ViscoStar
User's Guide

(M161048 Rev. A)

Wyatt Technology CORPORATION

Contents

1 Quick Start

This chapter provides an introduction to the instrument.

It also provides summarized setup procedures for first-time or experienced users who want to start the ViscoStar installation process before reading the entire manual.

Detailed descriptions and important use instructions concerning the ViscoStar are provided in the chapters that follow.

CONTENTS **PAGE**

1.1 About the ViscoStar

The ViscoStar measures specific viscosity. When coupled with a concentration detector such as the Optilab rEX, it can be used to derive the intrinsic viscosity. The intrinsic viscosity, in turn, can be used to derive the hydrodynamic radius and shape information about a molecule in solution.

The ViscoStar provides the best signal-to-noise ratio available in viscometers, and proprietary analysis corrects for peak broadening and pump pulses. To match chromatography conditions, the air-cooled Peltier allows you to control temperature from 4-60°C. The ViscoStar uses a high-speed 16 bit A/D system that is averaged internally to achieve better than 18 bits of effective resolution, giving it unmatched dynamic range.

The display screen on the front of the device allows you to control the instrument, view results, and monitor potential alarm conditions. Ethernet and USB access allow data to be fed across the network to software such as Wyatt's ASTRA software.

1.2 Setting Up the ViscoStar

This section describes basic setup procedures for those who want to begin using the ViscoStar as soon as possible.

More details about setting up the ViscoStar are provided in Chapter 3, “Installation and Setup”. (You may notice that the setup sequence in this chapter differs a bit from that in Chapter 3. The sequence in this chapter allows you to begin stabilizing the temperature earlier in the sequence.)

See Chapter 2, “Instrument Description” for descriptions of the front and rear panel controls and connections.

For instructions on navigating the LCD display tabs, see “Navigating the ViscoStar Tabs” on page 5-2.

Note:	The ViscoStar can be safely stacked above and below other instruments.

Follow these steps when you initially set up the ViscoStar:

1. **Plumb the instrument into the chromatography system.** See Section 3.1 “Connecting Plumbing” on page 3-2. Use standard HPLC tubing with 10-32 threads and 1/16 in. OD, 0.010 in. ID. Plumb the ViscoStar in one of following instrument sequences:
   - Chromatography → MALS → **ViscoStar** → RI detector → Waste
   - Chromatography → UV detector → MALS → **ViscoStar** → Waste
2. **Set the delay reservoir volume.** See Section 3.1.3 “Changing the Delay Reservoir Size” on page 3-3. This step is optional. The default delay volume of 27 ml is often appropriate.
3. **Attach back panel connectors.** See Section 3.2 “Connecting Power and Signals” on page 3-7. The power outlet is located on the ViscoStar rear panel (see Figure 2-4). The power supply is universal, for immediate use with 100–120 V or 220–240 V power at 50-60 Hz.
4. **Install software.** Install ASTRA on the PC to be used as the host computer. See the software manual for installation instructions.
5. **Connect communication cables.** See Section 3.2.3 “Communication Connectors” on page 3-11. Connect the ViscoStar directly to a computer or local area network (LAN) using the USB or Ethernet cable provided.
6. **Set the operating temperature.** See Section 4.1 “Powering On and Setting the Temperature” on page 4-2. The ViscoStar default temperature is 25°C, with a range of 4°C to 60°C. (If you are operating below 20°C, it is important that you see Section 3.3 “Dry Nitrogen Connection” on page 3-12 before cooling the instrument.)

7. **Purge the system with new solvent.** See Section 4.2 "Purging the ViscoStar" on page 4-2. Use at least 30 mL of co-miscible solvents step-wise as necessary to prepare the cell for data collection. Navigate to the IP Purge and DP Purge buttons using the front panel arrow keys (see Figure 2-3).

   The ViscoStar is shipped with Ethanol in the flow cell. A typical series of solvents (from polar to nonpolar) is shown below. Salt solutions should be considered separate steps from pure solvents.

   Water
   Methanol, Ethanol
   Isopropanol, Acetone
   Tetrahydrofuran
   Ethylacetate, Chloroform, Methylene chloride
   Toluene, Carbon disulfide
   Hexane, Petroleum ether

8. **Ramp the flow down to zero.**

Caution: Always ramp the flow rate slowly. If the transducers are subjected to a sudden change in pressure, they may be damaged. You should use a ramp rate of 1 ml/min/min or slower. If your pump does not support ramping, manually change the flow rate in increments of 0.1 ml/min, wait for 10 seconds, and change the flow rate again. A slow ramp rate also protects chromatography columns and helps prevent them from shedding particles.

9. **Zero the transducers.** See Section 4.3.1 "Zeroing the Transducers" on page 4-7
10. **Restart the flow.** Again, ramp the flow rate slowly. Let the graph stabilize after you reach your operating rate.
11. **Flow zero the DP transducer.** See Section 4.3.2 "Zeroing the Bridge" on page 4-8
12. **Inject sample into chromatography column and collect data.** See the *ASTRA V User's Guide* for more information on collecting data from the ViscoStar.

1.3 Using This Manual

The layout of this manual is as follows:

- **Chapter 2, "Instrument Description":** Describes how the ViscoStar works and the instrument's control interfaces and connections.
- **Chapter 3, "Quick Start":** Contains a summary of instructions for setting up the ViscoStar quickly and with minimal instruction.
- **Chapter 3, "Installation and Setup":** Provides detailed connection information for installation and setup.
- **Chapter 4, "Running the ViscoStar":** Describes using the ViscoStar as a viscosity detector for HPLC.
- **Chapter 5, "Using the LCD Display":** Provides detailed information on how to use the tabs on the LCD display to control, configure, and monitor the ViscoStar.
- **Chapter 6, "Service and Maintenance":** Provides maintenance and service instructions.
- **Appendix A, "Acronyms and Abbreviations List":** Contains a list of acronyms and abbreviations used in this manual.
- **Appendix B, "Troubleshooting":** Provides troubleshooting tips.
- **Appendix C, "Operating Principles and Theory":** Provides a basic and theoretical description of how the ViscoStar operates.
- **Appendix D, "ViscoStar Specifications":** Provides the specifications of the ViscoStar.
- **Index:** Provides lookup assistance.

1.4 Contacting Wyatt Technology

We solicit and encourage questions and comments about this manual and the ViscoStar instrument. Please contact:

Technical Support Department
Wyatt Technology Corporation
6300 Hollister Avenue
Santa Barbara, CA 93117

Telephone:	(805) 681-9009
FAX:	(805) 681-0123
E-mail:	support@wyatt.com

2 Instrument Description

This chapter provides an overview of the ViscoStar features.

CONTENTS **PAGE**

2.1 Measuring Viscosity

The ViscoStar measures specific viscosity.

- *Specific viscosity* (η_{sp}) is the increase in viscosity due to the sample in the solute.
- *Intrinsic viscosity* is the limiting slope as defined in Eq. 1. When the concentration is low, as it usually is for online chromatography, one can ignore the limit and use the approximation shown in Eq. 2. See Appendix C, "Operating Principles and Theory" for more details.

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} \qquad \textit{(Equation 1)}$$

$$[\eta] \cong \eta_{\mathrm{sp}}/c \qquad \textit{(Equation 2)}$$

The ViscoStar uses specific viscosity, along with concentration data from an RI concentration detector (such as the Optilab rEX) or a UV concentration detector, to calculate intrinsic viscosity.

Intrinsic viscosity, when combined with data from light scattering measurements, can be used to derive the hydrodynamic radius (r_h) and molecular shape information.

2.1.1 Pressure Transducers and the Viscometer Bridge

Small viscosity differences are detected by transducers that measure differential pressures. The ViscoStar uses magnetorestrictive pressure transducers to measure inlet pressure (IP) and differential pressure (DP or ΔP). These transducers contain two small cavities separated by a thin magnetic stainless steel membrane. When the pressure on one side of the membrane is higher than the other, the membrane flexes by a small amount. This change in position is measured by magnetic pickup coils in the transducer housing. The full scale travel for each membrane is less than 50 µm.

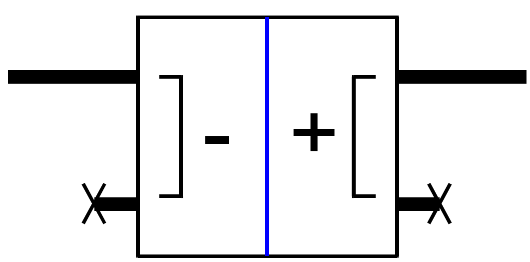


Figure 2-1: Transducer Flow Diagram

Since pump pulses generate pressure changes, viscometers measure differential pressure use a four-capillary bridge with a delay volume in one arm of the bridge. A simplified flow diagram for the bridge is shown in Figure 2-2. A more detailed flow diagram is provided in Figure 4-1.

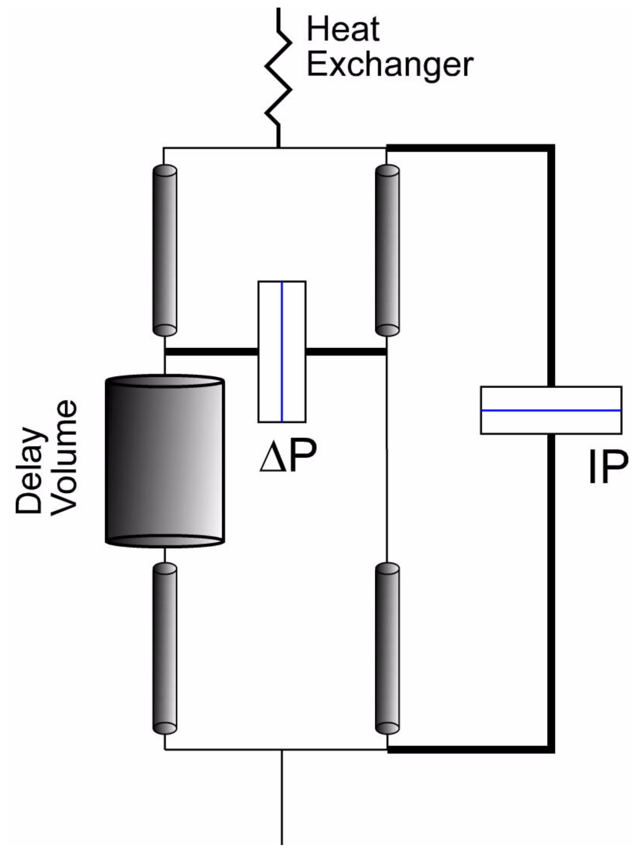


Figure 2-2: Four-Capillary Bridge Flow Diagram

The fluid flows into the bridge through the heat exchanger, which heats or cools the fluid to the set temperature. In the bridge, the fluid splits into the following two flow paths:

- **Right side of bridge:** This path goes through a capillary, is sensed by the positive side of the differential pressure transducer (ΔP), and then flows through another capillary. There is no delay volume.
- **Left side of bridge :** This path goes through a capillary, the negative side of the differential pressure transducer (ΔP), and then a delay volume.

The inlet pressure (IP) transducer measures the pressure of the fluid as it is pumped into the bridge. However, fluid does not flow through the IP transducer.

The paths rejoin after the bridge. The fluid then exits the ViscoStar and travels to the next analytical instrument in the flow path, if there is one.

The delay volume serves to delay the sample so that specific viscosity is calculated using the following equation:

$$\eta_{sp} = 4\Delta P / \left(IP - 2\Delta P \right) \qquad \textit{(Equation 3)}$$

For a more detailed theoretical description, see Appendix C, “Operating Principles and Theory”.

2.1.2 Transducer Protection System (TPS)

The two transducers have the same design, but their operational ranges are different because the membranes have different thicknesses. The full scale travel for each membrane is less than 50 μm.

The membrane in the DP transducer is approximately 60 μm thick. If the pressure difference across the membrane is small, then the membrane flexes elastically and returns to its undistorted state when the pressure is released. If the pressure is too high, the membrane can permanently deform, causing nonlinear behavior and a reduced operational range. Therefore it is very important that the transducers are never overpressured.

The IP transducer, has a thicker membrane and is therefore less susceptible to damage.

The rule of thumb is that the transducer will be damaged if it receives twice its rated pressure difference. The DP transducer has a pressure limit of 0.72 psi. It is common to exceed this pressure difference when changing solvents, if bubbles are accidentally introduced into the fluid stream, or if there is a plug somewhere in the tubing.

To prevent damage to the transducers, the ViscoStar is equipped with a Transducer Protection System (TPS) to monitor the transducers and prevent an accidental overpressure by automatically putting the system into a purge state in the case of overpressure.

When the TPS activates, the ViscoStar briefly displays a “Transducer out of Range” alarm on the Alarm tab, and then turns on both the IP Purge and DP Purge (on the Main tab). The ViscoStar also sends an alarm to the ASTRA software and to the Diagnostic Manager if they are currently collecting data.

Switching the IP Purge and DP Purge to ON is considered a “safe” state that should correct the “Transducer out of Range” condition. If you leave the system in run mode, and later find that the IP Purge and DP Purge are ON, it is a sign that the TPS was activated at some point.

2.2 Front Panel

The front panel, see Figure 2-3, provides fluid connections for the ViscoStar, along with the display panel and display controls for operating the instrument and monitoring data.

