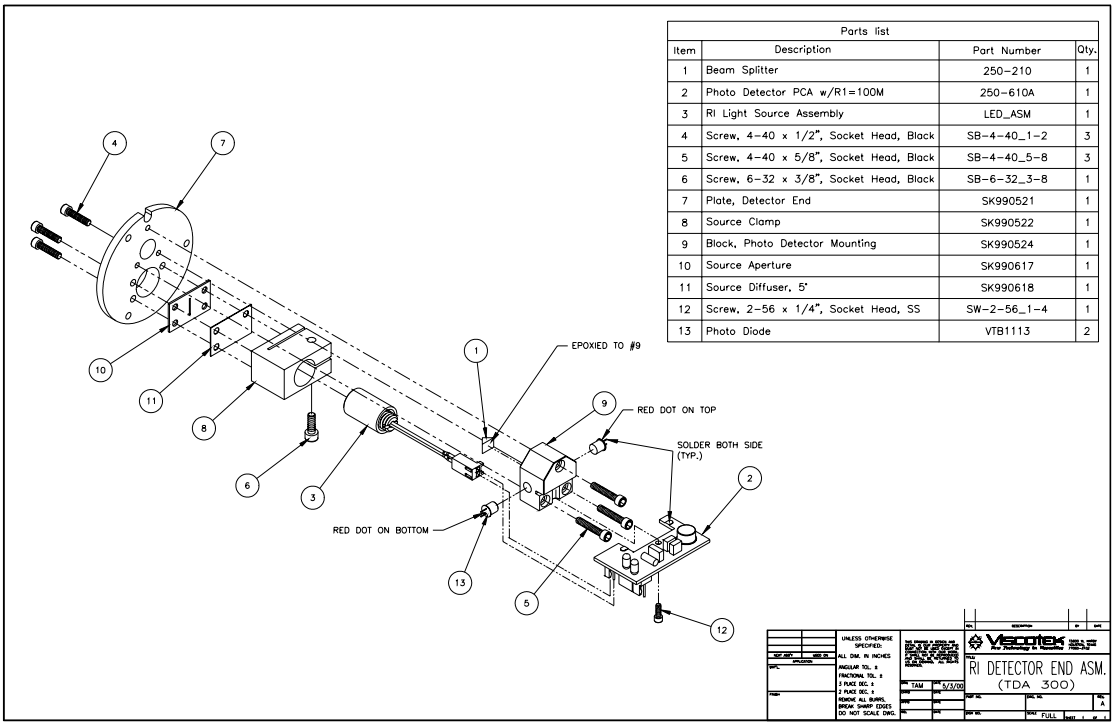


Figure 11 Source/Detector End of the RI Block Assembly

Is mounted in the center of the detector end plate. Below the source clamp, the detector block is mounted to the detector end plate, **Figure 11**. Inside the detector block is the beam splitter prism and the two photodiode detectors, located on either side of the detector block. The photodiode detectors are also mounted via their leads to the photodetector circuit board (PCB).

Cable connections are provided on the photodetector PCB for the LED light source and for the I/O cable to the RI Output PCB. Enclosing the source/detector end is the blackened aluminum endcap. A slit is provided in the cap for the I/O cable to exit.

RI OUTPUT

Outputs from the two photodetectors are differentially acquired on the TIA photodetector PCA. This differential signal is fed via the I/O cable to the main CPU board

RI output voltage is displayed on the front panel display meter when the detector is selected from the front panel board and is also available as DC output voltage on the back panel output connector when selected. The RI photoelectric signals are not adjustable by the user.

OPTICAL ZERO

The user can adjust this optical zero approximately by using the front panel switch to adjust the zero glass assembly. Please see section A.1.b General Layout for operation.

REFERENCE PURGE

The reference cell can be purged under normal flow conditions by switching ON the RI purge valve. Continue solvent flow for several minutes or until a stable RI signal is obtained.

The flow path during purge condition can be followed from the flow diagram. Flow is always first through the sample cell, then through a Tee to the top of the reference cell, through the purge valve, then out to the viscometer.

VAPOR SENSOR

The vapor sensor can be adjusted more or less sensitive depending on the solvent used. A level detector circuit (U21) monitors the chemical sensors output signal. The detection level is determined by potentiometer VR5 on the 301 CPU board. The factory setting for VR5 is 4.0 VDC. Clockwise rotation of VR5 increases the sensitivity of the detector. The voltage setting of VR5 can be measured across C1 on the CPU board, which is located directly below VR5. It should be noted that the chemical sensor has a built in heater, which requires a warm-up period of approximately 5 minutes. During the initial power-up state, the vapor detector output signal will be higher than its operating steady state level. Should a false alarm be detected, turn VR5 counter-clockwise to decrease the sensitivity of the detector.

A.3.b RI Specifications

Cell volume (μL)	12
Maximum backpressure on cell	30 kPa (5 psi)
Maximum Signal (V)	10 (nom.)
Baseline Noise (mV)	0.10
Baseline Noise (RIU)[15]	2×10^{-8}
Baseline Drift (mV/hr)[16]	5.0

[15] Refractive Index Units
[16] Measured under no flow or reference purge conditions.

A.4 VISCOMETER DETECTOR

A.4.a Theory

The viscometer detector utilizes the patented technology of the four-capillary bridge to minimize the influence of flow rate fluctuations and maximize the sensitivity of detection.

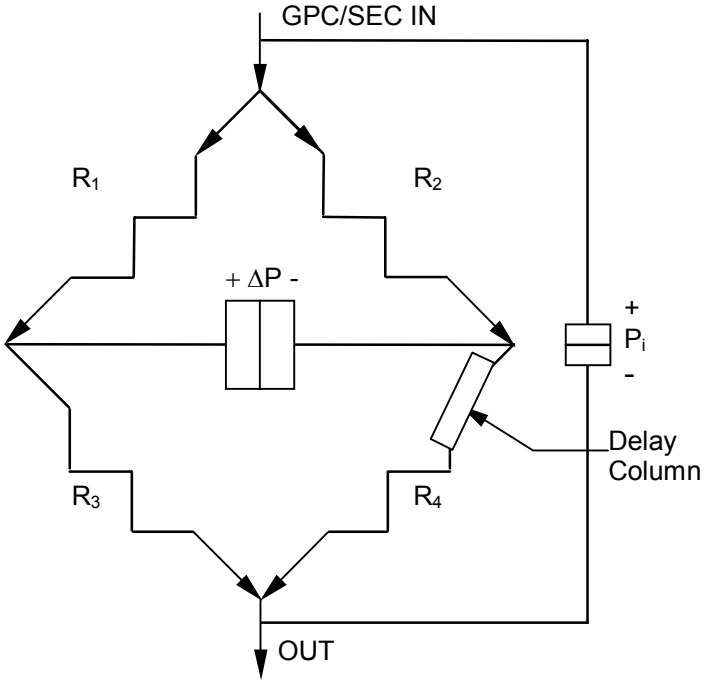


Figure 12 Four-Capillary Bridge

Consider that the four capillaries R_1 - R_4 have equal flow resistances, **Figure 12**. Then when pure solvent of viscosity η_0 is flowing in all four capillaries, the differential pressure $DP = P_2(+)$ - $P_2(-)$ will be zero, or have some near zero baseline value. Then as the polymer solution elutes through the bridge, the viscosity in capillaries R_1, R_2, and R_3 will increase to η, while the pure solvent remains flowing through capillary R_4 due to the delay volume column. Now it is found that DP is no longer zero, but increases in approximate proportion to the specific viscosity of the solution. The exact equation is shown below.[17]

