
6. CONNECTING COLUMNS

6.1. Purging the System

Before connecting columns it is necessary to purge the system of air and pre-equilibrate the fluid lines with the desired solvent.

1. Perform installation and connect Column Jumpers as described in Section 2.
2. Power up the system as described in Section 4.
3. Using appropriate solvent in the reservoirs, run a script (see Section 5) that uses all the valves and columns that will be used during the intended application. Plug any unused inlet and outlet lines using ¼-28 flat-bottom plugs. To purge all valves, valve block channels, inlet and outlet lines, connect all 4 inlet lines to their respective pumps and run the Step-PURGE script that is available in the Scripts folder on the desktop.
4. Run the script until no air bubbles are observed in any inlet line, outlet line, or column jumper.
5. Stop the script and close all valves by clicking the appropriate button in the Current Valve State window. Either connect the columns or shut down the system as described in Section 4.

6.2. Column and Back Pressure Considerations

An important consideration for the type of column to be used with the Semba Octave System is the expected back pressure that will be generated during operation. **The total back pressure limit for the instrument is 270 psi (assuming the Valve Pressure is set to this pressure); if the fluid pressure exceeds this value the valves will not function properly and separation will not be achieved.** Furthermore, the valve blocks could become contaminated and potentially damaged by the solvent/solutes.

Note that back pressures are additive. During typical applications no more than 3 columns are in any one zone, so in general if the back pressure generated by a single column with a given solvent and flow rate is less than 90 psi, it should be compatible with the system.

Back pressure on any of the pumps can be observed by pressing the Mode button on the pump keypad until the psi LED is lit. The pump then reads the back pressure on the display.

Most commonly, one or both of the Extract and Raffinate lines are connected through a detector and/or fraction collector. In Isocratic SMBC mode the Extract lines is commonly connected through pump 3 or 4 to control flow rate. Depending on the back pressure generated by the columns and flow configuration of the system, back pressure regulators (BPRs) may be added downstream to enable more precise flow control. As a rule, the pumps require a pressure differential of 25 -35 psi (inlet vs. outlet) to run accurately. Therefore, if the system generates 100 psi of backpressure at the Extract outlet, an additional 125-140 psi BPR should be inserted downstream from the Extract pump. This back pressure is not included for limit calculations because it is downstream of the pump.

Most small (1 – 5-ml) chromatography cartridges containing agarose or other polymer-based resins generate very low back pressure at the flow rates commonly used for separation (< 2 ml/min). In this case it is advisable to insert a 40 psi back pressure regulator (BPR) in all of the downstream outlet lines. Four 40 psi BPRs are supplied for this purpose. When installing the BPR use the supplied fittings and be sure that the arrow embossed on the unit points toward the outlet flow.

Note that some detectors can generate significant back pressure (40 – 60 psi at 1 – 2 ml/min) due to their small diameter flow cells or connecting tubing; this detector contributed back pressure must be considered when adding or omitting BPRs on the detector-monitored outlet lines.

6.3. Connecting Columns

6.3.1 Column Rack

The Chromatography Module is provided with a Column Rack, which accepts a variety of column shapes and sizes up to ¾" in diameter. To insert a column, pull out the spring-loaded drawer, place the column in the holder, and release the drawer. The column should be held firmly between the wedge-shaped "V" in the rack and the metal lip on the drawer.

6.3.2 Connection to Valve Block Ports using ShapeShifter™ Adapters

ShapeShifter Adapters (see Figure 10) are designed for use with small columns that can be supported via their 10-32 female column inlets. The adapters enable columns to be connected directly to the valve block column ports without the need for a separate inlet line, thus reducing the dead volume of the system.

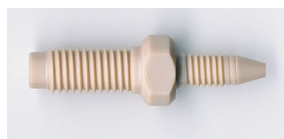


Figure 10. ShapeShifter Adapter

When using the ShapeShifter Adapters, replace the Column Rack with the Cover Plate provided with the system. This is accomplished by removing the 4 screws that hold the rack to the front plate of the Chromatography Module and re-attaching the Cover Plate using the same screws. Use care when removing the Column Rack; slowly pull the unit straight out of the cabinet, avoiding snags on the internal parts.

To connect the adapters, first connect the 10-32 coned end to the column inlet port (or union, if connecting "jumpers"), then use the column/union as a handle to connect the ¼-28 end of the adapter into the appropriate port on the valve block. Be careful not to over-tighten the adapter into the port to avoid damaging the valve block – a leak-free seal is formed with minimal torque applied.

The column outlet lines supplied with the system should be of sufficient length to use with the ShapeShifter Adapters and columns up to about 5 cm in length. See Figure 12 for a photo of the system with columns attached via ShapeShifter Adapters.

6.3.3 Column Connection Procedure

The main purpose of this stepwise column connection procedure is to insure that air is not introduced into the Octave flow channels or columns. Before beginning this procedure, make sure that the correct fittings for the column ends are available. Most column inlets have 10-32 female coned ports. Column outlets vary; in general, HPLC-type columns have 10-32 female coned ports, whereas bioseparation cartridges may have 10-32 male or M6 male connectors. Depending on the type of fitting present on the column, an adapter may be required at either end.

Figure 11 shows a diagram of the column connections for reference. See Figure 12 if using columns connected via ShapeShifter Adapters.