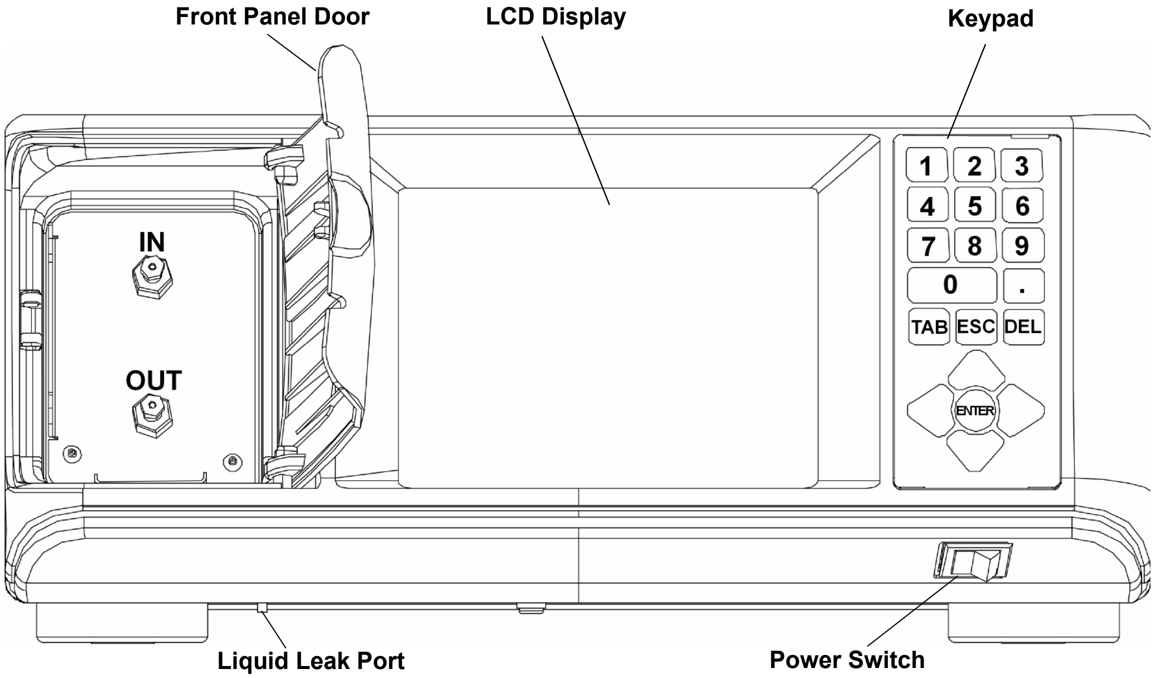


Figure 2-3: Front Panel

Front Panel Door: The front panel door opens to provide access to the IN and OUT fluid connections for the ViscoStar.

LCD Display: The LCD display provides a full-color, high-resolution user interface to the ViscoStar. It allows you to monitor, control and configure the instrument. Chapter 5, "Using the LCD Display" describes all of the tabs available on the LCD display and their functions.

Keypad: The keypad allows you to control the LCD display. "Navigating the ViscoStar Tabs" on page 5-2 describes how to use the keypad to navigate through the LCD display tabs.

IN/OUT Fluid Connectors: Fluid comes into the ViscoStar through the IN port, and exits through the OUT port. If the ViscoStar is stacked on top of the Optilab rEX, the drain system is designed to cascade so that only a single drain tube needs to be connected at the bottom of the instrument stack.

Liquid Leak Port: The liquid leak port provides an exit for any liquid that leaks from internal or external fittings. The Teflon tubing provided may be fit over this tubing and directed to a fluid waste bottle. When a liquid leak occurs, an internal liquid sensor activates an alarm shown on the LCD display (see “Alarms Tab” on page 5-5), and activates an Alarm Out signal that may be used to shut off a pump.

Power Switch: The power switch turns instrument power On and Off.

2.3 Rear Panel

The rear panel, see Figure 2-4, provides access for power, signal connections, the cooling fan, air filter, the exhaust fan, and a nitrogen purge port.

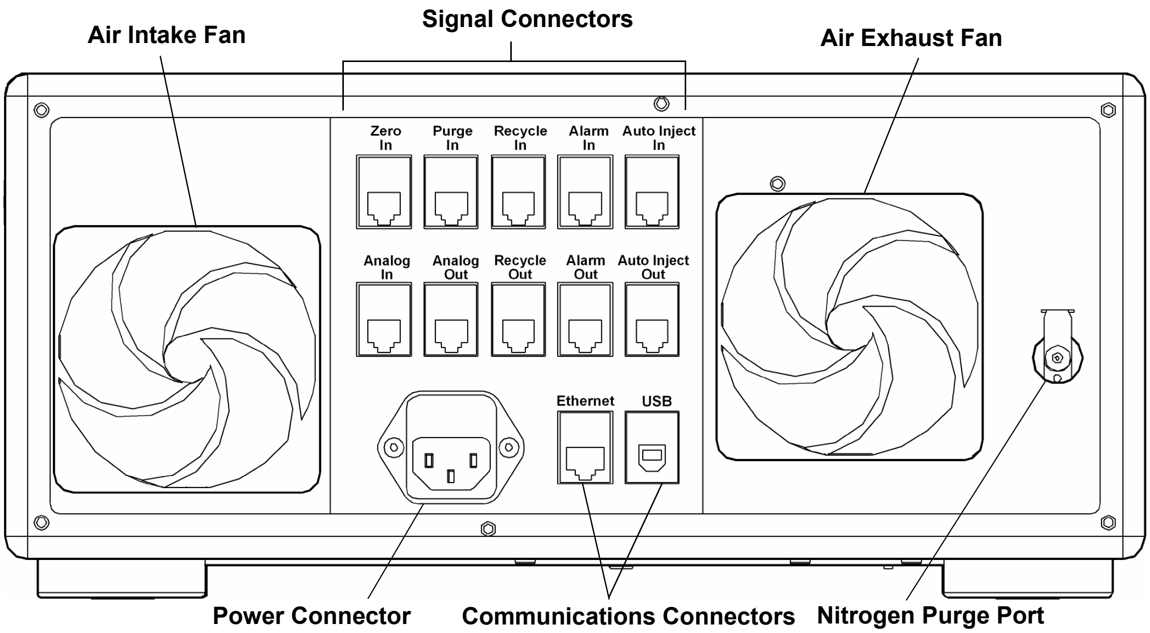


Figure 2-4: Rear Panel

Air Intake and Exhaust Fans: These fans help to keep the instrument electronics at a constant temperature.

Signal Connectors: There are 10 RJ-12 connectors that provide connections to Transistor-Transistor Logic (TTL) inputs and outputs, Analog input and output, Auto Inject input and output, and a solenoid drive output. For a description of the signal connectors, see “Connecting Power and Signals” on page 3-7.

Power Connector: Provides power to the instrument. See “Connecting Power and Signals” on page 3-7 for a complete description of the ViscoStar’s power requirements.

Communications Connectors: The Ethernet and USB connectors allow you to connect to a computer or an Ethernet network.

Nitrogen Purge Port: Provides the connection to purge the instrument with dry nitrogen in order to prevent condensation on the thermal bench when operating below ambient temperature. For detailed information regarding the nitrogen purge port, see “Dry Nitrogen Connection” on page 3-12.

3 Installation and Setup

This chapter describes the fluid, power, and signal connections used in setting up the ViscoStar.

CONTENTS **PAGE**

3.1 Connecting Plumbing

"Setting Up the ViscoStar" on page 3-2 contains an overview of the process for setting up the ViscoStar. The first step is to plumb the ViscoStar to other instruments in the system.

CAUTION: Be sure to wear gloves and other protection appropriate for the solvent you are using.

Before connecting tubing, fill the tubing with ethanol, which is the solvent the instrument is shipped with. See Section 4.2.1 "Changing the Solvent" on page 4-2 for important information about switching solvents.

Filling the tubing minimizes the air bubbles introduced into the system. Any bubbles will cause the system to have a large sensitivity to pump pulses. See Section 4.2.2 "Removing Air Bubbles" on page 4-4 for more information.

3.1.1 Plumbing Sequence

Plumb the ViscoStar in one of following instrument sequences:

- Chromatography → MALS → **ViscoStar** → RI detector → Waste
- Chromatography → UV detector → MALS → **ViscoStar** → Waste

"MALS" is a multi-angle light scattering device. For example, a DAWN EOS or miniDAWN. QELS may optionally be included as part of the MALS instrument.

In the first configuration, the RI detector is plumbed after the ViscoStar. As the sample exits the ViscoStar, it is diluted by approximately a factor of 2. (See **Appendix C, "Operating Principles and Theory"**). Therefore the RI detector does not measure the same concentrations that flowed through the MALS and ViscoStar instruments. To correct for the change in concentration, you will determine the exact dilution ratio in Section 4.3.3 "Measuring the System Dilution Factor" on page 4-9.

In the second configuration, the ViscoStar is the last instrument in the chromatography chain. Since the concentration is measured before the ViscoStar, you do not need to correct for the dilution.

3.1.2 Plumbing Fittings

The ViscoStar accepts standard HPLC fittings: 1/16 in. outer diameter tubing with 10-32 threads. Use the following tubing sizes with the ViscoStar:

- **Inlet:** 0.010 in. (0.254 mm) ID
- **Outlet to downstream RI detector:** 0.010 in. (0.254 mm) ID
- **Outlet to waste:** 0.010 in. (0.254 mm) ID or larger to waste bottle or solvent recycle valve.

The ViscoStar is plumbed with 0.010" exit tubing to allow other analytical instruments to be plumbed downstream. The use of small bore exit tubing minimizes the interdetector band broadening.

3.1.3 Changing the Delay Reservoir Size

The ViscoStar has three delay reservoir volumes: 15 ml, 8 ml, and 4 ml. These volumes are, respectively, 180 mm, 90 mm, and 55 mm in length. The ViscoStar is shipped with these volumes plumbed in series so that the total delay volume is 27 ml (15 + 8 + 4). This ensures the maximum separation between the positive peaks from the sample and the negative peak that occurs when the delay volume empties.

The delay reservoir volume can be changed by moving plumbing fittings. By bypassing the 15 ml delay reservoir, you set the delay volume to 12 ml (8 + 4). By bypassing the 15 and 8 ml reservoirs, you set the delay volume to 4 ml.

The best delay volume for your use depends upon how long it takes the peaks to elute from your chromatography column(s). Samples with small hydrodynamic volumes—such as proteins—undergo substantial diffusion while in the delay reservoir, and the negative peak that occurs when it empties is substantially broadened and takes longer to empty than an equivalently narrow peak with a larger hydrodynamic volume.

If you use the largest delay volume, you should wait until approximately 60 ml has passed through the ViscoStar before injecting a new sample into the column. This ensures that all of the sample has been purged from the delay reservoir. (Since each arm of the viscometer receives approximately 50% of the flow, the large delay volume fills and empties after 30 ml passes though the instrument.)

If you use a column set with a small exclusion volume, the peaks typically elute much sooner. For example, suppose the peaks elute within 10 ml of the injection. Although the large delay volume would still work, you would need to wait longer than necessary between injections. To speed up the system throughput, you can use a smaller delay volume configuration.

The following three figures illustrate the effect of changing the delay volume. These images show the results for a mixture of 200 kD and 30 kD polystyrene in toluene, using the three different delay volume configurations.

Figure 3-1 shows a long wait for the negative peak after injection when all three delay volumes are used.

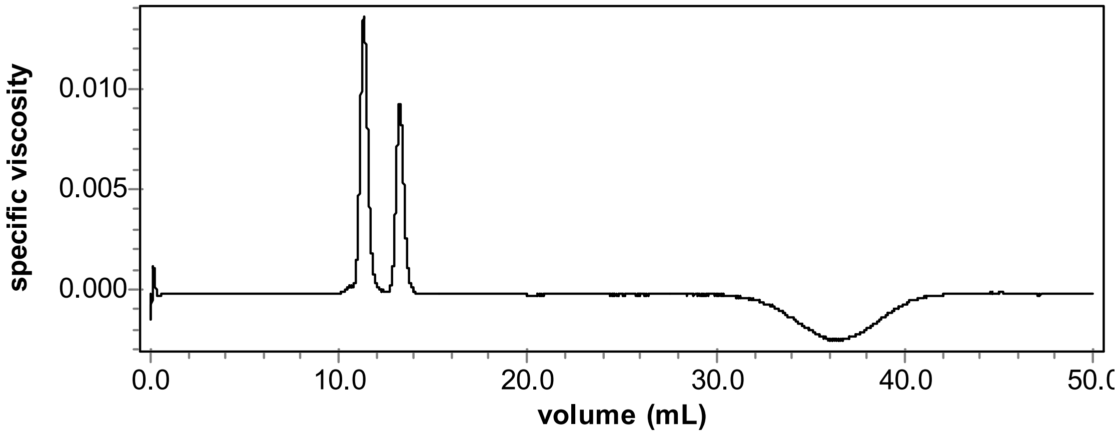


Figure 3-1: Long Wait After Injection with Three Delay Volumes

Figure 3-2 shows a moderate wait for the negative peak when two delay volumes are used.

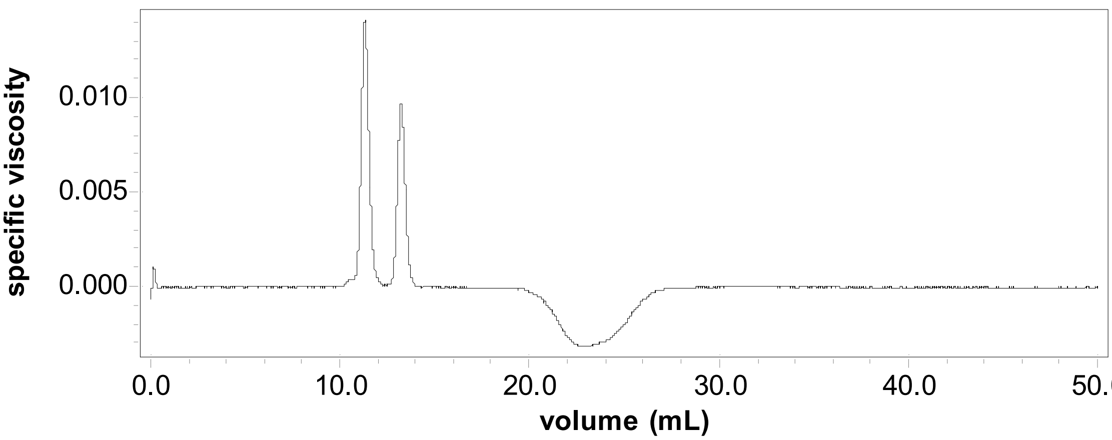


Figure 3-2: Shorter Wait Using Two Delay Volumes

Figure 3-1 shows the shortest wait for the negative peak when using a single delay volume.