[A.4-1] $$\eta_{sp} = \frac{4DP}{IP - 2DP}$$

where DP = Pressure across middle of bridge = $P_2(+) - P_2(-)$

IP = Inlet pressure through bridge top to bottom = $P_1(+) - P_1(-)$

[17] See Haney, M. A. , American Laboratory, 17 (4), 116-126 1985, for the complete derivation.

η_{sp} = Specific viscosity of the solution = $\frac{\eta - \eta_0}{\eta_0}$

DP is typically measured in Pa; IP is typically three orders of magnitude larger than DP and is measured in kPa. Thus, Equation [A.4-1] can be approximated as

[A.4-2] $$\eta_{sp} \approx \frac{4DP}{IP}$$

The inlet pressure is more or less constant, being primarily a function of the flow rate of the solvent and its viscosity. The pressure DP provides the "viscometer chromatogram," as it is nearly proportional to the change in viscosity, *i.e.*, specific viscosity. Note that the IP will sometimes show a slight change as the sample elutes. This is essentially a "single capillary viscometer" response to the sample. The full power of the bridge to cancel flow rate pulsation and noise is seen in the DP signal.

DELAY COLUMNS

It is clear that the delay column is an important component of the bridge, as it provides the reference solvent to the reference capillary R_4 during the period of elution of the polymer. If the delay volume provided by the delay column is well-matched to the elution profile (chromatogram), the polymer solution will not break through into capillary R_4 until well after the sample has eluted and DP has returned to baseline, **Figure 13**.

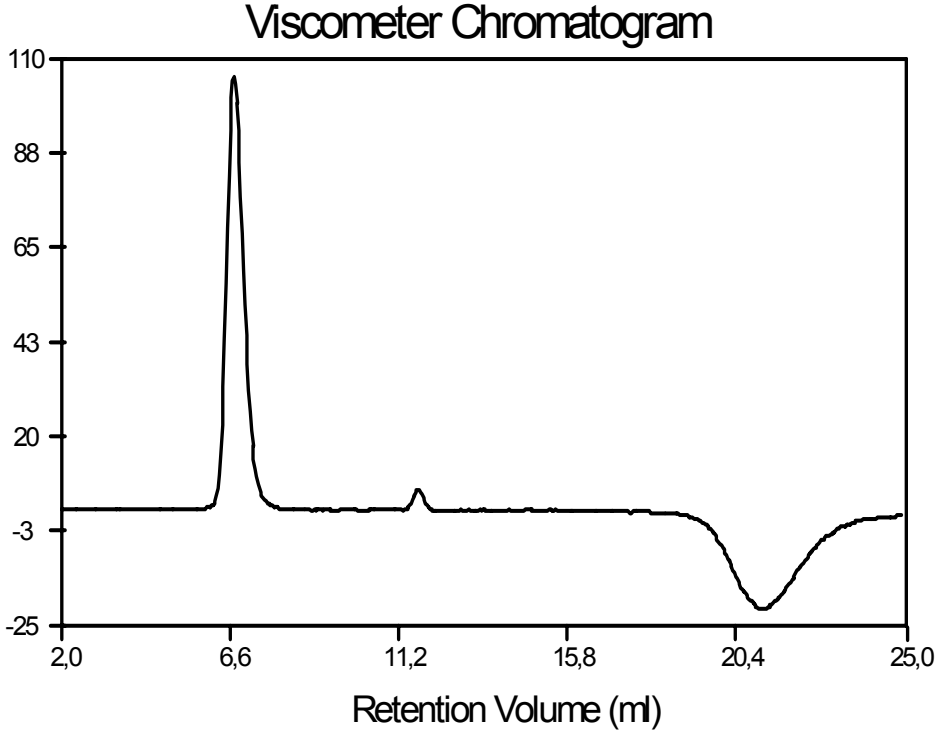


Figure 13 *DP* Chromatogram Showing Ideal Delay Volume

However, if the delay volume is too small, the breakthrough will occur too early, interfering with the DP chromatogram, as shown in **Figure 14** below.

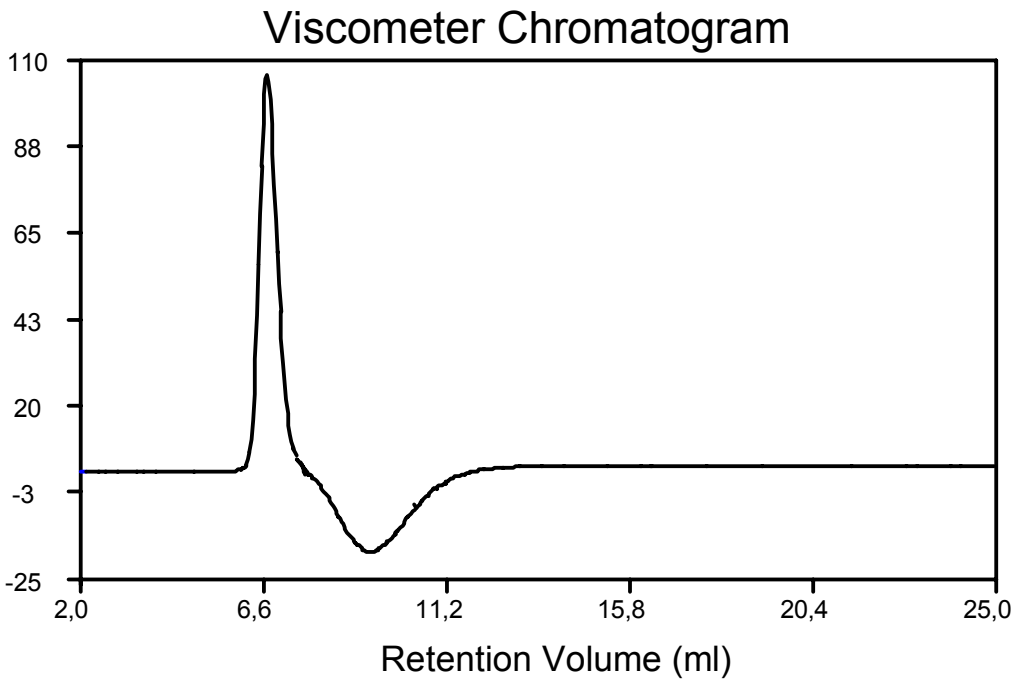


Figure 14 *DP* Chromatogram Showing Delay Volume Too Small

The third possibility is that the delay volume is too large, a situation that will result in overlap of the breakthrough negative DP peak with the chromatogram of a subsequent injection. This may always be prevented by delaying the start of the next injection, but at a loss in sample throughput.

