V. TROUBLESHOOTING AND BASIC MAINTENANCE

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Troubleshooting

General

A large percentage of problems with any HPLC pumping system arise from one of two causes: dissolved gas in the mobile phase, or clogging of the filters with impurities. Both causes are preventable, and this prevention can go a long way toward providing reliable, trouble-free system operation.

Always degas the mobile phase thoroughly. A widely used method for degassing the mobile phase is by bubbling a stream of helium through the eluant. The solvent reservoir provided with the µPro use a bottle cap assembly designed for helium degassing.

Clean or replace the inlet filters and/or solvents regularly. This is especially important if you work with aqueous buffers, in which baterial growth can clog inlet filters within a matter of days. If your µPro pump is fitted with a dynamic mixing chamber, the mixer contains a filter, which can become clogged over time. Monitoring your system pressure will provide you with a good diagnostic tool for determining if the filter in the mixer needs replacement.

Pumping at low flow rates has some added special challenges. Temperature control of the syringes is critically important and if your µPro is fitted with a heater, it should be turned on. If your system is fitted with mechanical valves, you may find the inherent leak rate of the valves results in imprecise flow (for superior precision at low flow rates, we recommend high pressure active valves).

Baseline Problems

Baseline problems can be caused by virtually any component in the HPLC system. For purposes of diagnosis and troubleshooting we can categorize baseline problems as: Spikes, which are high frequency excursions (typically a second or less) from the baseline. Noise, which are intermediate frequency excursions (typically a few seconds to a few minutes) from the baseline, which can often be mistaken for small peaks in the sample. Drift, which is long term (minutes to hours) changes in the baseline level.

Regardless of the nature of the noise problem, the first test is simply to shut the pump off (or set the flow to zero), then let the system equilibrate for a few minutes. If the problem persists, then it is almost surely not related to the pumping system.

If the problem disappears, then pump problems are still a possibility (although not the only one). The nature of the noise will guide us in searching for a possible cause.

Spikes

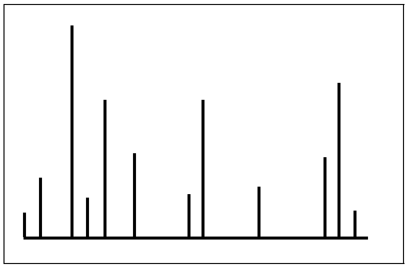

Figure 5.1: Spikes

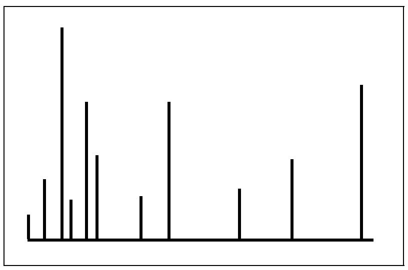

Figure 5.2: Decreasing frequency of spikes suggest that trapped air is being

Carefully examine the outlet tubing of your detector for any sign of small bubbles emerging from the detector. These are an indication of air in the mobile phase (the spikes are generated as the air bubbles pass rapidly through the detector). The question that needs to be answered is "where is the air coming from?".

If you have recently injected air into the system (from an empty loop for example), you may have to equilibrate the column long enough to re-dissolve the trapped air. If you have recently changed the column or guard column you may have to equilibrate the column long enough to re-dissolve the trapped air.

Some of the fluid transfer lines are clear plastic and careful examination may lead to a clue as to where the air is coming from. Proper tightening of the fittings is important in preventing air from being introduced into the system.

Noise

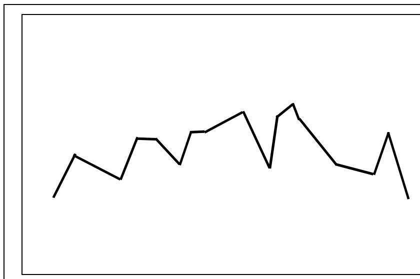

Figure 5.3: Noise

The most likely cause of pump-related noise is variation in output flow rate. Generally, flow variations of sufficient magnitude to cause baseline noise will be accompanied by fluctuations in system pressure. Carefully watch the pressure readout on the LCD display. Note whether the baseline excursions are correlated with pressure fluctuations. If they are not, then the most likely cause of the problem is contaminants in the solvent eluting from the column.

If the baseline noise is accompanied by pressure fluctuations, the nature of the noise and the fluctuations can provide important diagnostic information. Random noise, correlated with changes in pressure suggest air bubbles lodging in the check valves (if your µPro is fitted with mechanical valves). If re-priming the pump does not cure the problem, the culprits may be dirty, worn, or damaged check valves (again, if your µPro is fitted with mechanical valves). To ascertain if this is the case, replace the check valves with known good check valves. If replacing a check valve cures the noise problem, you may try cleaning the old check valves and re-installing them.