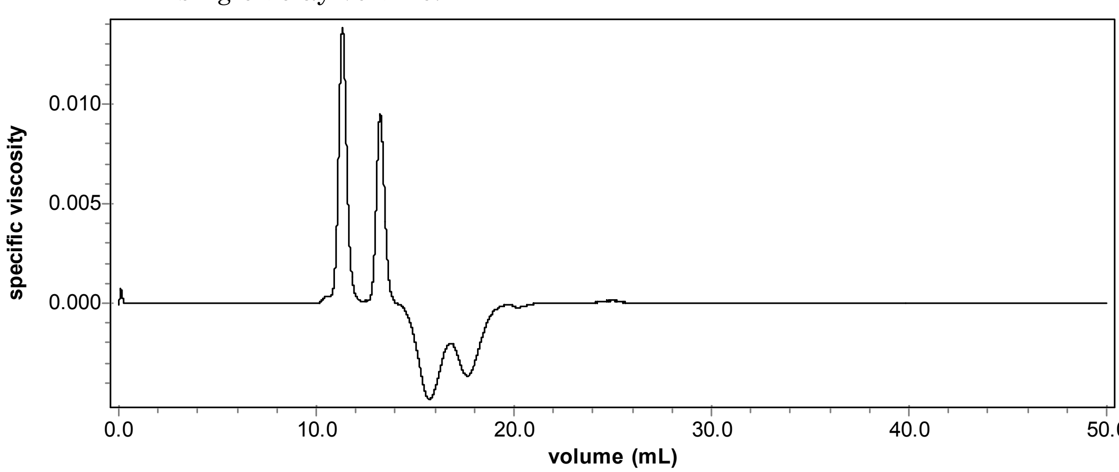


Figure 3-3: Shortest Wait Using One Delay Volume

To change the delay volume, follow these steps:

1. Disconnect the power cord from the instrument.
2. Using a 2mm ball driver, remove the four screws that secure the cover to the instrument.
3. Lift the cover at the front and pull forward to remove.

CAUTION: Be sure to wear gloves and other protection appropriate for the solvent you are using.

4. After removing the outer cover, use a 2.5 mm hex driver to remove the top of the thermal box shown in Figure 3-4. Then, remove the foam insulation and the inner thermal box cover.

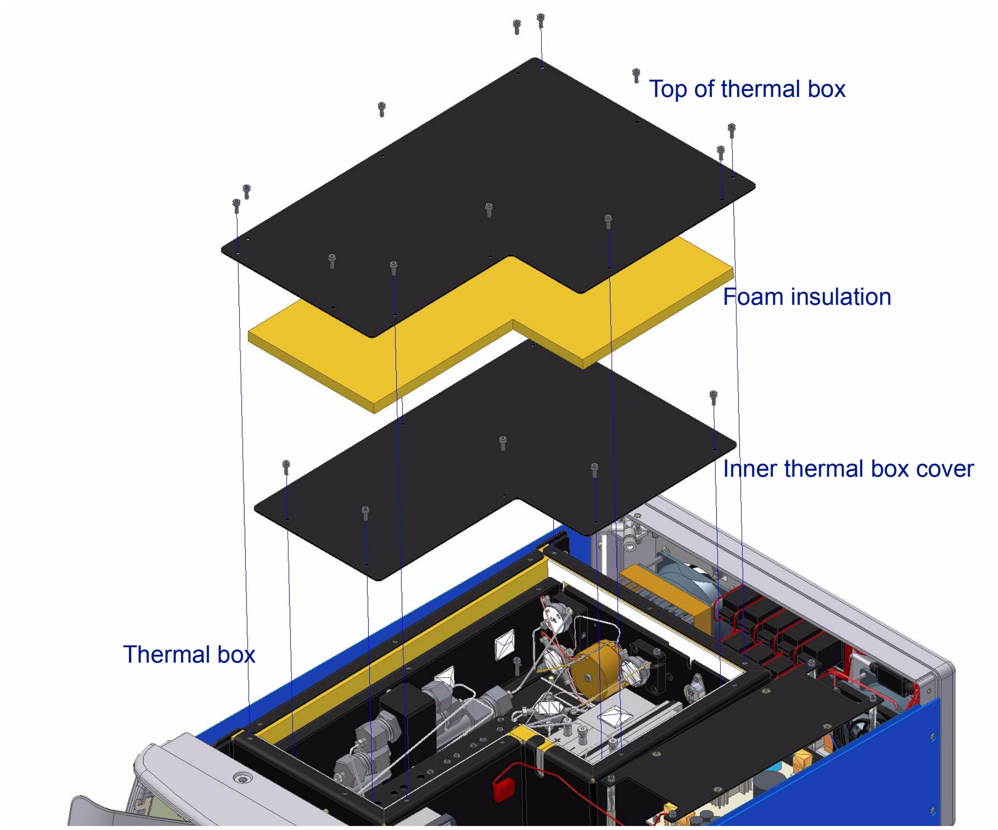


Figure 3-4: Removing the Thermal Box Covers

5. To connect to a different location, replace the tubing shown connected to Location A with the tubing provided in the ViscoStar kit. Connect the tubing to a location marked A, B, or C in Figure 3-5. When you disconnect the large column, plug the ends of the tubings that are disconnected with the female plugs also provided in the ViscoStar kit.
   - Location A sets the delay volume to 27 ml.
   - Location B sets the delay volume to 12 ml.
   - Location C sets the delay volume to 4 ml

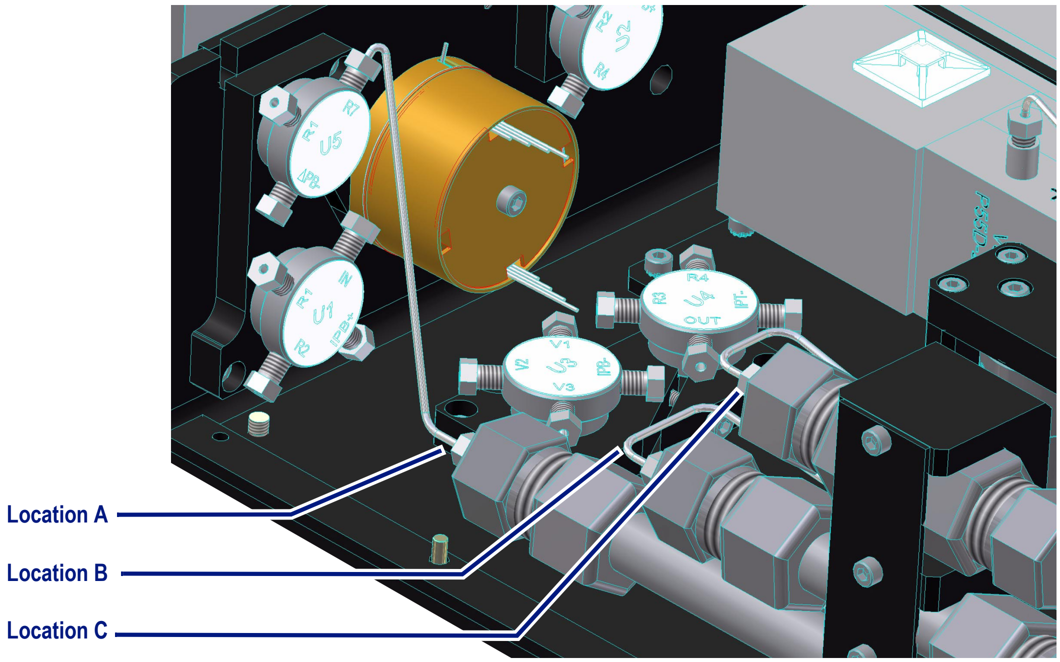


Figure 3-5: Plumbing Inside the ViscoStar

6. If any solvent leaks, use absorbent paper or cotton to soak up as much as possible from the liquid leak well.
7. Install the cover and secure it with the four screws you removed.

3.2 Connecting Power and Signals

The second step in the installation process is to make back panel connections.

All power and signal connections to the ViscoStar are made using the interfaces on the rear panel of the instrument. Refer to Figure 3-6 to view the connectors on the rear panel of the ViscoStar.

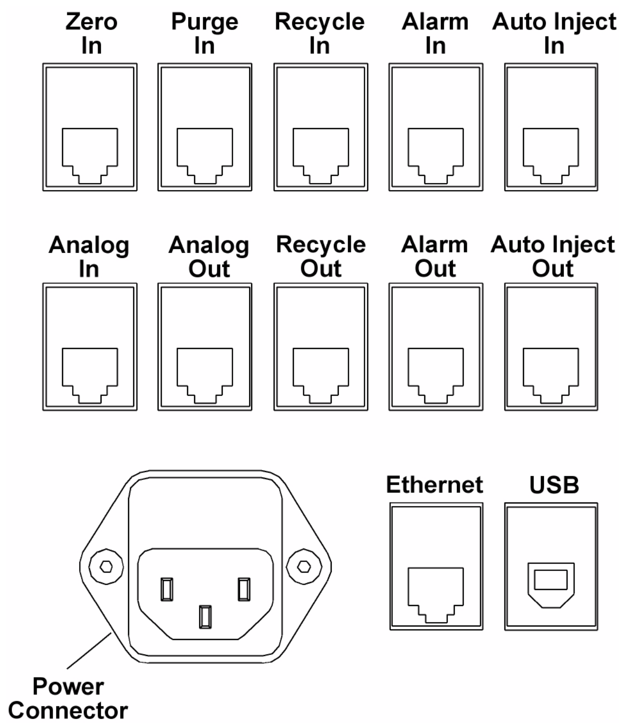


Figure 3-6: Rear Panel Power and Signal Connections

3.2.1 Connecting Power

The power input accepts 90–250 VAC at 50–60 Hz. The power input module contains two fuses, each rated at 2 Amps, one for the line voltage in, and the other for the line voltage return.

3.2.2 Connecting Signals

The instrument rear panel contains 10 RJ-12 connectors for TTL inputs and outputs, analog input and output, auto inject input and output, and a solenoid drive output.

Cables not supplied by Wyatt Technology may use a different color code scheme or have a different correspondence between color and pin number. If you are using non-Wyatt Technology supplied cables, please refer to Figure 3-7 and Table 3-1 to determine the correct pin number and wire usage. We recommend using only cables supplied by Wyatt Technology; wiring the connection incorrectly could damage your instrument.

Pinouts for the RJ-12 connectors used in Wyatt Technology-supplied cables are shown in Figure 3-7.

Pin	Wire Color
1	White
2	Black
3	Red
4	Green
5	Yellow
6	Blue

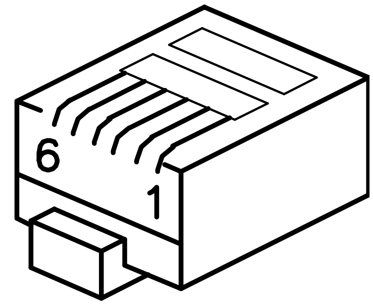


Figure 3-7: RJ-12 Connector and Pinouts

Several general-purpose cables are supplied for TTL inputs and outputs, analog inputs and outputs, auto inject input and output, and solenoid drive output.

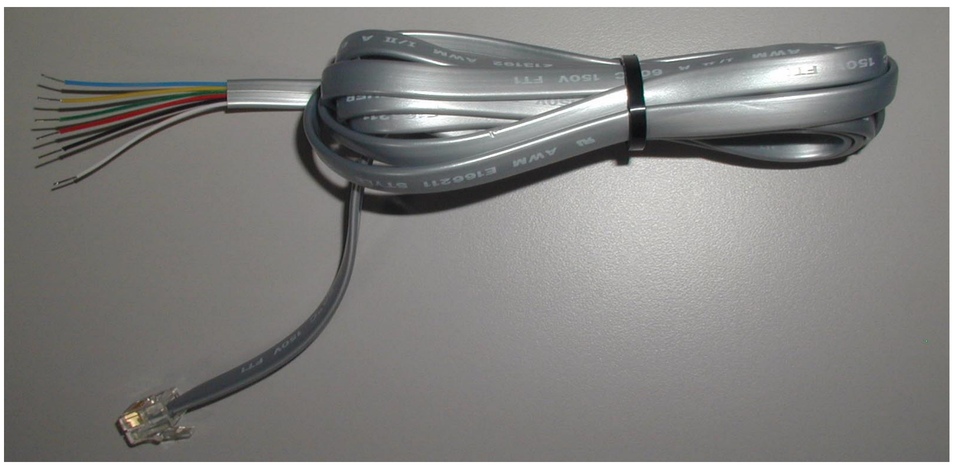


Figure 3-8: General-Purpose Cable

When you connect your ViscoStar to other instruments, see Table 3-1 for RJ-12 connector signal information.

Table 3-1: RJ-12 Signal Function

ViscoStar Connector	**RJ-12 Connector Wire Signal**					
	White	**Black**	**Red**	**Green**	**Yellow**	**Blue**
Zero In			Signal	Signal Ground		
Purge In			Signal	Signal Ground		
Recycle In			Signal	Signal Ground		
Alarm In			Signal	Signal Ground		

ViscoStar Connector	RJ-12 Connector Wire Signal					
	White	**Black**	**Red**	**Green**	**Yellow**	**Blue**
Auto Inject In			Signal	Signal Ground		
Analog In			Positive	Negative		Ground
Analog Out	Positive	Negative				Ground
Recycle Out	Signal	Signal Ground				
Alarm Out	Signal	Signal Ground				
Auto Inject Out	Contact	Contact				

The Zero In, Purge In, Recycle In, Alarm In, and Alarm Out signals all follow the TTL voltage convention. In the TTL convention, 5 V on the signal line is interpreted as a High signal, and 0 V on the signal line is interpreted as a Low signal.

Zero In: TTL input on red (signal) and green (signal ground). When the signal on this line transitions from 0 V to 5 V, the last measured value is considered to be zero value. That value is subtracted from subsequent data before it is output for the DP transducer. This is equivalent to activating the Flow Zero DP button on the front panel display.

Purge In: TTL input on red (signal) and green (signal ground). When the signal on this line transitions from 0 V to 5 V, the internal purge valves are actuated. While the signal on this line is held at 5 V, the purge valves remain actuated. When the signal on this line transitions from 5 V to 0 V, the purge valves are de-actuated. This is equivalent to activating both the IP Purge and DP Purge buttons on the front panel display. (See “Main Tab” on page 5-3.)

Recycle In: TTL input on red (signal) and green (signal ground). When the signal on this line transitions from 0 V to 5 V, the instrument actuates an external solenoid valve by supplying power to the Recycle Out connector. When the signal transitions from 5V to 0V, the Recycle valve is de-actuated.