So it is important that the delay volume be matched to the chromatographic columns being used for the separation. Viscotek provides two columns for this purpose: small (one to two columns), and large (three to four columns). They are easily changed out whenever the SEC columns are increased or decreased.

A.4.b Components

CAPILLARY BRIDGE

The four capillaries R_1 - R_4 are stainless steel 1/16"od x 0.01"id x 24"l. They are wrapped together in a harness and suspended in the center panel module. The inlet tubing is also 0.01"id tubing. Most other tubing connections to the bridge (to transducers, purge ports, viscometer exit tubing) are 0.04"id, **Figure 15**.

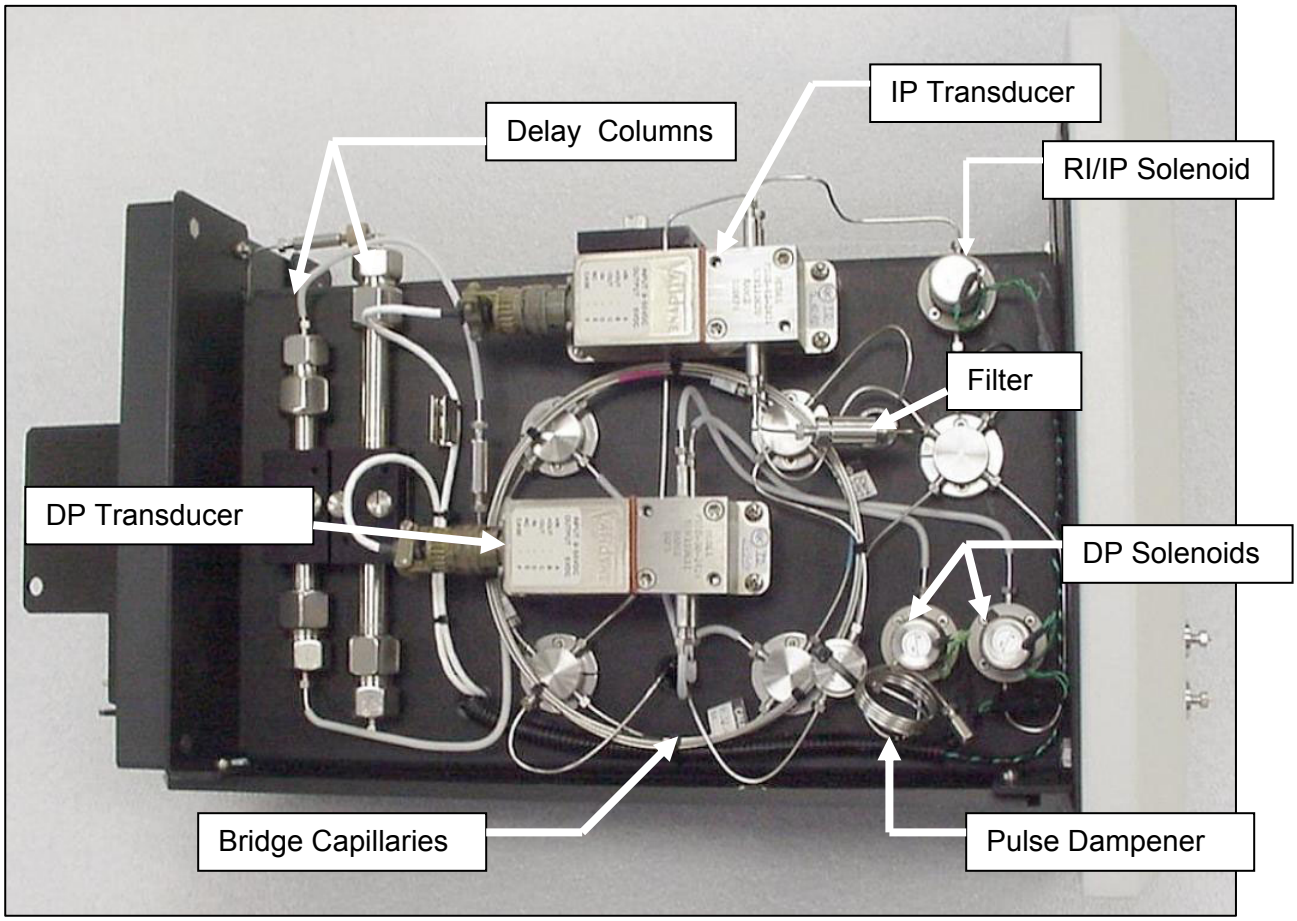


Figure 15: Viscometer components

PRESSURE TRANSDUCERS

The DP and IP transducers are of the magnetic reluctance type. They are mounted on the inside center panel of the detector module. Connections via 0.04"id ss tubing go to the transducers and to the purge valves. The IP transducer is positioned in series with the RI reference purge and is thus purged with the RI.

PURGE SOLENOIDS

The transducers must be purged completely of air bubbles in order to obtain an optimum baseline. This is accomplished by using the purge switches located on the front panel while flow is on and allowing solvent to drain to waste for a few minutes.

When the dP purge valves are open, GPC flow is diverted through the DP(+) and DP(-) transducer cavities through the purge valves in preference to flowing through the bridge, due to the high resistance of the bridge. The flow splits more or less equally between the DP(+) and the DP(-) purge ports. One may conclude that air bubbles are purged from the transducers when the reading on DP becomes steady for several minutes while purging. Note: In order to purge the DP transducer, GPC flow must be turned on.

It is also advisable to purge the transducers after changing mobile phase solvents, although there may be some cases where the solvent is corrosive to the transducers and may best be excluded. In this case, the operation of the viscometer will be satisfactory after sufficient purging without opening the purge valves, provided air bubbles do not get into the DP transducer cavity spontaneously.