1. Ensure that the column inlet and outlet lines are installed correctly with their adapters as column jumpers (see Figure 4, Section 2.8) and that the system has been purged as in Section 6.1, noting the labels on the valve block face plates and column holders.

2. Power up the system as described in Section 4.
3. Start the SembaPro application and close all the valves (see Section 4).
4. After making sure the Feed pump (Pump 1) is primed and a reservoir of appropriate solvent is in place, adjust the flow rate in the Pump Control window to 1 ml/min (or set a low pump flow rate that will not result in excessive back pressure with the columns being connected).
5. Make sure that the Raffinate outlet line is not plugged or otherwise blocked.
6. Remove the adapter from the column jumper for Column 1, leaving the inlet (or ShapeShifter Adapter) and outlet lines connected to the valve blocks.
7. In the Current Valve State window, Open the Column 1 Feed and Raffinate valves and turn on the Feed pump in the Pump Control window.
8. After a few seconds the stream should emerge from the Column 1 inlet line (or ShapeShifter Adapter). Attach the first column while the pump is running to avoid trapping air bubbles in the column inlet.
9. After a few seconds the stream should emerge from the end of the column. When no more bubbles are seen, connect the outlet line to the outlet port on the column and place the column in the Column Rack (if using it). The Raffinate outlet should flow almost immediately. After ensuring that no bubbles are in the line, turn off the pump and close the Column 1 Feed and Raffinate valves.
10. Repeat this procedure for the other 7 columns, opening the relevant Column Feed and Raffinate valves for the column being connected prior to starting Pump 1.
11. The system is now ready to be run or shut down as described in Section 4.

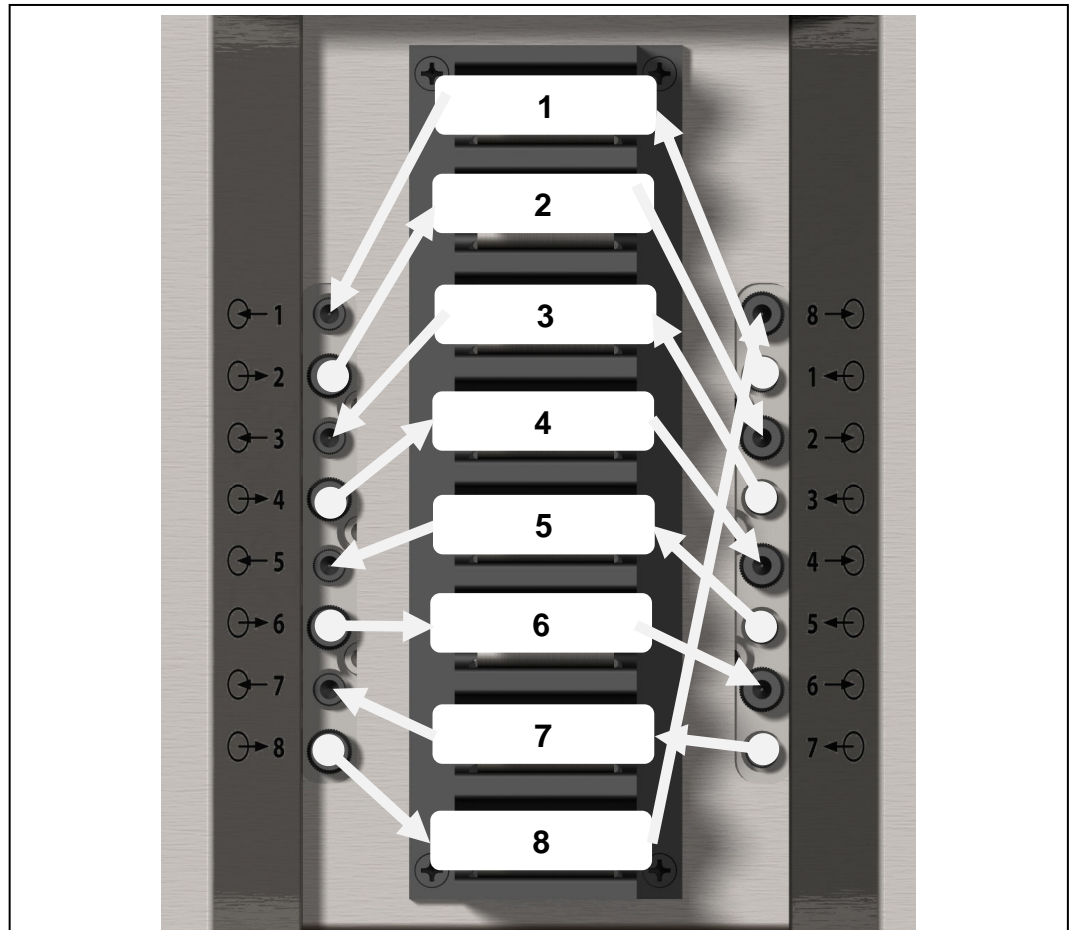


Figure 11. Column connections with Column Rack

Columns 1-8 are shown in position in the Column Rack. Inlet/outlet lines are indicated. Note that the outlet of Column 8 connects to the top port in the right valve block. All inlet lines and all outlet lines should be equivalent length to maintain consistent zone volumes. Lines are shown here as different lengths for clarity.