Alarm In: TTL input on red (signal) and green (signal ground). On the instrument LCD display Alarms tab (see “Alarms Tab” on page 5-5), you may select the active state of this input. If you select “Active High”, the instrument considers a transition on this line from 0 V to 5 V to be an Alarm In event. If you select “Active Low”, the instrument considers an Alarm In event to occur when the signal on this line transitions from 5 V to 0 V. When an Alarm In event occurs, the Alarm signal flashes on the LCD display, and an Alarm Out signal is transmitted (see Alarm Out).

Auto Inject In: TTL input *or* contact closure input between wires red (TTL signal) and green (TTL signal ground). The red signal line is internally held at 5 V. When the red signal line is brought to 0 V, the instrument receives an Auto Inject signal. The signal line may be brought to 0 V by simply connecting the red and green wires together (hence the term contact closure). Most auto injectors supply a contact closure signal, which simply brings the two wires into contact with each other.

In general, the same auto injector contact closure signal cannot be sent to multiple instruments, since different instruments may be trying to maintain different internal voltages to sense the contact closure, and the instruments could be in conflict with each other.

The ViscoStar provides an Auto Inject Out signal that simply retransmits the Auto Inject In signal as a contact closure output (see Auto Inject Out), allowing you to bring an auto injector contact closure signal to the ViscoStar, and then send the signal to another device. The receipt of this signal by the ViscoStar causes a mark to appear in the data on the LCD display Main tab, changes the state reported on the LCD display System tab Auto Inject input field, and causes a signal to be sent with the digital data stream indicating the time of receipt of the signal.

Analog In: Analog input Positive on red, Negative on green, and Ground on blue. The ViscoStar digitizes an analog input in the range of ±10 V with a resolution better than 38 μV (18 bits).

Analog Out 1 and 2: Analog Out 1 is white (+) and black (-). Analog Out 2 is red (+) and green (-). Ground is blue. The ViscoStar supplies an analog output in the range of ±10 V with a resolution better than 38 μV (18 bits). There are two analog outputs. Analog output 1 sends the IP transducer signal on a scale of -10 to +10 V; Analog output 2 sends the DP transducer signal on a scale of -10 to +10 V. The output of each transducer is scaled to fit the full range of pressures possible with that transducer within the +/-10 V range. The conversion between voltage on the AUX outputs and pressures is shown in the Calibration Constants area on the System tab. For each transducer, the formula is as follows:

$$P = \text{"Calibration Constant"}\ V + \text{Offset} \qquad \textit{(Equation 1)}$$

where P is the pressure on the transducer and V is the voltage measured on the AUX output line. The IP transducer output is Analog Out 1. The DP transducer is Analog Out 2.

Recycle Out: The solenoid valve drives current on the white and black wires (the current direction is irrelevant for the solenoid). This signal may be connected to a user-supplied solenoid valve or a Wyatt Technology Recycle unit, which contains an internal solenoid valve that switches between waste and recycle. When this connector is actuated (via the System tab or the Recycle In input), the connector supplies current to drive a 12 V solenoid valve. The valve is actuated with 12 V (up to 1 Amp, depending upon resistance of the solenoid), held for 0.1 second, and then dropped down with 12 V across an internal 51 Ohm resistor.

Alarm Out: TTL output on white (signal) and black (signal ground). On the instrument LCD display Alarms tab (see “Alarms Tab” on page 5-5), you may select the active state of this output. If “Active High” is selected, the instrument keeps this signal at 0 V for no alarm state, and brings the signal to 5 V in the event of an alarm state. If “Active Low” is selected, the instrument keeps this signal at 5 V for no alarm state, and brings the signal to 0 V in the event of an alarm state. In this context, an alarm state occurs if the internal liquid leak sensor detects liquid, or the internal vapor alarm detects organic solvent vapors, or the rear panel connector Alarm In signal is active (see Alarm In).

Auto Inject Out: Contact closure output between wires white and black. This signal is a retransmit of Auto Inject In (see Auto Inject In). Internal to the instrument, the white and black wires are normally not in contact with one another. Upon actuation of this contact closure retransmit, the white and black wires are brought into contact with each other inside the instrument.

3.2.3 Communication Connectors

The rear panel has one Ethernet connector and one USB connector, allowing digital communication to an external computer.

Ethernet: Standard Ethernet cable for connecting the instrument to an Ethernet network, see Figure 3-9.

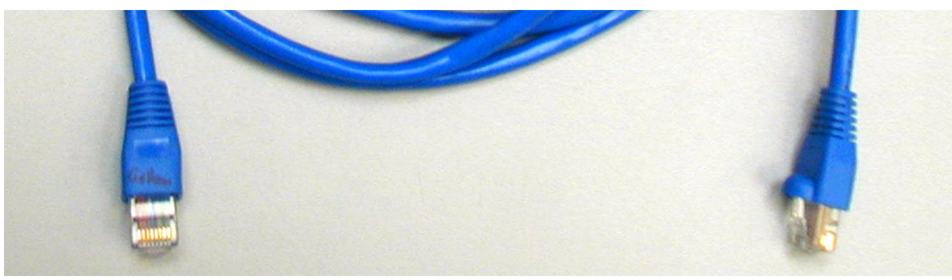


Figure 3-9: Ethernet Cable

USB: Standard USB cable for connecting the instrument directly to a computer, see Figure 3-10.

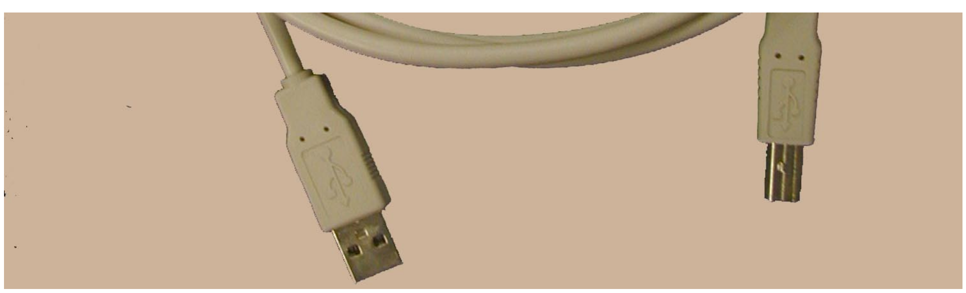


Figure 3-10: USB Cable

3.3 Dry Nitrogen Connection

If you will operate below ambient temperature, a dry nitrogen purge is essential to prevent condensation.

To prevent condensation, attach a dry air or nitrogen line to the Nitrogen Purge fitting on the rear panel of the ViscoStar (see Figure 2-4). (Other dry gases, such as carbon dioxide can also be used.) This provides a continuous flow of dry gas into the interior of the instrument.

Use a right-angle male connector and a 10-foot length of Polyethylene tubing, provided with the ViscoStar, to connect to the Nitrogen Purge fitting.

The system is designed to run with a maximum applied pressure of 80 psi, but can be run at lower pressures to reduce the flow rate into the instrument. The pressure in the dry air or nitrogen line should be 20–80 psi (0.14–0.28 MPa). With a pressure of 25 psi, the dry gas flows into the temperature regulated box at a rate of approximately 38 mL/minute. A standard high pressure gas tank contains about 8,600 L of gas at STP, and so a tank should supply a single ViscoStar for approximately 150 days.

The ViscoStar has an internal pressure sensor that measures the pressure of the dry gas being supplied. The measured dry gas pressure may be viewed on the LCD display Alarms tab. If the dry gas pressure drops below 20 psi (0.14 MPa), then the temperature control set point cannot be set below 20°C. If the system temperature is set below 20°C and the dry gas pressure subsequently drops below 20 psi (for example, if a nitrogen tank is fully depleted), the temperature control set point is automatically changed to 20.5°C.

4 Running the ViscoStar

This chapter provides details about preparing samples and running the ViscoStar.

CONTENTS **PAGE**

4.1 Powering On and Setting the Temperature

The power switch for the ViscoStar is located on the front of the instrument in the lower right hand corner (see Figure 2-3). Start by powering on the instrument using this switch.

After powering on, set the temperature control set point from the LCD display Main tab as described in “Main Tab” on page 5-3. The ViscoStar has a temperature range from 4°C to 60°C.

Operation below ambient temperature requires the flow of dry gas into the temperature-controlled environment, as described in “Sample Preparation” on page 4-12.

After setting the temperature control set point, wait approximately 30 minutes for the instrument to stabilize. If the temperature set point is quite different from room temperature, the instrument stability continues to improve for several hours. Flushing the instrument, as described in “Conditions for Operation” on page 4-10, may be performed during this warm up period.

If the LCD display hangs (freezes) or crashes, see “Microsoft Windows Encounters Problems” on page B-3 for information about rebooting.

4.2 Purging the ViscoStar

After you have set the operating temperature, you can begin flowing solvent through the system as described in this section. Temperature stability is not necessary until you are ready to zero the instrument and collect data.

4.2.1 Changing the Solvent

When you change from one solvent to another, you must flush the delay reservoir with the new solvent. The instrument is shipped with ethanol in the delay reservoir and lines.

Caution: When changing solvents, use a very low flow rate until the delay volume is purged. A large viscosity change will cause the TPS will switch to the “safe” state to prevent damage to the differential transducer. This safe mode bypasses the delay volume to be purged.

The differential transducer, owing to its high sensitivity, can be damaged by relatively small pressure differences. If the viscosity of the new solvent is much different from that of the old solvent, the differential transducer can easily be overpressured unless the flow rate is quite small.

Follow these steps to change solvents:

1. Plumb the system with the new solvent. Always flush the ViscoStar with co-miscible solvents stepwise to prepare the cell for data collection. The ViscoStar is shipped with ethanol in the delay reservoir and lines. A typical series of solvents (from polar to nonpolar) is shown below.

Note:	Salt solutions should be considered separate steps from pure solvents.

Water
Methanol, Ethanol
Isopropanol, Acetone
Tetrahydrofuran
Ethylacetate, Chloroform, Methylene chloride
Toluene, Carbon disulfide
Hexane, Petroleum ether

2. Navigate to the DP Purge button on the Main tab of the LCD display, and press ENTER. Purge the differential transducer for at lease 10 ml. Then, press ENTER to disable the purge.

   If this purge mode is enabled, the button is yellow and reads "DP Purge On". If the purge mode is disabled, the button is green in color and reads "DP Purge Off".

Hint:	When flushing solvents, always actuate the IP Purge and DP purge buttons several times to help release any fluid trapped in the flow valves.

3. Navigate to the IP Purge button on the Main tab of the LCD display, and press ENTER. Purge the inlet transducer for at lease 10 ml. Then, press ENTER to disable the purge.
4. Flow at a slow flow rate in run mode until the delay volume is completely flushed.

   Flush with at least 4 times the delay volume. For example, if you are using the 15 ml delay volume, you should flush at least 60 ml through the system. Flushing with an even larger volume is preferred.
5. If the TPS puts the system into "safe" mode, reduce the flow rate and continue flushing.

Hint:	It sometimes helps to increase the system temperature to reduce the viscosity of the solvents and therefore allow a greater flow rate before the TPS activates.

6. After the delay volume has been flushed out, you can increase the flow rate.

Caution: Always ramp the flow rate slowly. If the transducers are subjected to a sudden change in pressure, they take some time to stabilize. If the flow rate is ramped slowly instead of changed abruptly, this effect is minimized. Moreover, if there are bubbles trapped in the plumbing, rapidly ramping the flow rate can cause large differential pressures, resulting in damage to the transducer.

4.2.2 Removing Air Bubbles

The bridge viscometer depends on the accurate cancellation of the pressures across the two arms of the bridge. Gas bubbles, being much more compressible than the fluid, upset this delicate balance. If air is trapped in one of the valves or in one of the delay volumes, the bridge will act erratically, and will show a large sensitivity to pump pulses.

There are several ways to combat the problem of bubbles. The following preventative measures are important.

- Never leave the entrance or exit fittings open to the air for longer than is absolutely necessary.
- When the ViscoStar is not to be used for some time, leave it connected to a chromatography system with a slow flow rate, or disconnect the inlet and exit tubings and replace them with plugs.
- Always use an online degasser in the chromatography system. Degassed fluid allows small trapped bubbles to slowly dissolve and be eliminated from the system. An online degasser also makes the baseline of refractive index detectors much more stable and improves the reproducibility of the chromatography.

The system is designed so that all potential bubble traps in the system can be flushed with fluid. If a bubble is trapped in either of the transducers, purging that transducer as described in Section 4.2.3 “Purging the Transducers” on page 4-4 should eliminate it.

4.2.3 Purging the Transducers

The ViscoStar has two purge modes to purge the transducers.

- **IP Purge:** Purges fluid and any trapped bubbles in the inlet pressure (IP) transducer.
- **DP Purge:** Purges fluid and any trapped bubbles in the differential pressure (ΔP) transducer.

Buttons for activating these purge modes are provides on the Main tab of the LCD display. To activate a purge mode, navigate to the button you want and press **Enter**.

When you activate the **IP Purge** button, valve V1 in Figure 4-1 is closed, and fluid flows through both sides of the IP transducer and to the instrument OUT port. This is different from the normal state, in which fluid pressure affects the transducer, but fluid does not flow through the two halves of the transducer.

When you activate the **DP Purge** button, valves V2 and V3 in Figure 4-1 are closed, and fluid flows through both sides of the ΔP transducer and to the instrument OUT port. This is different from the normal state, in which fluid pressure affects the transducer, but fluid does not flow through the two halves of the transducer.

Typically you use the manual IP purge and DP purge separately. If the TPS discovers a pressure overflow condition, it activates both the IP and DP purge simultaneously.

Figure 4-1 shows details of the flow path through the bridge and transducers.