DELAY COLUMN

The delay column is mounted on the Viscometer Mount using the mounting clamp(s) provided. It is connected on its inlet end in the bridge using 0.04"id SS tubing. On its outlet end it is connected to R4 using a length of 0.04"id tubing and a 1/16" union (U1).

Any air bubbles in the delay column will affect the DP signal stability the same as if they were in the DP transducer. Therefore, it is equally essential that the delay column be purged of any air. Normally, the continuing flow of mobile phase will keep the column free of air bubbles. However, if some air is introduced into the system, it will take the equivalent of a chromatographic run to purge them out. During this time, the viscometer signal may be noisy.

VISCOMETER SIGNAL OUTPUT

The transducer signals from both the IP and DP transducers are amplified, and scaled. No adjustments of amplification or scale are available to the user. Output on the back panel is fixed according to the following scale:

Transducer	Output sensitivity	Maximum Output
DP	1.0 mV/Pa	5000 Pa
IP	1 mV/kPa	100 kPa

Note: the front panel display will over-range at 1999mV with a full scale voltage setting of 2.5V. The instrument will over-range at 100mV and 500mV, as well if those full scale gain settings are chosen.

The IP signal is an absolute value and therefore *must* be precisely zeroed *with the flow off*. Routine checking of the IP zero is a necessary practice. To zero the IP signal, turn the GPC flow off, push the select button until the IP channel is selected. Push the zero button.

A.4.c Viscometer Specifications

Capillary Dimensions	0.01”id x 24”L
Bridge Balance	1 %
Maximum Flow Rate (H_2O)	1.5 mL/min
Maximum Flow Rate (THF)	3 mL/min
DP Noise Maximum*	0.3 mV (0.3Pa)
DP Noise Typical*	0.2 mV (0.2Pa)
DP Drift Typical**	1.5 mV/hr

* Assuming variations in IP of less than 1%.
** With flow off or with negligible variation in solvent composition and IP.

B. INSTALLATION

B.1 INSTALLATION KIT FOR MODEL 302 TDA

Quantity	Item
1	Power Cable
1	12-Pin Phoenix Connector
1	Viscotek Screwdriver
1	Allen Head Wrench, 0.050"
1	36" SS Tubing, 1/16"x 0.01"id
1	36" TFE Tubing, 1/16"x 0.01"id
1	36" TFE Tubing, 1/16"x 0.03"id
10	Valco Nuts, 1/16"
10	Valco Ferrules, 1/16"
3	Valco Cap, 1/16"
2	Valco Plug, 1/16"
1	Valco Union, 1/16”x 0.03”id
1	RALLS Filter:
	2 Teflon O-ring P/N FIL060
	2 SS Frit - 0.5 micron P/N 728025
	10 Filter Membranes:
	5 - 2046 nylon (Aqueous)
	5 - 2013 PTFE (Organic)
1	PS 90 Narrow Calibration Standard
1	PS 280 Broad Calibration Standard
1	Instrument Manual
2	4 Ft. Analog Signal Cable (1 Pr..Shielded)
1	10 Ft. Trigger Cable (1 Pr.)
1	10 Ft. Serial Cable
1	10 Ft. Diagnostic Cable

B.2 CONNECTION TO SEC SYSTEM

The Model 302 TDA is designed to be directly to the outlet of a properly functioning HPLC pump by the minimum length possible of 0.01”id SS tubing.

1. Check the power receptacle on the back and make sure that the voltage set is correct for the AC power source (110V/220V) being used. Connect the power cord and turn on the main power switch on.

2. The detector is normally checked out and shipped with THF solvent. If possible, it is best to use THF for the initial checkout at the site. If another mobile phase is to be used, be certain that it is miscible with THF before proceeding.

3. With the detector drawer removed from the oven module, flush fresh solvent from the pump to the connection provided to the inlet of the separation columns.

4. If the instrument is a 302 TDA with a LS detector verify that a RALLS filter is properly installed below the column mounting platform, **Figure 16**.

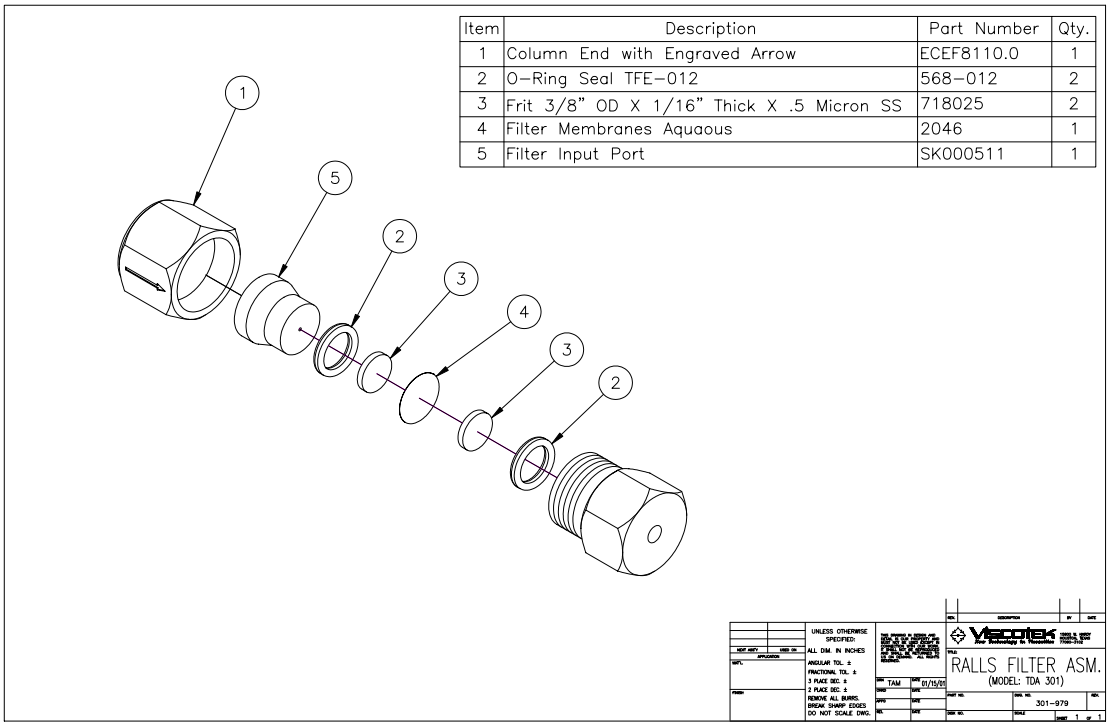


Figure 16 Light Scattering Filter Assembly

5. Verify that the pneumatic pulse dampener is empty by removing the s cap and loosening the nut at the SS tee. Solvent will drain from the tube. See **Figure 17** for proper assembly.