Drift

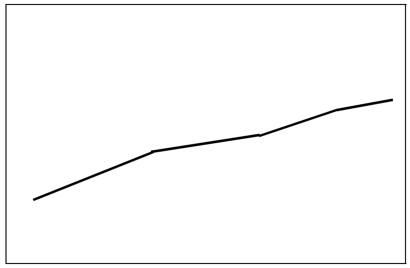

Figure 5.4: Drift

Drift problems are rarely the result of pumping system malfunction. The most common causes of drift, especially in gradient operation, are the use of solvents which do not provide equal detector response, contamination in one or more of the solvents, or temperature fluctuations.

When drift problems are pump-related, they will be accompanied by long-term changes in pump output. Monitor the solvent flow at the detector outlet. Long-term changes in solvent flow suggest losses due to leakage in the system.

Check all connections and fittings for evidence of leaks. If any leakage is found, correct by gentle tightening or replacement of the fitting, as required.

If no evidence of leakage at system connections is found, you may have a piston seal leak and need to replace the piston seal.

Retention problems

Retention problems, like noise and drift, are more often due to column/eluant/sample chemistry problems than to pumping system malfunctions. In those cases where the problems are pumping system-related, the root problem is either variations in output flow or variations in eluant composition.

Isocratic Systems

Variations in output flow are associated with variations in system back pressure. Monitor the system pressure over several runs while periodically checking the solvent flow rate at the detector outlet. If variations in pressure and/or flow are found, diagnosis and troubleshooting of the problem is similar to that described for noise problems. If a variation in pressure and/or flow occurs at the same point in time after refilling the syringe(s), a likely cause is a scratch in the syringe cylinder.

If the output flow is constant, then retention problems may be cause by variations in eluant composition. This is readily diagnosed by connecting a solvent bulk-property detector (refractive index detector, or, for aqueous systems, a conductivity detector) in series with the system. If the baseline of this detector remains flat, the problem may be assumed to be in some other component (column contamination, temperature control, etc.).

Although mixer volume is not usually critical in isocratic separations, it may be a limiting factor in cases where solvents differ greatly in viscosity (isopropanol/water, for example). There are several different mixer sizes available for the µPro and trying another size may yield better results.

Gradient Systems

The major cause of retention time irregularities in gradient elution is variation in system equilibration prior to injection. In many cases, a “dummy” gradient (no sample) should be run prior to the first sample to ensure that each injection is made into a system that has been equilibrated in the same way for the same time.

Variations in system pressure are of limited diagnostic help in gradient chromatography because of the large changes in viscosity (and hence, system back-pressure) that can occur as solvents such as methanol and water are blended (the viscosity of a mixture can actually be higher than the viscosity of either of the pure solvents).

Basic Maintenance

General

The piston seal and the dynamic mixer (if your µPro pump system is fitted with a mixer) are the only components which are likely to need periodic attention. If your µPro pump is fitted with mechanical valves, these may also require some attention. Maintenance of these items is simple.

Piston Seal Replacement

To replace the piston seals:

1. Remove the retaining ring from the piston head by using a small screwdriver to pry the ring open.
2. Remove the seal retainer with your fingers.
3. Remove the primary piston seal with your fingers.
4. Place the new primary piston seal on the small shaft of the piston head (with spring side of seal facing toward the seal retainer).
5. Place seal retainer over the primary piston seal.
6. Re-install retaining ring, using a small screwdriver to pry the ring open and snap it around the piston head shaft.
7. Remove the piston head assembly from the piston shaft. The piston head assembly is unscrewed from the piston shaft by using a 1/16” dowel through the hole in the piston head assembly and stabilizing the piston shaft with a 1/16” dowel (for the 2mL syringe) or a 5/16” wrench (for the 10mL syringe).
8. Remove the secondary piston seal with your fingers from the piston head assembly.
9. Install a new secondary piston seal, with the spring side of seal facing toward the piston shaft.
10. Reverse step 7 to re-install the piston head assembly on the piston shaft.
11. Screw the piston shaft back into the syringe assembly.
12. Reinstall the syringe cylinder and syringe spacer.

Mixer Filter Replacement

Figure 5.6 shows an exploded view of the mixer assembly.

To replace the filer you will remove the cartridge holder, remove the internal components and replace the filter.

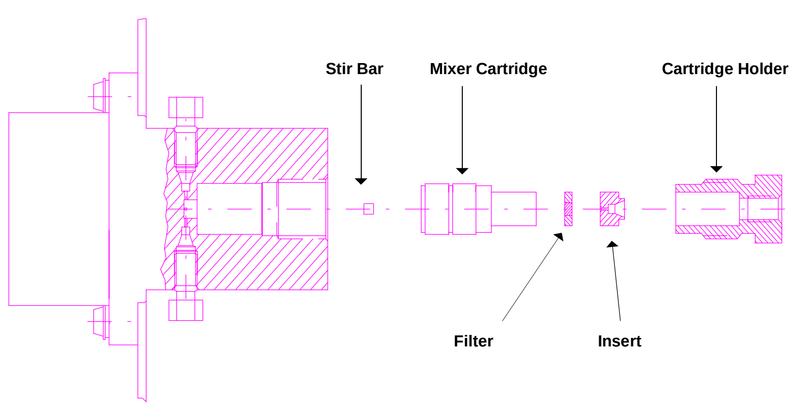

Figure 5.6: Mixer Assembly

1. Using a 1/2” wrench, turn the hexagonal portion of the cartridge holder in a counter-clockwise direction. Usually the cartridge and stir bar will remain in the mixer body. The