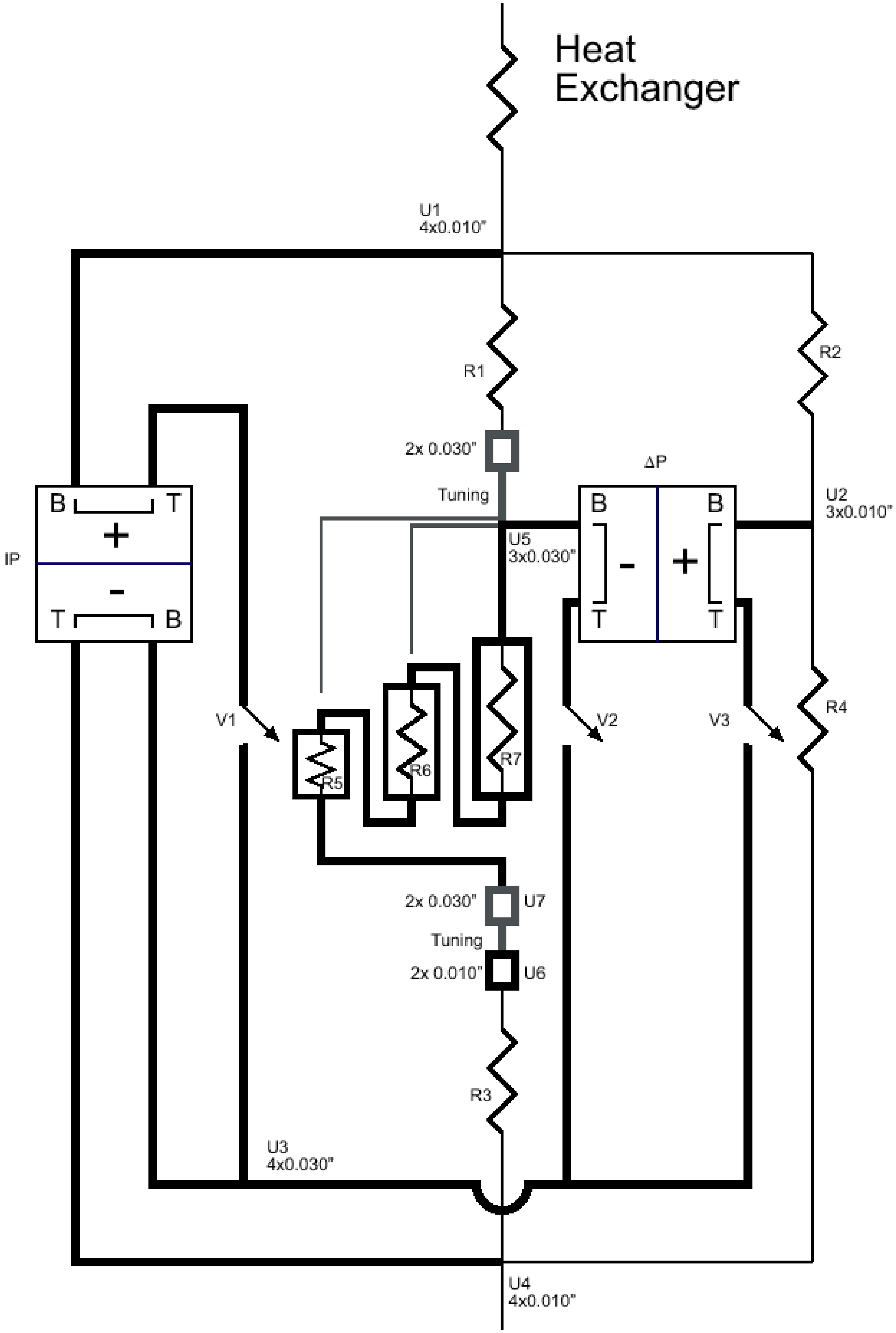


Figure 4-1: Detailed ViscoStar Flow Path

In this figure, R1 through R4 are the bridge capillaries, R5 through R7 are the delay volumes, V1 through V3 are valves that are closed when purge modes are activated, U1 through U5 are multi-port unions. T and B in the IP and ΔP transducers indicate the top and bottom of the transducer flow path, which is important for determining which way bubbles rise. Thick lines indicate places where 0.040" tubing is used. Thin lines indicate places where 0.010" tubing is used.

A tuning capillary is installed by WTC during manufacturing to balance the bridge—insuring that the DP signal is close to zero when solvent flows in all arms of the bridge. The two tuning capillary locations marked in Figure 4-1 are possible locations for the tuning length. A tuning capillary is installed in one (not both) of these locations.

4.3 Zeroing the Instrument

There are two types of zeroing in the ViscoStar:

- **Zeroing the transducers** adjusts the transducer output so that when no flow is applied, they read zero. You should zero the transducers whenever you change the plumbing configuration within the ViscoStar—that is, whenever you change the delay volume plumbing.
- **Zeroing the bridge** adjusts the output of the DP transducer to account for bridge imbalance. You should zero the bridge on a regular basis. Once per week is a suggested interval for zeroing the bridge.

4.3.1 Zeroing the Transducers

The pressure transducers are calibrated at the factory so that when there is no applied pressure across their membranes they accurately read zero pressure. This zero offset may slowly drift with time. Moreover, the transducers are both differential transducers. This means that when the same pressure is applied to both sides a transducer, it should read zero pressure difference. In practice, there is a weak dependence on the absolute pressure.

To correct for the difference from zero, follow these steps:

1. Plumb the system and eliminate any bubbles.
2. Flow for some time to ensure stable baselines.
3. When you are confident that the system is stable, turn off the applied flow and let the system equilibrate for 30 minutes or longer.

4. On the System tab of the LCD display, activate the Zero button. This step zeros the pressure offset for both the IP and DP transducers.

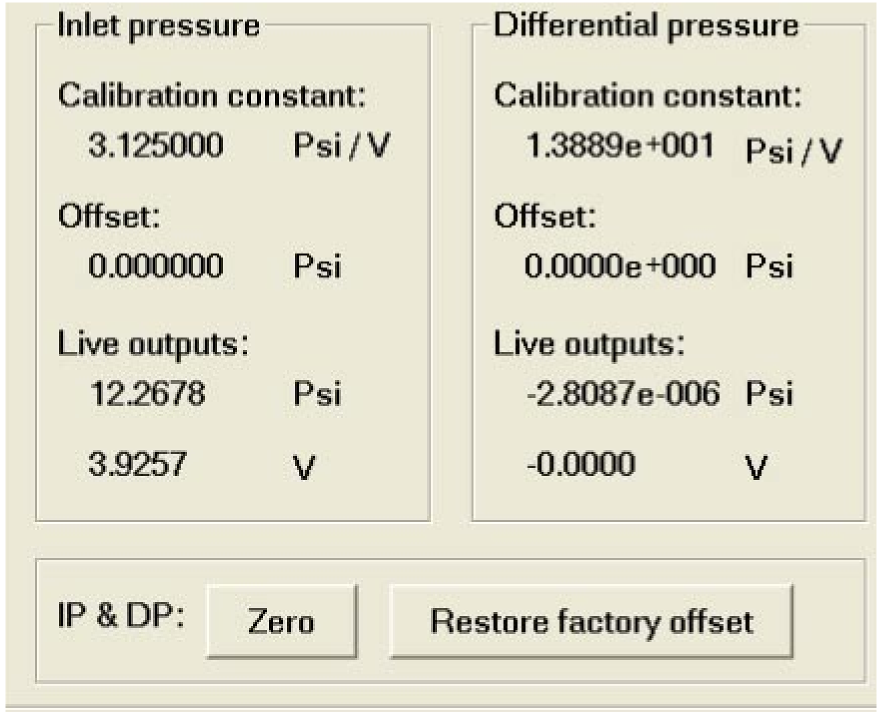


Figure 4-2: Zero Button on System Tab

Zero the transducers any time you change the plumbing configuration within the ViscoStar—that is, whenever you change the delay volume plumbing. Also zero the transducers periodically (around once per week) in case the transducer zero position has drifted since manufacture.

4.3.2 Zeroing the Bridge

In addition to zeroing the transducers themselves, there is almost always some small imbalance in the four-capillary bridge. This bridge imbalance may change over time or with operating temperature. As the system ages, samples that coat the tubing will slowly build up, affecting the bridge balance.

To measure the zero the bridge, follow these steps:

1. Follow the procedure in “Zeroing the Transducers” on page 4-7 to zero the transducers with no flow.
2. Increase the flow to 1 ml/min.
3. Look at the specific viscosity reported on the graph on the Main tab of the display.

   The specific viscosity with only pure solvent flowing is a measure of the bridge imbalance. If, at 1.0 ml/min, the reported specific viscosity is 0.01, then the bridge is balanced to 1%.

4. Navigate to the **Flow Zero DP** button on the Main tab and press ENTER. This button subtracts the DP offset so that the reported specific viscosity is 0.

   The Flow Zero DP button has been so named to remind you that unlike the Zero button on the System tab, this button should be used only when solvent is flowing. Also it only affects the DP transducer signal.

Another way to look at the bridge imbalance is to measure the DP signal with pure solvent. The DP transducer has a range of 0.72 psi. If at your operating flow rate, the DP signal reads 0.1 psi, the maximum peak that can be accomdated is now only 0.62 psi, instead of the full scale of 0.72 psi.

All ViscoStar instruments are balanced to better than 1% at the factory.

In the literature, there are statements that indicate that if the bridge is not perfectly balanced, there will be excessive pickup of pump pulses (which are supposed to be cancelled by the bridge). In practice, the additional pump pickup is minimal. The biggest problem caused by an improperly balanced bridge is that the maximum signal achievable before the DP transducer goes off scale may be reduced. (In fact, since most peaks are positive, having the bridge slightly imbalanced to the negative side, increases the range).

4.3.3 Measuring the System Dilution Factor

If an RI detector (or any other instrument) is plumbed after the ViscoStar in the flow sequence, the sample that exits the ViscoStar is diluted by approximately a factor of 2. Therefore the RI detector does not measure the same concentrations that flowed through the MALS and ViscoStar instruments.

To correct for the change in concentration, you will determine the exact dilution ratio. If the two arms were identical, the dilution factor would be 50%. In practice, the two arms are never *exactly* identical. The dilution ratio will also change over time as samples that coat the tubing slowly build up.

This procedure will be described in more detail in the *ASTRA V User's Guide* since the dilution factor does not affect the operation of the hardware, but is essential in the data analysis. Briefly the steps to determine the dilution factor are:

1. Inject a known quantity of sample into the system.
2. Measure the known recovered mass in the positive peak with the concentration detector.
3. Use the discrepancy to calculate the dilution factor. The dilution factor is simply the ratio of the recovered mass to the injected mass.

This method assumes that none of the sample is retained in the chromatography columns and that 100% is recovered. In practice, this assumption can be problematic, but the dilution factor, once determined, should be sample-independent since it only depends on the relative flow impedance of the two arms of the bridge.

We recommend that you measure the dilution factor with several standards. If the same dilution factor is measured, column absorption is likely not a problem. Also be aware that preparing a sample of a known concentration can be difficult if the sample has adsorbed water, salts, or other impurities.

4.4 Conditions for Operation

In order to use the ViscoStar successfully, the following are recommended:

- Solvents should be pre-mixed and stored in a covered reservoir. For the lowest possible baseline noise, a stirrer should be used in the solvent flask. The solvent should be degassed by vacuum, sonication, or boiling, and blanketed with an inert gas—such as helium or argon—that has a low solubility in the solvent. An in-line degasser is strongly recommended.
- Thermal control and/or insulation of the system components are important in order to obtain good results, particularly in:
    - the column(s)
    - the eluent before the column(s)
    - the ViscoStar itself

  “Main Tab” on page 5-3 outlines how to control the ViscoStar temperature. The ViscoStar is extremely stable thermally, so unless the ambient temperature fluctuates considerably, the ViscoStar should be able to achieve a stable temperature.
- A pump with a constant flow rate is required, and regularly checking the pressure is an important routine in HPLC. A slowly increasing or unstable pressure is an indication that the chromatography conditions are changing, most often due to:
    - blockage in the solvent reservoir filter
    - blockage in the in-line filter after the pump
    - blockage in the column
    - malfunctioning pump
    - air or gas in the system

These problems often create an unstable baseline due to an unstable column environment. The instabilities result in fluctuating solution density, changing the system pressure. Remember since the ViscoStar measures pressures, an unstable flow will adversely affect the results. The result is a signal with oscillations, spikes, or drift.

- Many chromatography pump systems have “silk” settings for adjusting the piston stroke for solvent compressibility. It is recommended that you set these properly. See your chromatography pump documentation.
- A high-pressure pulse dampener before the sample injector, although not required, is recommended. Although the ViscoStar's bridge design cancels most pump pulses, the more stable the flow rate, the better.
- When changing solvents, special care should be taken to avoid leaving residues of the old solvent in the chromatography system. It is best to work with pre-conditioned chromatography columns and to change solvents with the columns by-passed. “Purging the ViscoStar” on page 4-2 describes solvent change procedures.

The pump pressure should be noted regularly, and the system should be checked for short-term variations in flow rate, eluent, or operating temperature. If variations are found, appropriate action should be taken as recommended in the user’s manual for the questionable component.

We also strongly recommend that the chromatography pump have a pressure limit set to approximately 100 psi over the expected working pressure. If a plug develops somewhere in the tubing downstream of the ViscoStar, the pressure in the instrument could potentially get very high, and cause damage to the transducers and valves. By setting a pump pressure limit, this can be avoided.

For further information regarding injection procedures, pumps, thermostats, etc., refer to the various instruction manuals for these devices and to the broad literature on HPLC.

4.5 Sample Preparation

To avoid contamination of the column and obtain the best possible separation, it is important to have a clean sample solution. Additionally, the following guidelines may be helpful in sample preparation:

- Solvent used for sample preparation should have the same composition as the eluent solution. Only HPLC-grade solvents should be used.
- Sample dissolution may be encouraged by shaking or swirling, homogenizing, or sonication. Care should be taken to avoid precipitation from excessively high concentrations. Several hours of dissolving time may be required for the sample to fully dissolve.
- Particle removal by filtration (0.2–0.5 μm filter) and the use of a guard column is highly recommended.
- Degassing improves the quality of the solvent/sample analysis. Degassing also causes bubbles to dissolve. It is strongly recommended.

4.6 Making Measurements

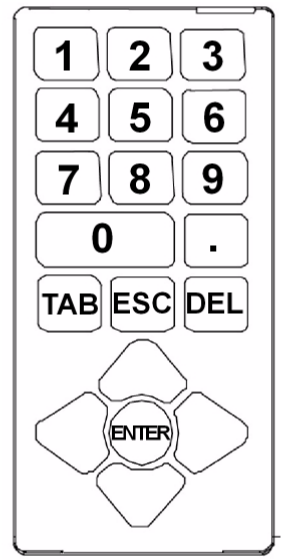
1. Prior to injecting a sample, confirm that the baseline and temperature are stable.
2. Flow zero the instrument by navigating to the LCD display Main tab and pressing ENTER on the Flow Zero DP button.
3. Start the data collection in ASTRA V. See the *ASTRA V User's Guide* for more detail on data collection.
4. Inject the sample.
5. Wait until the positive sample peak occurs and the negative flush peak elutes from the system before making another injection.