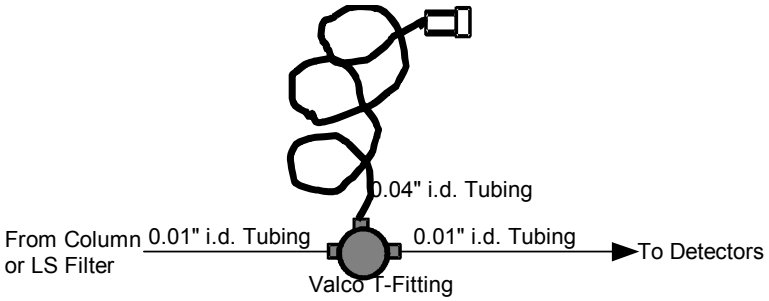


Figure 17 Pneumatic Pulse Dampener

6. Connect a waste line to the outlet connection on the front of the detector.

7. With the solvent flowing slowly, install the separation columns. Increase the solvent flow to normal flow-rate for at least three column volumes before slowing the flow-rate.

8. Connect the outlet of the SEC columns to the tubing that leads to the LS filter.

9. Turn on the mobile phase flow to normal running flow-rate and allow the system to purge for 20 minutes.

10. Insert the detector drawer into the oven module. Apply power and program temperature to working value. Allow the detectors to stabilize. This could take several hours.

11. Purge the Refractometer detector for approximately 5 minutes or until the displayed value becomes stable.

12. Purge the Viscometer detector for approximately 5 minutes or until the displayed value becomes stable.

13. Turn off the flow; wait several minutes and then zero the Inlet Pressure transducer. Resume normal flow.

14. Run a "monitor Baseline" to verify proper detector operation.

15. Run a test sample using the PS90K narrow calibration standard provided. If using another solvent, use an appropriate narrow standard for that solvent. Use one SEC column only for this test.

B.3 INSTALLATION OF OTHER DETECTORS

If a UV detector is to be used, plumb the detector before the 302 TDA module. Any other detector can be plumbed after the 302 TDA module. Connect any analog signal cables to the analog signal terminal strip located on the back of the 302 TDA.

B.4 CORROSIVITY OF SOLVENT AND COMPATIBILITY WITH INSTRUMENT

The Model 302 TDA detector array will not function properly for prolonged periods of time in the presence of aqueous halides at low pH values. If the mobile phase involves a salt such as sodium chloride, the pH must be buffered toward neutral conditions. Failure to do this will cause damage to both pressure transducers.

If a halide must be used unbuffered or at a low pH, the instrument should be first purged without the presence of the salt. The salt may then be run for short periods of time without purging the differential transducer lines. Do not purge the differential pressure lines when operating in this mode. Purge the entire unit with fresh aqueous solution without halide as soon as data acquisitions are complete.

Likewise, the instrument operates best when changed between miscible solvents only. Failure to go through a proper intermediate may result in damage to the instrument or unstable baselines for a prolonged period of time.

C. OPERATION

C.1 PURGING THE SYSTEM

C.1.a Air Bubbles

The presence of air bubbles in any of the detectors will greatly increase the level of baseline noise in the signal. It is imperative that bubbles be kept out of the detectors. There are basically two ways for air bubbles to get into the system: Purging is accomplished by operation of the Refractometer and Viscometer Purge valves as previously described.

1. **By Entry from the Outside**
   This ordinarily happens only when the detector is disconnected and then reconnected. Air may enter by siphoning of solvent from the detector, or from air in the connecting tubing. To minimize this, always immediately plug off the Sample In port when disconnecting the connecting tubing. Then before reconnecting the tubing, be sure to purge the tubing of any air that may have entered.

2. **By Spontaneous Bubble Formation in the Solvent**
   If the solvent is not thoroughly degassed before use, it can occasionally form bubbles spontaneously on the wall of any static cavity. The best way to prevent this from happening is to use only filtered / vacuum degassed mobile phase solvent. For this purpose, **an in-line solvent degasser before the HPLC pump is most convenient and highly recommended.** Air bubble problems seldom occur in systems operating with an in-line degasser. Sparging is not a recommended method for degassing solvent.

C.1.b Changing Mobile Phase Solvent

The other reason for purging is upon changing mobile phase solvent composition.

1. Be certain that the new mobile phase is miscible with the old solvent that is in the detector. In most cases, the detector is shipped in THF, which is miscible with virtually all organic solvents and also with water. However, it is not miscible with all aqueous salt solutions. The best practice when going from THF to an aqueous salt solution is to go through an intermediate flush with distilled/deionized water.

2. To make a change of mobile phase in the columns, disconnect the detector from the system while making the solvent changeover in the columns. Reconnect the detector only after the columns are sufficiently purged and equilibrated with the new mobile phase. The reason for this is that columns frequently discharge residues upon solvent changeover and these residues can sometimes coat the capillary walls of the viscometer bridge and deposit debris on the surface of light walls causing high background and noise.

3. To replace the columns with another set that is already equilibrated to the new mobile phase solvent:

- Remove the old column set and replace with an in-line HPLC filter, if one is not already in place. The in-line filter will prevent particle contamination from the pump entering the detector and possibly plugging up the viscometer capillary tubing.

- Change to the new mobile phase solvent, purging continuously through the detector with both the purge ports open. Continue until at least 60 mL of new solvent has purged through the detector.

- Install the new columns and continue the detector purge for at least two column-volumes more.

- Close purge ports and examine the baselines on the detector.

C.2 ADJUSTING ZERO OFFSETS OF DETECTOR SIGNALS

C.2.a Adjusting IP Zero

The IP zero can be adjusted by selecting the appropriate channel and pushing the zero button on the front panel. No additional offset should be required. **It is very important to zero the inlet pressure with flow off only.**

Note: The IP measurement is treated as an "absolute" measurement by the data system, unlike the RI and DP signals which are baseline corrected. For this reason, ***the IP must never be zeroed with the flow on****.*

C.2c Adjusting LS Zero

The LS zero can also be adjusted by selecting the LS signal with the select button on the front panel and pushing the zero button. This provides offset up to the full scale collection setting established in the TriSEC software. Typically, this signal is zeroed with the flowrate turned on and the laser has been on for at least 45 minutes. *Note: the typical zero reading is 10 millivolts after pressing the zero button.*

C.2.d Adjusting RI Zero

The RI can be zeroed by first selecting the RI signal with the select button on the front panel, and then by moving the RI zero switch either up or down depending on the algebraic sign of the signal. Typically, this response is zeroed with the flowrate turned on, the detector has been sufficiently purged, and after the temperature has stabilized overnight.