stir bar should be removed when installing the 5µL mixer If they do not, re-insert the stir bar in the small cavity in the mixer body. The cartridge can be installed in the cartridge holder once the filter has been replaced and the whole assembly reinstalled in the mixer body.

2. Remove the insert and filter from the cartridge holder (a 1/16” dowel may be needed for this step).
3. Reinstall the insert and new filter into the cartridge holder.
4. Reinstall the cartridge holder into the mixer body, tighten to finger tight and then using a 1/2” wrench to tighten an additional 1/4 turn (tighten to 70 inch pounds).
5. Reinstall the cartridge holder into the mixer

Mechanical Valve Repair

On occasion the mechanical valves (if your µPro is fitted with mechanical valves) may require attention. Small amounts of particulate matter may clog the valve, preventing their proper operation. Additionally, excessive force used in tightening the valves to the head or fittings to the valves may fracture the sapphire seats, requiring replacement.

Removal of Existing Valves

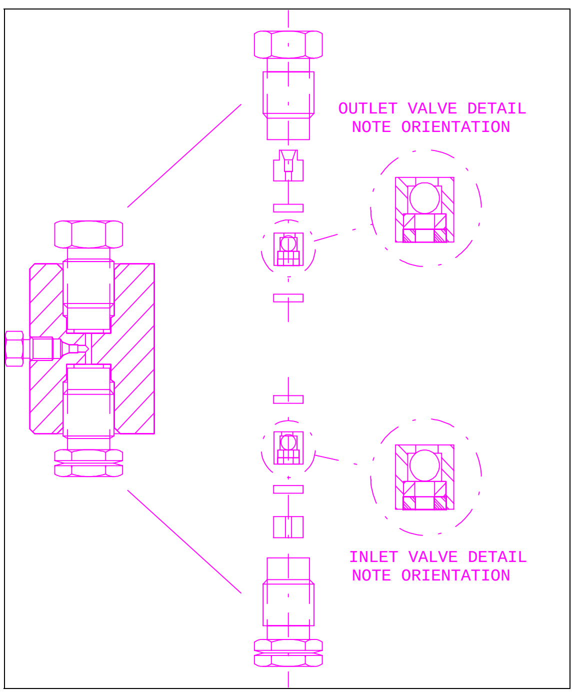

Figure 5.7: Mechanical Valves

The valves are the two hexagon-shaped components on the round head (see Figure 5.7). Inlet valves can be distinguished from outlet valves by the groove on the hexagonal part of the valve. Remove the valves with a 1/2” wrench, turning in a counter-clockwise direction.

Installation of New Valves

1. Inlet valves can be distinguished from outlet valves by the groove on the hexagonal part of the valve.
2. Unscrew the shipping nut and remove the metal shipping washer, as these are not used for installation of the valves.
3. Insert the new valve into the round head and tighten by hand until just finger-tight. Then use a 1/2” wrench and tighten 1/4 turn more. You should tighten valves to the point at which no further leakage occurs, or not more than 4.5 ft./lbs.

OVER TIGHTENING THE VALVES CAN CAUSE THE SAPPHIRE SEATS TO CRACK.

STABILIZE THE VALVE WITH A 1/2” WRENCH WHEN ATTACHING YOUR FITTINGS TO PREVENT FURTHER VALVE TIGHTENING. DO NOT TIGHTEN FITTINGS TO BEYOND 40 IN./LBS., OR THE POINT WHERE NO FURTHER LEAKAGE OCCURS.

Replacing Valve Cartridges

1. Remove the valve from the round head.
2. Using the smaller dowel pin provided (1-1/4” long by 1/8” diameter), press out the internal components of the valve assembly using a steady pressure. Do not hammer parts through

with the dowel pin or hammer on the dowel pin. Do not allow the valve parts to fall out of the valve onto a hard surface.

3. Reassemble the valve by placing the valve insert in the valve housing using the larger (3/16") dowel pin provided. Make sure it is oriented correctly (see Figure 5.7). Press a new Kel-F seal into place. Slide the valve cartridge into the valve housing making certain it is oriented correctly. Press another new Kel-F seal into place. The Kel-F seal will extend approximately 0.020-0.030" from the valve housing.

4. Reinstall the rebuilt valve into the round head and tighten by hand until just finger-tight. Then use a 1/2" wrench and tighten 1/4 turn more. You should tighten valves to the point at which no further leakage occurs, or not more than 4.5 ft./lbs.