5 Using the LCD Display

This section describes the keypad controls and the tabbed interface on the LCD display on the front panel of the instrument. These tabs allow you to monitor, configure, and control the function and operation of the ViscoStar.

CONTENTS **PAGE**

5.1 Navigating the ViscoStar Tabs



The keypad consists of number keys, TAB, ESC, DEL, ENTER, and arrow keys.

You use these keys to move around the LCD display on the instrument front panel and to enter values where applicable.

To select a tab on the LCD display:

1. Press the TAB key until the cursor is positioned on a tab title at the top of the screen
2. Use an arrow key to move to the desired tab. Each tab is displayed as you use arrow keys to move to another tab.

Once the desired tab is displayed, use the keys as follows:

- **TAB key:** Move forward to the next field within a tab.
- **ESC key:** Exit a pull-down list or to cancel an entry.
- **DEL key:** Remove the previous character from a numeric entry field. (Same as Backspace on a regular keyboard.)
- **ENTER key:** Save an entry made using the number buttons, “click” a button, select an option, or choose an item in a pull-down list.
- **Up and Down Arrow keys:** Move to a different selection within a pull-down list or radio field.
- **Right and Left Arrow keys:** Move to another tab, or move the cursor within a numeric field.

Note:	The TAB key moves *only* in the forward direction.

The sections that follow describe the ViscoStar LCD display tabs.

5.2 Main Tab

The Main tab allows you to view either the specific viscosity (η_{sp}) or the instrument temperature through time.

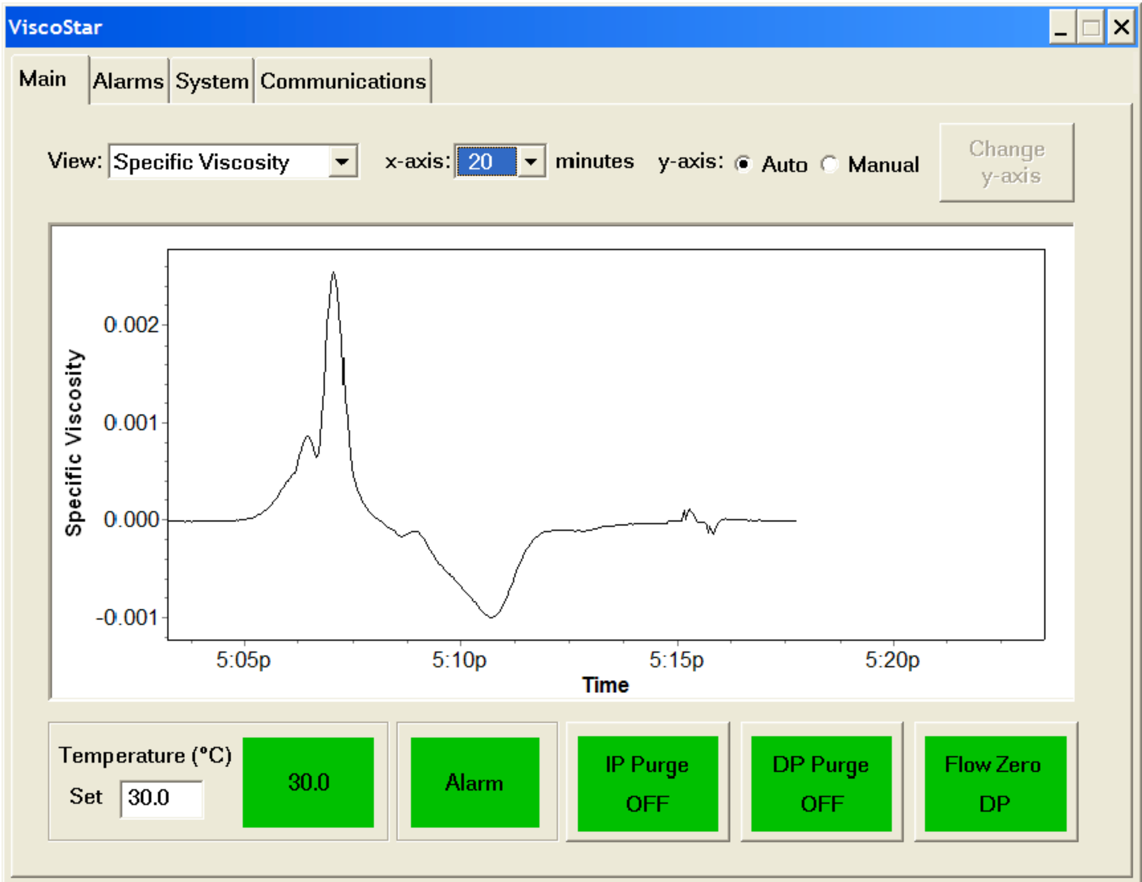


Figure 5-1: Main Tab

View: The viewing options are IP, DP, Specific Viscosity, Bench Temperature, Chassis Temperature, Aux Input, and N2 Pressure. The option you select is shown over a period of time.

x-axis: Selects the length of time to be displayed on the screen on the x-axis.

y-axis: Options are Auto and Manual. Auto automatically determines the range to display on the y-axis. To customize the y-axis, select Manual, tab to the Change y-axis button and press ENTER. Enter the desired numeric values using the keypad in the pop-up dialog, tab to the OK button, and press ENTER.

Temperature (°C) Set: Enter the value for the temperature that the instrument is to maintain. The value in the colored box to the right is the current internal temperature of the instrument. The box is yellow until the temperature stabilizes. When the temperature has remained within ±0.05°C of the Temperature (°C) Set value for a minimum of 15 minutes, the box is green.

Alarm: Flashes red if an instrument alarm is active, and remains solid green if no alarms are active. If any alarm condition is detected, the Alarm box flashes on the Main tab and the Alarms tab. Navigate to the Alarms tab to determine the nature of the alarm. See Section 5.3 “Alarms Tab” on page 5-5.

IP Purge: This is a toggle button that turns the Inlet Pressure (IP) transducer purge valve On or Off. In the ON state, the purge valve purges the IP transducer. If the purge valve is OFF, the transducer measures fluid pressure, but fluid does not flow through the + side of the transducer. See Figure 4-1 and Section 4.3.1 “Zeroing the Transducers” on page 4-7.

DP Purge: This is a toggle button that turns the Differential Pressure (ΔP) transducer purge valves On or Off. In the ON state, the purge valves purge the ΔP transducer. If the purge valves are OFF, the transducer measures fluid pressure, but fluid does not flow through either side of the transducer. See Figure 4-1 and Section 4.3.1 “Zeroing the Transducers” on page 4-7.

Flow Zero DP: Press ENTER on this button to cause the current value measured by the bridge to be considered the “zero” value. See Section 4.3.2 “Zeroing the Bridge” on page 4-8.

5.3 Alarms Tab

The Alarms tab allows you to view the current state of ViscoStar alarms, and to set the voltage state of external alarms. If any alarm is detected, the Alarm button flashes on the Main and Alarms tabs.

If no alarm conditions are detected, the status areas show OFF in a green box. If an alarm condition is detected, the status areas show Alarm in a flashing red box.

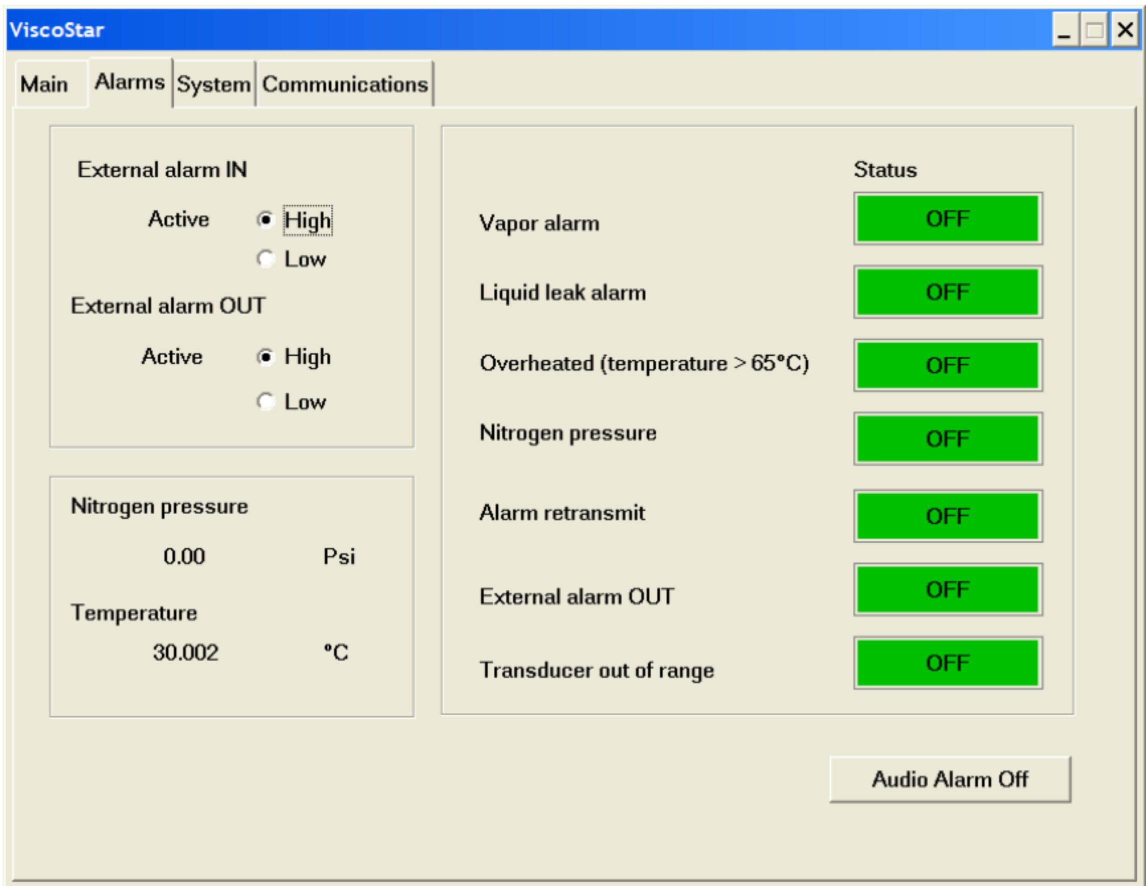


Figure 5-2: Alarms Tab

External alarm settings are used when alarms from additional instruments or systems are daisy chained through the ViscoStar. Connections are made at the rear panel using the Alarm In or Alarm Out connectors.

External alarm IN: The External alarm IN is an input used to capture alarms from additional devices, and then retransmit that alarm as an External alarm OUT.

For example, multiple instruments in a chromatographic system typically have liquid leak detectors. It is desirable that the solvent pump be shut off in the event of a liquid leak in any one of those instruments. Since the alarm outputs from each instrument cannot all be connected to the same pump shut-off signal line, the alarm outputs from the instruments need to be logically ORed with each other. The External alarm IN is logically ORed with the ViscoStar internal vapor, liquid leak, and overheating alarms, and then retransmitted as the External alarm OUT.

Different devices have different conventions for alarm states. Some maintain a 5 V state on a wire to indicate no alarm, and bring the voltage to 0 V to indicate an alarm condition. This is called the “Active Low” convention. Others use 5 V to indicate an alarm condition; this is the “Active High” convention.

Select High or Low depending on the voltage output convention of the connecting device. If the connecting device expects to receive a low voltage to signal an alarm, select Low. Voltage signal ranges are from 0 V to 5 V.

External alarm OUT: This output signal is the logical OR of the External alarm IN, the Vapor alarm, and the Liquid leak alarm. If connecting a device to the ViscoStar External alarm OUT, select High or Low depending on the voltage input of the external device. If the connecting device expects to receive a low voltage to signal an alarm, select Low. Voltage signal ranges are from 0 V to 5 V.

Nitrogen pressure: Displays the gas pressure in psi of the gas line attached to the back panel nitrogen purge port (see Figure 2-4). For subambient operation, this pressure must be greater than 20 psi.

Temperature: Displays the current temperature in degrees Centigrade (°C) inside the ViscoStar.

Alarm status area: This area displays a red box for the following items if an alarm is active. Otherwise, it shows green for each item.

- **Vapor alarm**: Displays an alarm indication when organic solvent vapors are present in the instrument chassis.
- **Liquid leak alarm**: Displays an alarm indication if the sensor located inside of the ViscoStar comes in contact with any liquids.
- **Overheated (temperature > 65°C)**: Displays an alarm indication if the temperature rises above 65°C. If this condition occurs, the heater/cooler is disabled.
- **Nitrogen pressure**: Displays an alarm indication when the temperature drops below 20°C and the nitrogen pressure drops below 20 psi (0.14 MPa). If this alarm occurs, the temperature is set to 20.5°C. Once the temperature reaches that point, this alarm indicator goes back to green.
- **Alarm retransmit**: Displays an alarm indication if the instrument detects a voltage signal on the rear panel Alarm In connector from an external device as configured by the External alarm IN Active High or Low button. See Section 3.2.2 “Connecting Signals” on page 3-7.

- **External alarm OUT**: Displays an alarm indication if the instrument is sending an alarm out through the rear panel Alarm Out connector. The voltage state of the External alarm OUT signal is configured by the External alarm OUT Active High or Low button.
- **Transducer out of range**: Displays an alarm indication if the pressure measured by a transducer is out of the safe range for that transducer. The Transducer Protection System (TPS) automatically triggers the IP and DP purges immediately if this condition occurs. This alarm indicator goes off when the TPS activates. If you see this alarm persist, the TPS failed to activate. If this occurs, one of the capillaries is plugged, and the instrument must be returned to WTC for service.

Audio Alarm On/Off: Navigate to this button and press ENTER to toggle the Audio Alarm On or Off.

5.4 System Tab

The System tab allows you to configure a waste or recycle activation, set the time of day, view the instrument's serial number and the Auto Inject input status, and modify calibration constants and analog out parameters.