C.3 CHECKING PERFORMANCE

There are a few measurements necessary to define in order to properly check the performance of the detectors.

C.3.a Measuring Capillary Bridge Balance

An important operating variable for the viscometer is the balance of the capillary bridge. At the factory, the bridge is balanced to 1% or better. During use, the balance can deteriorate due to partial plugging of one of the capillaries. The balance is determined by measuring the pressures at baseline condition and using the ordinary formula to compute the *apparent* specific viscosity due to the bridge imbalance. As seen earlier:

[A.2-1] $$\eta_{sp} = \frac{4DP}{IP - 2DP}$$

where DP = Pressure across middle of bridge = $P_2(+) - P_2(-)$

IP = Inlet pressure through bridge top to bottom = $P_1(+) - P_1(-)$

η_{sp} = Specific viscosity of the solution = $\frac{\eta - \eta_0}{\eta_0}$

The procedure to check the balance is simple:

1) Turn the flow on and allow to run a few minutes.
2) Turn the flow off (disconnect at the inlet to the viscometer detector).
   - Zero the IP signal.
3) Turn the flow back on again.
   - Record the initial jump of the DP signal and its settling value (Pa). An initial jump value greater than 150 Pa indicates air is in the system.
   - Record the IP value (kPa). If air in the system has been ruled out as the problem, compute the balance as below.

For example, if the inlet pressure IP is 30 kPa and DP reads 20 mV = 40Pa (with a sensitivity of 0.5mV/Pa), the apparent specific viscosity with the flow through the balance is easily determined:

$$\eta_{sp} = \frac{4DP}{IP - 2DP} = \frac{4 \times 0.040}{30 - 2(0.040)} = 0.00535$$

Guideline: If the balance gets worse than 3% (η_{sp} > 0.03 at baseline), steps should be taken to clean the bridge, or replace it if necessary.

C.3.b Measuring Baseline Noise

The measurement of baseline noise can be done most rigorously using the actual digital values of the signal to compute the rms or standard deviation about the mean. There may be some applications where this degree of rigor is necessary, but in most chromatography situations it is adequate to estimate the noise graphically as shown in **Figure 19** below.

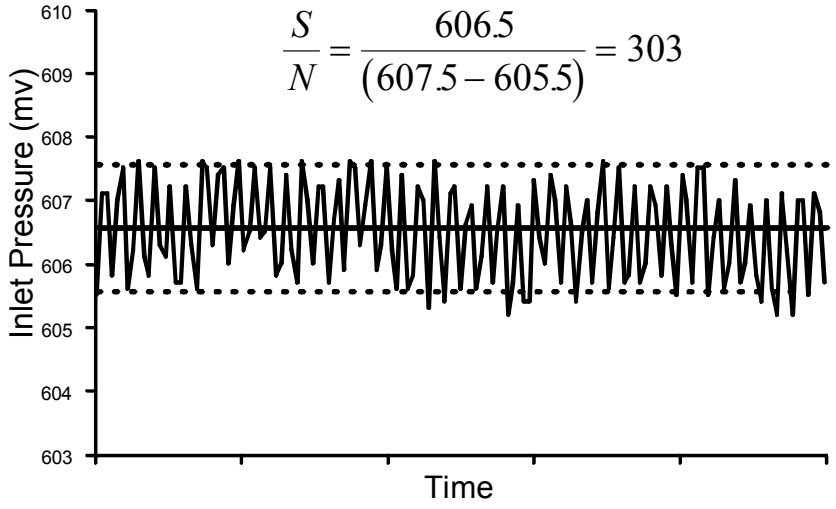


Figure 19 Method of Estimating Baseline Noise

Observe that the noise is estimated not by the peak-to-peak method, which is inappropriate for random noise, but by a visual average of the highest and lowest excursions.

Specifications*: *DP baseline noise should be < 0.5 mV
LS baseline noise should be < 1.0 mV

C.3.c Computing Pump Pulsation Noise

The pulsation of the pump is an important factor in the viscometer baseline noise. Remember that the bridge cancels most of the flow rate variations, but it cannot cancel all of them. The actual pulsation noise of the pump would have to be measured at the pump head itself using the pressure transducer normally provided with the pump. This may be a very desirable thing to do, but it is not necessarily a good measure of the pulsation that is transmitted to the detectors. The columns absorb some of the pump pulsation, for example. If the pneumatic pulse dampener is installed in front of the viscometer, it will absorb a good deal more.

For general purposes, the best measurement of the actual pulsation noise experienced by the viscometer detector comes from the noise on the IP transducer signal. At the baseline condition (with solvent in all four capillaries) this signal is directly proportional to the flow rate through the viscometer bridge. A typical IP signal at baseline is shown in **Figure 20**.

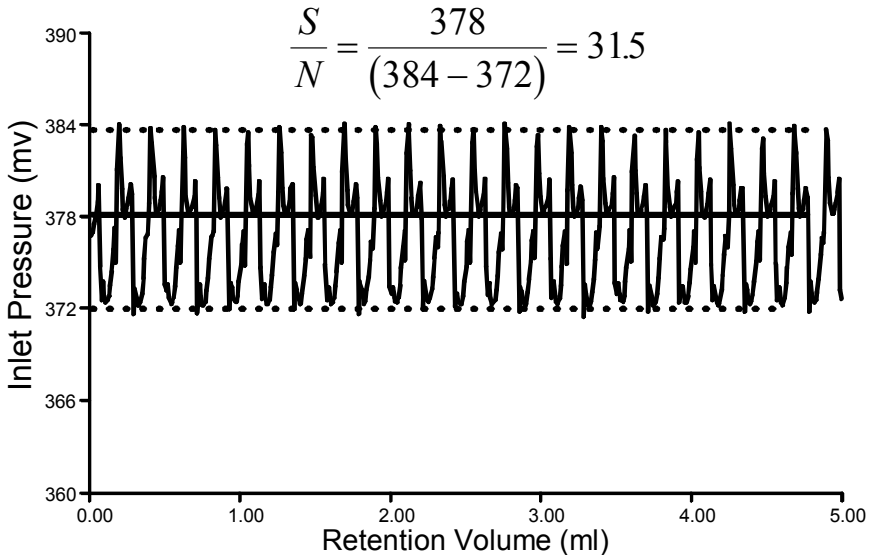


Figure 20 Measurement of Pump Pulsation from the IP Signal/Noise

In most (but not all) cases, the pump pulsation is regular, so one can measure the noise using the peak-to-peak method as shown.

Guideline: The IP signal/noise ratio will need to be greater than 200 in order to obtain optimum S/N on the DP signal.

C.3.d Checking Background Light Scattering

From time to time the background scattering should be checked following this procedure: Flush out the detector with THF. Turn the laser off. Zero the scattered signal. Turn the laser back on and read the background signal for both the RALLS and the LALLS. The background signal for the LALLS should be around 15% less than the RALLS signal. Run a fresh Polystyrene standard of 90,000 Da. Calibrate both detector signals. The LS calibration constant for the LALLS detector should be 50% less that the RALLS detector. If either of these values are significantly different, then you may have a contaminated optics system. Please contact the Viscotek Technical Service and Support department for further instructions.

C.3.e Standard Sample Analysis

Periodically it is a good idea to check the performance of the detector by running a polymer standard sample and comparing it to that obtained upon installation. The recommended standard for organic solvent use is one of the narrow MWD polystyrene standards. For aqueous solvent use, one of the narrow MWD polyethylene oxide or polyethylene glycol standards are recommended. Choose a standard somewhere in the molecular weight range of 20K - 100K. Before running the standard, check the baseline performance as described under sections C.3.a-c. If the detector baselines are within specifications, run the standard sample and calculate the intrinsic viscosity. Intrinsic viscosities of the standards should follow the Mark-Houwink relationships below.

$$[\eta] = KM^a$$

	a	K
PS	0.71	1.28×10^{-4}
PEO/PEG	0.65	5.88×10^{-4}

Remember that the molecular weights of standards are often in error by 5 - 10 % and in some cases can be off more than that. So run more than one standard before concluding that the viscometer is in error.

The sensitivity/response of RI detectors varies too much to specify exactly what response should be obtained for a standard sample. It is best to reference to the standard sample run upon installation.

The viscometer sensitivity factor can be checked by using the PS280K standard, which has an IV value of 0.87.

D. TROUBLESHOOTING

D.1 PROBLEMS IN THE VISCOMETER BASELINE

Noise in the viscometer (DP) baseline can be characterized as either *random*, *pulsation*, or *sporadic* type.

D.1.a Pulsation Noise in the DP Baseline

Pulsation noise in the DP baseline is a common problem. In most cases it arises from the pump pulsation, but it may possibly come from electronic sources. To eliminate the latter possibility, turn off the pump flow. If the pulsation noise on the DP goes away, electronic sources are clearly eliminated. Assuming it is pump-related, there are three possible causes to consider:

1. **Air bubbles in the DP transducer and/or the delay column.**
   Any air in the DP transducer cavities or in the delay column will amplify normal pump pulsation response to unacceptable levels. To test for air bubble in DP or column:

   a) Shut off flow and remove the pneumatic pulse dampener from its tee, inserting a plug instead.

   b) Restore flow to viscometer and wait until baseline is reestablished.

   c) Open the plug on the pulse dampener tee while observing the DP baseline. If the DP returns to near zero with less than 50 Pa overshoot, there is no air in the DP transducer or in the column. If more than 50 Pa of overshoot is observed, an air bubble may be present.

   To eliminate air bubbles, simply open the detector purge ports and allow at least one column volume of solvent to flow through.

2. **Excessive imbalance in the capillary bridge.**
   The ability of the bridge to cancel pump pulsations is directly proportional to the degree of balance. Normal balance is better than 1% as calculated in Section C.3.a and is unacceptable above 3%. The higher the imbalance, the lower the pump pulsation will need to be.

3. **Excessive pump pulsation, as measured by the IP signal/noise in Section C.3.c.**
   If this is excessive (IP S/N < 200), check the operation of the pneumatic pulse dampener. Drain the tube with the flow off, recap, and measure IP noise again. Finally check the operation of the pump itself.

D.1.b Random (White) Noise in the DP Baseline

Random noise in the DP transducer is usually electronic rather than flow related. To confirm that this is the case, simply turn off the pump flow. If the noise continues, it is electronic in nature. Normal random (white) noise as measured in section C.3.b should be 0.3 mV or less. If this level is exceeded with the flow off, check the DP transducer. To check this as a possible source of noise, remove the cable from the DP transducer. If the noise disappears, the transducer is at fault and will need to be replaced. If the noise does not disappear, electronic noise elsewhere in the output board circuit is indicated.

D.1.c Sporadic Noise in the DP Baseline

Sporadic noise in the DP baseline, such as a sudden decrease in the baseline, or a spike, etc., is usually caused by flow problems such as a faulty pump seal, check valve, or bad column frit, or occasionally by the passage of an air bubble through the system. This can be confirmed by observing the IP baseline overlaid together with the DP baseline. If the IP baseline shows a large change coincident with the change in the DP baseline, some fault in the flow stability is indicated.

D.2 PROBLEMS IN THE LIGHT SCATTERING BASELINE

D.2.a General Instrument Function

If there is any question as to the instrument's function, or if the instrument has been moved around, the response of the instrument should be compared to the results of a known standard, as described in Section C.3.e.

D.2.b Baseline Noise

If there is any question about the source of baseline noise, the detector should be taken off-line and pure clean solvent (methanol or THF works well) should be put in the cell of the light scattering unit with a syringe. Once the fresh solvent has thoroughly flushed out the system, the baseline noise should be less than 2 mV peak to peak on average. If the instrument shows

more noise than expected, excluding any occasional fluctuations, the cell may need cleaning. Please contact the Viscotek Technical Service and Support department for further instructions.

D.3 PROBLEMS IN THE REFRACTOMETER BASELINE

D.3.a General Instrument Function

Noise in the Refractometer baseline can be characterized as either random, pulsation, or drift type.

D.3.b Baseline Noise

Random white noise generated by the refractometer is generally the lowest level of the triple detector configuration. It's value is always less than 0.1 mv and often approaches that of the individual bits. Pulsation noise is sometimes found in the refractive index detector signal, however, when it occurs, generally the viscometer signal has already become unusable since it is much more sensitive to solvent compressibility changes than even the RI. The most frequently encountered form of baseline noise in the RI signal is that of drift. The RI is extremely sensitive to compositional changes in the solvent and to thermal variation. Often when mixed solvent systems are used it is necessary to continuously stir the mixture to minimize the drift caused by the continuous change in composition (possibly caused by the more rapid evaporation of one component over another). The Model 300TDA is temperature controlled to minimize thermal effects encountered in the RI.

> *Note: Thermal equilibration is normally slow and will may require several hours to accomplish. We recommend leaving the oven On for periods of time when the detectors are not being used continuously, but are needed frequently, to minimize start-up time for analysis.*

APPENDIX I. CHANGING THE INTERNAL VISCOMETER DELAY COLUMN

Note: Wear safety glasses, gloves, and use any other necessary protective equipment when changing the delay column, as you will be in direct contact with the solvent.