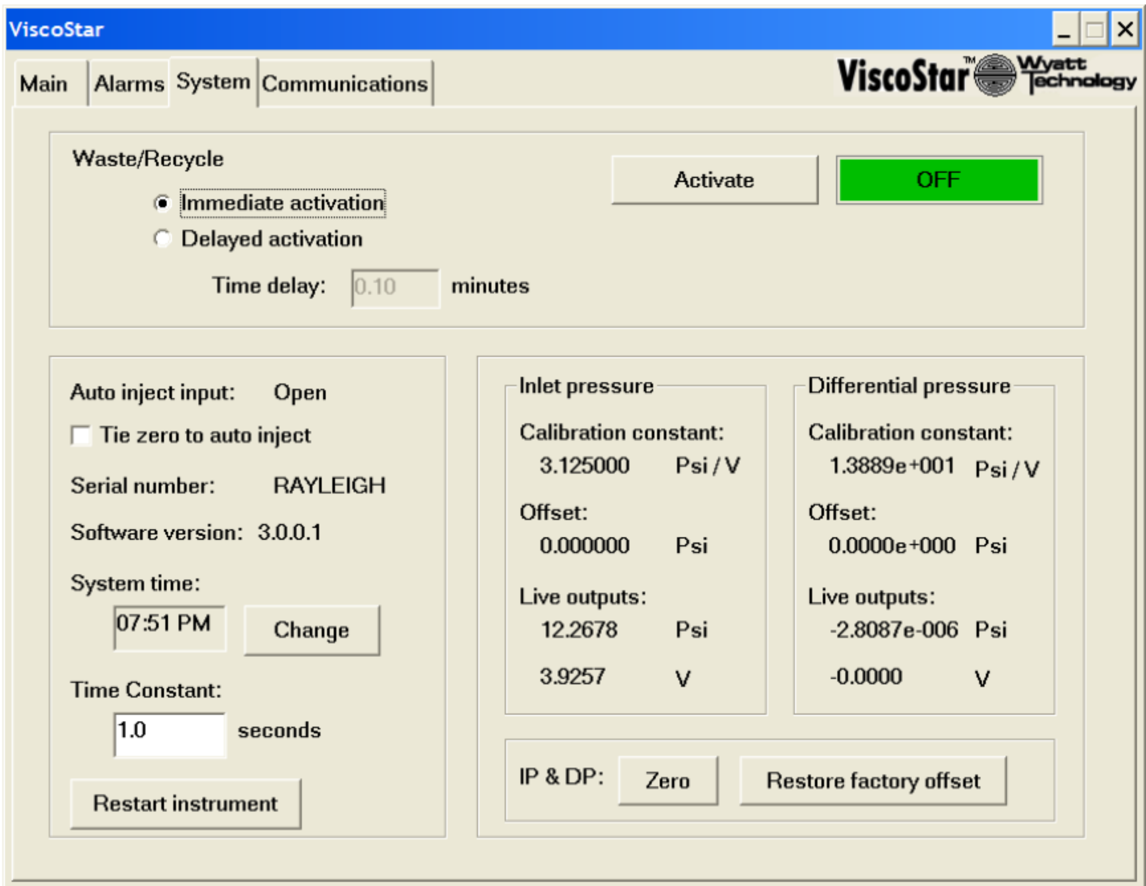


Figure 5-3: System Tab

Waste/Recycle: The Activate button toggles the state of the solenoid valve connected to the Recycle Out connector on the rear panel. If Immediate activation is selected, the valve toggles immediately. If Delayed activation is selected, the valve toggles after the number of minutes you enter in the Time delay field has elapsed. If the box shows OFF, the valve is de-actuated. If the box shows ON, the valve is actuated. When the timer for the delay is running, a Cancel button is shown, the box says PENDING, and a countdown timer below the box shows the time left before activation.

Auto inject input: Displays the status of the input signal from the rear panel Auto Inject In connector. The Auto Inject In is a contact closure signal, which may be Open, indicating no contact between the signal wires, or Closed, indicating that the signal wires are physically connected.

Tie zero to auto inject: When this box is checked, the autoinject input causes a Flow Zero DP command to be performed when the autoinject signal indicates that an injection occurred. This can be used to guarantee that the system is always zeroed after every injection.

Serial number: Displays the serial number of your instrument.

Software version: Displays the version number of the firmware running on the ViscoStar. After a firmware upgrade, you must restart the instrument.

System time: Displays the time of day used by the instrument to display times in the graphs on the Main tab. To change the time of day, navigate to the Change button and press ENTER. Use the dialog to enter the new time. Note that this time is not used to timestamp the data. Instead, the data is timed by an internal timer that is not based on the time of day.

Time constant: Time over which data is averaged before being presented. Time constants must be multiples of the minimum time constant, which is 0.1 seconds.

Restart instrument: You can activate this button to restart the instrument and reboot the Microsoft Windows operating system used for the LCD display.

Calibration Constants: This area displays the values of calibration constants that can be used to convert the back panel AUX out voltages to pressures. For each transducer, the formula is as follows:

$$P = \text{"Calibration Constant"}\ V + \text{Offset} \qquad \textit{(Equation 1)}$$

where P is the pressure in psi on the transducer and V is the voltage measured on the AUX output line in volts. The IP transducer output is Analog Out 1. The DP transducer is Analog Out 2.

Live outputs: This area displays the actual pressures currently measured by the transducers and the corresponding voltages being sent to the AUX out connectors on the back panel.

IP & DP Zero: This button sets an offset value for the IP and DP transducers to cause the current pressures to be zero. See Section 4.3.1 “Zeroing the Transducers” on page 4-7.

Restore factory offset: This button restores IP and DP transducer zero settings originally made when the instrument was manufactured.

5.5 Communications Tab

The Communications tab allows you to set parameters regarding communication with computers. The ViscoStar is equipped with both Ethernet and USB communication:

- **Ethernet communication** allows the instrument to be presented to any computer on a local area network.
- **USB communication** limits access to the ViscoStar to a local computer physically connected to the instrument via a USB cable. The USB interface uses USB Virtual Ethernet communication, which emulates all aspects of Ethernet communication over the USB connection. To use USB on a local computer, the USB Virtual Ethernet software, which is shipped with Wyatt Technology's ASTRA software, must be installed on that computer.

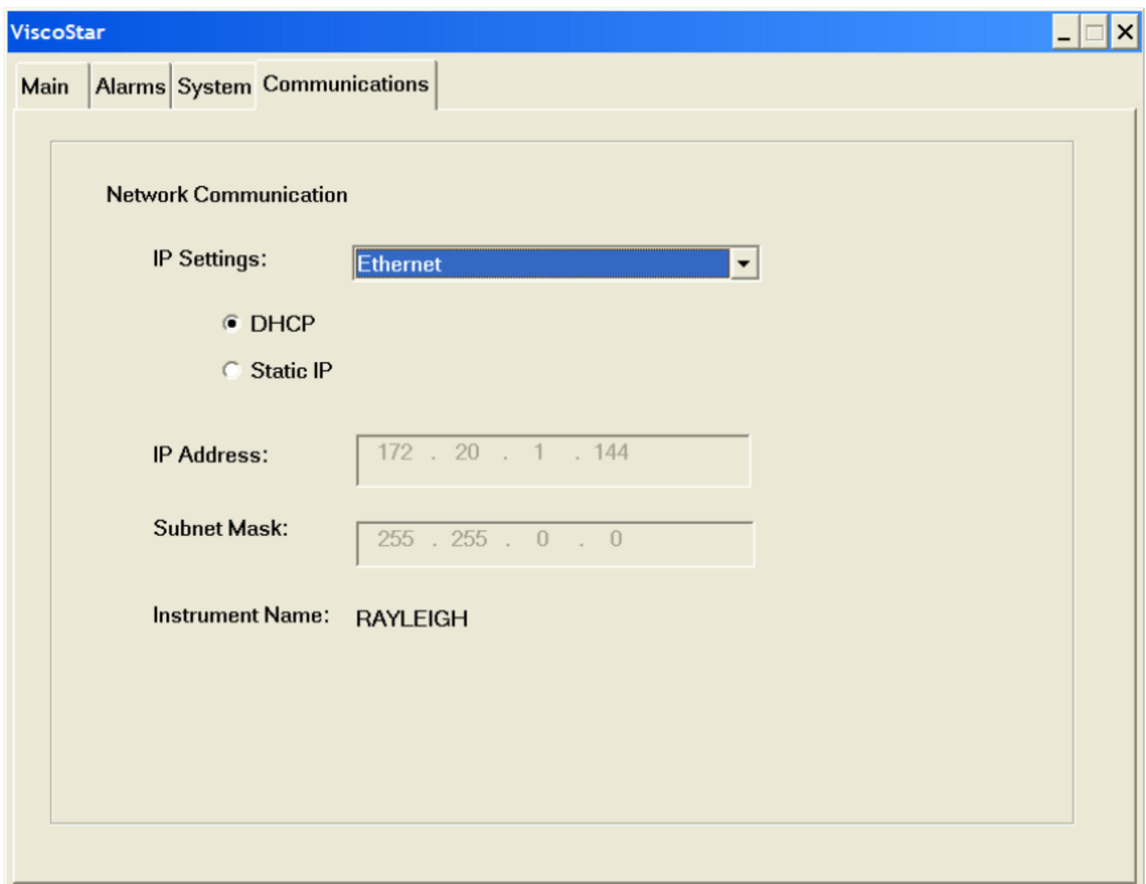


Figure 5-4: Communications Tab

IP Settings: Select Ethernet to view information concerning the local area network. Select USB to view information concerning the USB Virtual Ethernet.

DHCP/Static IP: For either Ethernet or USB, select DHCP to have the Ethernet network or USB Virtual Ethernet automatically assign an IP address to the instrument. Select Static IP to manually assign a fixed IP address and subnet mask.

IP Address / Subnet Mask: If you select DHCP, these fields display your automatically assigned IP Address and Subnet Mask values. If you select Static IP, enter the IP Address and Subnet Mask values. Contact your network administrator for valid static IP Address and Subnet Mask values.

Instrument Name: Shows the instrument name used on an Ethernet network.

6 Service and Maintenance

This chapter provides detailed instructions on minor maintenance tasks that can be performed by the user on the ViscoStar.

CONTENTS **PAGE**

6.1 Cleaning After a Fluid Leak

Liquid from leaks of internal or external fittings is directed to a liquid leak well inside the instrument. If a liquid leak occurs, an optical liquid sensor in that well senses the presence of the liquid and activates an alarm that may be seen on the LCD display (see “Alarms Tab” on page 5-5 for a description of the Alarms tab), and activates an Alarm Out signal that may be used to disable a pump.

The well in which the liquid collects has two chambers. The chamber directly beneath the liquid leak detector, which is also the one that is more difficult to access, is the chamber that needs to be emptied and cleaned (see Figure 6-1).

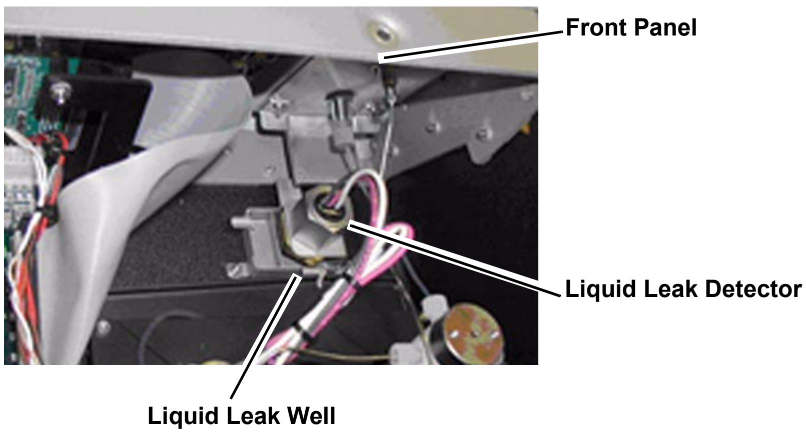


Figure 6-1: Liquid Leak Chamber

To clean a liquid leak, follow these steps:

1. Disable the pump or otherwise prevent additional liquid from being sent into the ViscoStar.
2. Disconnect the power cord from the instrument.
3. Using a 2mm ball driver, remove the four screws that secure the cover to the instrument.
4. Lift the cover at the front and pull forward to remove.

CAUTION: Be sure to wear gloves and other protection appropriate for handling the solvent that has leaked.

5. Using absorbent paper or cotton, soak up as much as possible from the liquid leak well.

   When the liquid leak well has been emptied, the liquid leak alarm should turn off. A slight wetting of the well should not be sufficient to set off the liquid leak alarm.

6. If the liquid leak well contains residue from the leak, use a syringe to flush the liquid leak well with a pure solvent that is co-miscible with the solvent that has leaked (see Section 4.2 “Purging the ViscoStar” on page 4-2 for a list of solvent miscibilities).
7. Repeat step 5 to remove any pure solvent you used to clean the liquid leak well.
8. Install the cover and secure it with the four screws you removed in step 3.

6.2 Cleaning the Air Intake Filter

Periodically remove and clean the foam filter for the instrument’s air intake fan. The air intake fan is located on the rear panel of the instrument next to the power connector on the left (see Figure 2-4).

1. Lift the filter bracket off of the rear panel of the instrument and remove the air filter from the filter bracket.
2. Wash the air filter with mild soap and water.
3. When dry, place the air filter back into the filter bracket.
4. Attach the filter bracket with filter back onto the rear panel of the instrument.

6.3 Changing a Fuse

The fuses are located in the power connection socket (see Figure 2-4).

In order to change a fuse, you need the following items:

- Tool for prying the AC Power module cover off, such as a small-bladed screwdriver.
- Fuses from the spares supplied in the accessory kit.

To replace a fuse, follow these steps:

1. Disconnect the power cord from the instrument.
2. Open the cover of the AC Power module using a small blade screwdriver or similar tool.
3. Replace the old fuses with new ones. Use only 2 Amp, 240 V fuses.

Note: Be sure to use the correct fuse for the voltage.

4. Replace the cover of the AC Power module.
5. Reconnect the power cord.

A Acronyms and Abbreviations List

This appendix contains a list of acronyms and abbreviations used in this manual.

CONTENTS **PAGE**

A.1 Acronyms and Abbreviations

The following acronyms and abbreviations are used in this manual:

Item	Meaning
°C	Centigrade
AC	alternating current
Amp	Ampere
cm	centimeters
DC	direct current
DHCP	Dynamic Host Configuration Protocol
g	gram
HPLC	high pressure liquid chromotography
Hz	Hertz
ID	inside diameter
in.	inch
IP	Internet Protocol
kPa	kilopascal
LAN	local area network
MALS	multi-angle light scattering
mg	milligram
MHz	Megahertz
µL	microliter
mL	milliliter
µm	micrometer
mm	millimeter
MPa	Megapascal
mV	millivolt
nm	nanometer
OD	outside diameter
ppm	parts per million
psi	pounds per square inch
RI	refractive index
RIU	refractive index unit
STP	standard temperature and pressure = 0 °C and 1 atmosphere of pressure
TTL	transistor-transistor logic
V	Volt
VAC	Volts alternating current
VDC	Volts direct current

B Troubleshooting

This appendix provides details about troubleshooting procedures for the ViscoStar.

CONTENTS **PAGE**

B.1 Measuring Bridge Balance

To measure the bridge balance, first zero the transducers, as described in Section 4.3.1 “Zeroing the Transducers” on page 4-7. Restart the flow with pure solvent and let the system equilibrate for at least 30 minutes. The bridge imbalance is simply the specific viscosity reported with purge solvent. This value should be independent of the applied flow rate since both IP and DP should be proportional to the flow rate. In practice, however, more accurate results are obtained at higher flow rates since the intrinsic transducer becomes relatively less important when the IP and DP signals are large.

B.2 Temperature Will Not Set Below 20 C

For sub-ambient operation, dry gas must be slowly flowed into the cool thermal environment to prevent water vapor from condensing on the electronics or optics.

The ViscoStar contains a gas pressure sensor to measure the pressure of the dry gas supplied to it. It will not set the temperature set point below 20 °C unless at least 20 psi of pressure is sensed. If dry gas of sufficient pressure is supplied to the ViscoStar and the temperature is set below 20 °C, but the gas pressure subsequently drops below 20 psi, then the temperature set point is automatically set to 20 °C.

B.3 Noisy Baseline

If the baseline noise of the instrument seems excessive, try the following:

- Purge both the IP and DP transducers for extended periods of time. They should be purged one at a time. That is, either IP Purge or DP Purge should be on, but not both. This ensures the maximum flow rate through the transducers.
- A nitric acid flush can be performed as a last resort. To perform a nitric acid flush, follow these steps:
    - **a.** Flush the system with miscible solvents until the system is filled with water.
    - **b.** Set the flow rate to 0.1 ml/min.
    - **c.** Using a syringe filled with 25% nitric acid, flush the system in run mode.
    - **d.** Slowly ramp the flow rate to 0.5 ml/min. If the transducer protection system activates, reduce the flow rate and increase it again slowly.
    - **e.** Flush at least 100 ml of 25% nitric acid through the system.

f. Flush the system with water overnight.

g. Flush the system with miscible solvents until you have returned to your chosen solvent.

If the noise no longer is present, then the noise could have its origin in the chromatographic system, or could be due to a small bubble in the flow cell that is forced into the measurement region only under flow conditions.

If the noise is intermittent during flow conditions, or remains in the absence of flow, the noise may be due to an internal leak. Even leaks slow enough that they don't trigger the leak or vapor sensors can be enough to cause a noisy baseline. Remove the top covers and inspect the fittings for leaks. If you are using aqueous buffers with salts, a telltale sign of a slow leak is a small white crust around a chromatography fitting. If you are using an organic solvent, leaks are harder to detect. Slow leaks can often be detected by vapors concentrated inside the closed thermal bench. Each bench is extensively leak-tested at WTC, so pay particular attention to the fittings you removed when changing the delay reservoir configuration.

If the baseline noise persists, contact Wyatt Technology Corporation.

B.4 Microsoft Windows Encounters Problems

If the Microsoft Windows operating system on the LCD display encounters a problem that causes it to freeze or crash, the ViscoStar reboots the operating system within one minute. You can use the power switch on the front panel to turn the ViscoStar off and on if you want to reboot the machine yourself.

Experiments in progress when the operating system crashes must be restarted.

If the system does not successfully restart, the factory default firmware can be run. During the bootup process, at the screen with the Wyatt logo and a color sweep on the bottom, press the ESC key. This causes the factory default firmware to run.

C Operating Principles and Theory

This appendix provides a basic overview of the theory and operation of the ViscoStar.

CONTENTS **PAGE**

C.1 Theory of Bridge Design

For laminar flow, the mass flow rate through a tube is given by Poiselle's law:

$$Q = \frac{\Delta p}{R\eta} \qquad \textit{(Equation 1)}$$

where, Q is the mass flow rate, η is the fluid viscosity, and Δp is the pressure across the tube, and R is the flow impedance through the tube defined by Eq. 2:

$$R = \frac{8l}{\pi r^4} \qquad \textit{(Equation 2)}$$

where r is the radius of the tube, and l is the length of the tube. Consider the flow through the left hand side of the bridge, Q_1. We have:

$$Q_1 = \frac{IP - P_1}{\eta R_1} = \frac{P_1}{\eta_0 R_3} \qquad \textit{(Equation 3)}$$

where η is the viscosity of the sample and η_0 is the viscosity of the solvent. Similarly the flow through the right side of the bridge is given by

$$Q_2 = \frac{IP - P_2}{\eta R_2} = \frac{P_2}{\eta R_4} \qquad \textit{(Equation 4)}$$

Solving Eqs. 3 and 4 for IP gives Eqs. 5 and 6:

$$\frac{IP}{P_1} = \left(\frac{\eta}{\eta_0}\frac{R_1}{R_4} + 1\right) \qquad \textit{(Equation 5)}$$

$$\frac{IP}{P_2} = \frac{R_2}{R_4} + 1 \qquad \textit{(Equation 6)}$$

The ratio of the pressure difference across the bridge to the applied pressure is given by Eq. 7:

$$\frac{\Delta p}{IP} = \frac{P_2 - P}{IP} = \frac{1}{\frac{R_2}{R_4} - 1} - \frac{1}{\frac{\eta}{\eta_0}\frac{R_1}{R_3} + 1} \qquad \textit{(Equation 7)}$$

If the capillaries are all equal, $R_1 = R_2 = R_3 = R_4$, the results are independent of the bridge capillary impedance and Eq. 7 can be solved for the specific viscosity as follows:

$$\eta_{sp} = \frac{\eta}{\eta_0} - 1 = \frac{4\Delta p}{IP - 2\Delta p} \qquad \textit{(Equation 8)}$$

Eq. 7 also defines the bridge balance condition. If $R_1 R_3 = R_2 R_4$, then $\Delta P = 0$ for pure solvent ($\eta = \eta_0$).

Note that the specific viscosity measurement only depends on the two pressure measurements Δp and IP. The measurement is independent of the flow rate. However, both Δp and IP are directly proportional to the applied flow rate. The noise in the measurement, for a system with minimal pump pulses, is nearly independent of flow rate. This means that the signal to noise ratio of Eq. 8 improves directly proportional to the applied flow rate. If one measures samples with small intrinsic viscosities or low concentrations, it is advantageous to use faster flow rates subject to the limitations of keeping the signals on scale and not compromising the separation efficiency of the chromatography.

C.2 Calculating Intrinsic Viscosity

As shown in the previous section, the ViscoStar directly measures the specific viscosity, but since this quantity is roughly proportional to the sample concentration, it is much more useful to compute the intrinsic viscosity which is defined as the limit of:

(Equation 9)

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

Of course, all real instruments measure the specific viscosity at finite concentrations. The concentration dependence of the specific viscosity can be described by a series expansion known as the Huggins equation:

(Equation 10)

$$\eta_{sp} = [\eta]c + k'[\eta]^2 c^2 + O\left(c^3\right)$$

The coefficient k' is the Huggins constant. For random coil polymers in good solvents, the Huggins constant typically has a value between 0.3 and 0. In SEC, the concentration of the sample is usually so dilute that one can ignore the concentration square terms and use the approximation:

(Equation 11)

$$[\eta] \approx \eta_{sp} / c$$

where the concentration is derived from a concentration detector such as the Optilab rEX or a UV absorption detector.

C.2.1 Intrinsic Viscosity and Molecular Parameters

The simplest model of the intrinsic viscosity is due to Einstein and Simha (A. Einstein, *Ann.Physik*, 19, 289 (1906); 34, 591 (1911) and R.Simha, *J. Phys. Chem.*, 44, 25 (1940); Science 92, 132 (1940)). They considered the case of noninteracting rigid particles. They found that the viscosity can be related to the volume fraction of the fluid occupied by the particles. They found:

(Equation 12)

$$\eta = \eta_0 (1 + \gamma\phi)$$

where φ is the volume fraction and $\gamma = 2.5$ for spheres and larger for nonspherical particles.

If the weight concentration of the molecule is c, then the number of molecules per unit volume is $N_A\, c/M$, where N_A is Avogadros number and M is the molar mass as measured by light scattering. Therefore Eq. 12 can be written in terms of the measured intrinsic viscosity as:

$$[\eta] = \gamma\, N_A\, V_h / M \qquad \textit{(Equation 13)}$$

where V_h is the hydrodynamic volume of the molecules. Note that M/V_h is the molecular density, so in some sense, the intrinsic viscosity is measuring the molecular density.

The intrinsic viscosity often differs from the bulk density due to molecular shape, molecular density, and the effects of adsorbed or immobilized solvent on the surface of molecule. This so-called hydration layer moves with the molecule, so it affects measurement of the molecular density. In addition, when the molecule has an extended shape, penetration of non-immobilized solvent into the interior of the molecule similarly affects this measurement.

If we set γ=2.5, this can be used to define the equivalent spherical volume of a nonspherical molecule. Similarly, it can be used to define the hydrodynamic volume r_h as:

$$r_h = [3\ V/(4\pi)]^{1/3} \qquad \textit{(Equation 14)}$$

When defined in this way, r_h is the radius of a sphere with the same intrinsic viscosity as the molecule under study.

C.3 Flory-Fox Relation

While the Einstein-Simha relation can be used to define the hydrodynamic radius for solid molecules with adsorbed solvation layers, it not simply related to the molecular size of extended molecules such as random coil polymers. Several models have been developed to consider the effect the hydrodynamic drag on the intrinsic viscosity.

One of the most successful models comes from Flory and Fox who modeled the random coil as a series of "beads on a string" or a "jointed chain". The string is flexible, but beads are rigid. Flory and Fox considered that hydrodynamic friction causes the solvent near the center of the molecule to move with the same velocity as the center of mass, but solvent near the edges is free to flow into and out of the molecule. This led them to a relationship between the intrinsic viscosity and the mean square radius of the polymer chain in a theta solvent. Their model is:

$$[\eta] = \Phi \langle r^2 \rangle^{3/2} / \mathrm{M} \qquad \textit{(Equation 15)}$$

where $\langle r^2 \rangle$ is the mean squared end-to-end distance of the chain, and Φ is a universal constant having the value 2.87×10^{23}. In practice, this constant varies somewhat from polymer to polymer with an experimental value closer to 2.5×10^{23}.

The Flory-Fox relationship is valid for polymers in theta solvents. Ptitsyn and Eizner considered the modification required to model other solvents. They found the following relationship:

$$[\eta] = \Phi(\varepsilon) \langle r^2 \rangle^{3/2} / \mathrm{M} \qquad \textit{(Equation 16)}$$

$$\Phi(\varepsilon) = \Phi_0 (1 - 2.63\,\varepsilon + 2.86\,\varepsilon^2) \qquad \textit{(Equation 17)}$$

where now Φ is a function of the polymer-solvent iteration parameter ε, and Φ_0 is the Flory-Fox constant. When ε=0, it reduces to the theta solvent result.

The ε parameter is experimentally measurable with a Mark-Houwink analysis. To perform a Mark-Houwink analysis, the data for a random coil polymer is fit to:

$$[\eta] = KM^a \qquad \textit{(Equation 18)}$$

where M is the molar mass. The K and a are fit parameters, which depend upon the polymer, solvent, and temperature. Traditionally, this data is also plotted as Log[η] vs. Log[a]. If the data is fit well, this should be a straight line. The slope parameter a is related to ε by:

$$\varepsilon = (2a - 1)/3 \qquad \textit{(Equation 19)}$$

D ViscoStar Specifications

This appendix provides specification details for the ViscoStar instrument.

CONTENTS **PAGE**

D.1 Specifications

Table D-1: Sizes and Temperatures

Maximum Flow Rate:	1-3 ml/min (solvent dependent)
Holdup Volumes:	15 ml, 8 ml, 4 ml (user selectable)
Capillary Dimensions:	0.01" ID x 26"L
Bridge Capillary Volume:	80 µL
Operating Temperature Range:	4-60 °C
Ambient Temperature Range:	0-40 °C
Temperature Stability:	< 0.01 °C

Table D-2: Measurement Specifications

Measurement Precision:	greater than 18 bits
Linearity:	0.5% of full scale
Integration Time:	0.1 sec to infinity, increments of 0.1 sec
Sample Shear Rate:	3000 Hz

Table D-3: Pressure Specifications

Maximum Absolute Bridge Pressure:	0.69 MPa (100 psi)
Damage Causing Bridge Pressure:	220 kPa (31.9 psi)
Maximum Differential Pressure:	5 kPa (0.73 psi)
Damage Causing Differential Pressure:	10 kPa (1.46 psi)
Maximum Differential Pressure Noise:	< 0.15 Pa (2.25 E-5 psi)
Maximum Differential Pressure Drift:	< 2.5 Pa/hour (3.6 E-4 psi/hr)
Maximum System Backpressure:	0.69 MPa (100 psi)

Table D-4: inputs and Outputs

Power Input:	90-250 VAC 50-60Hz (universal)
Input Signals:	Autoinject contact closure input with retransmit. External recirculation valve option for software control of solvent recirculation.
Output Signals:	2 analog: absolute pressure and differential pressure
Computer Communication:	USB and Ethernet
User Controls:	LCD front panel control including button array for menu navigation
Protection:	Transducer Protection System (protects from over pressure)
Alarms:	Vapor alarm for volatile organic solvents. Leak alarm. External alarm input (remote instrument can signal alarm).

Index