1. Disconnect and plug off the Sample inlet port.
2. Set the SEC Pump to the normal running flowrate.
3. Connect the new delay column to the SEC outlet line and let it purge for twenty minutes.
4. Disconnect the new delay column and reattach SEC outlet line to the instrument inlet port.
5. Turn off the main power switch.
6. Remove the detector drawer and connects it electrical umbilical cord.
7. Set the SEC Pump flow to 0.2 mL/min.
8. Remove the delay column from the clamp ring holders.
9. Place a towel underneath the delay column.
10. Disconnect the delay column by removing the 1/4” fittings with a wrench.
11. Replace the delay column with the new one, being careful not to allow any air into the system.
12. Cap off the old delay column so that no air will be introduced to it during storage.
13. Increase pump flow-rate and check for leaks.
14. Turn On Main Power and run Baseline monitor.
15. Verify baseline noise level (may be as much as twice normal due to air exposure).
16. Turn Off Main Power and disconnect umbilical.
17. Replace detector drawer.
18. Turn On Main Power.
19. Let temperature equilibrate before running baseline monitors to verify proper noise levels.
20. Run a control Standard.

APPENDIX II. CLEANING THE VISCOMETER DETECTOR IN SITU

The viscometer bridge can occasionally accumulate dirt or debris, affecting the performance of the detector. The symptom is bridge imbalance, as measured in section . A flush with diluted nitric acid will often clean the system and restore optimum performance.

1. Turn Off the Main Power.

2. Disconnect the tubing at the Sample Inlet Port.

3. Remove the detector drawer and connect the electrical umbilical.

4. Remove the viscometer delay column from the bridge circuit, plugging each end to prevent air from entering. Replace it with a union.

5. Likewise, bypass the SEC columns, LS and RI dectectors from the chromatographic circuit, replacing them also with a shunt of clean tubing. **It is very important that both the RI and LS detectors be bypassed when using dilute acid for cleaning. Failure to do this could damage the detectors.**

6. Exchange the mobile phase with a miscible solvent and eventually distilled H_2O. Purge the detector with at least 100 mL.

7. Exchange the mobile phase with 50% HNO_3 (6M) and purge the detector with at least 200 mL.

8. Take the mobile phase back through the above stages in identical reverse order.

9. Replace the separation columns and other detectors. Test for leaks.

10. Replace the detector drawer and temperature equilibrate. Run a control.

11. Recalibrate the system.

APPENDIX III. SOLVENT CHANGEOVER PROCEDURE

It may become necessary to change from one solvent to another depending on the application. In most cases, going from either organic to organic, or aqueous to aqueous solvents does not pose any problems. However, going from organic to aqueous or visa versa can cause excessive noise in the baselines, drifting, and sometimes plugging of the TDA if one is not careful in choosing the right solvents for changeover. Therefore, it is important to follow this procedure for changing over to different solvents to help prevent possible downtime due to problems.

1. First, remove all analytical columns and replace them with a zero dead volume union.
2. Replace the membrane in the RALLS filter with a new one.
3. Change the solvent reservoir from the organic solvent to 30% methanol/70% water. Note: it may be necessary to go to a different organic solvent than the original in order to become miscible with the water/methanol solvent.
4. Flow the solvent through the system at 1.0 ml/min. Pump at least 200ml through the system.
5. Purge both the RI and the DP with at least 20 ml of solution.
6. Stop the flow and replace the solvent reservoir with whatever new solvent is to be used.
7. Flow the solvent through the system at 1.0 ml/min. Pump at least 200 ml through the system.
8. Purge both the RI and DP with at least 20 ml of solution.
9. Examine the signals from the TDA through the front panel. They should be stable. If not, repeat steps 7 and 8 until stable.
10. Connect the analytical column but run the column exit to waste and not into the TDA.
11. Flow solvent through the column for at least FIVE column volumes.
12. Connect the column waste to the TDA.
13. Flow solvent at the appropriate flow rate and let the detectors equilibrate.

If you have any questions about the changeover procedure, please contact the Technical Service Department at Viscotek Corporation.

APPENDIX IV. WARRANTY INFORMATION

The Viscotek Corporation warranty provides that all products that it sells are of good quality and workmanship and are produced under good quality control conditions. These products are suitable for their intended purpose during the warranty period when used in accordance with the instructions set forth within this manual.

Disclaimer

Viscotek will have sole discretion to make reasonable efforts to correct any defects that are found to be from the manufacturing process including, if necessary, replace the product.

Warranty Service

Warranty service is performed at no charge in one or all of three conditions:

- A Technical Service and Support representative will be dispatched to the customer site.
- Replacement parts will be sent at no charge to the site for customer installation
- The product can be returned the Viscotek Corporation for depot repair.

Warranty service will not be provided on weekends or legal holidays. In addition, warranty service will be provided only if the customer notifies the Technical Service and Support department within the stated warranty time frame.

Warranty service will not be provided when:

- Any product or parts have been serviced by someone outside of the Viscotek Corporation unless otherwise directed.
- Parts that are identified as not being used by the Viscotek Corporation in the manufacture of the instrument.
- Physical misuse including dropping the unit or excessive torquing of fittings or physical breakage of any electrical connectors.
- Products that fail due to poor operating procedures.
- Failure of parts due to chemical decomposition, poor facilities, or environment.
- Any expendable items such as filters, gaskets, frits, and fittings.

Warranty Period

The warranty begins when the product is installed by a qualified Viscotek technician or in the case of customer installation 21 days after shipment from the Viscotek facility.

The Model 302 TDA will be warranted free of defects for a period of one year from time of installation.

At no time will the warranty period exceed 15 months from date of shipment. Any part that is replaced within the warranty period is warranted for the rest of the original warranty time period.

Returns

Any Viscotek product that needs to be returned to depot must have authorization from the Viscotek Technical Service and Support department or the Viscotek Sales department. At that time a RMA number will be assigned to the repair and the product can b e shipped back to depot for service. Any non-warranted product will require a purchase order before service can commence. Any product returned to Viscotek for evaluation is subject to a bench set up fee of $500. This is not refundable. Viscotek will not be liable for damage due to improper packing or shipping.

TO OUR CUSTOMERS:

Viscotek strongly believes in the quality of its instrumentation and its customer service. As a part of this ongoing commitment, Viscotek offers telephone, telefax and e-mail support for users of its instrumentation. If there are any questions about the material contained in this manual, or if there are any questions in general about the instrumentation or the ability of the instrumentation to perform sample analyses, please do not hesitate to call us at (800) 375-5966 or fax us at (281) 931-4336. Furthermore, Viscotek gladly welcomes any comments from users, either positive or negative, which can be forwarded directly to our Technical Service and Support department. These comments are always valuable to us and enable us to continue to deliver higher customer satisfaction standards.