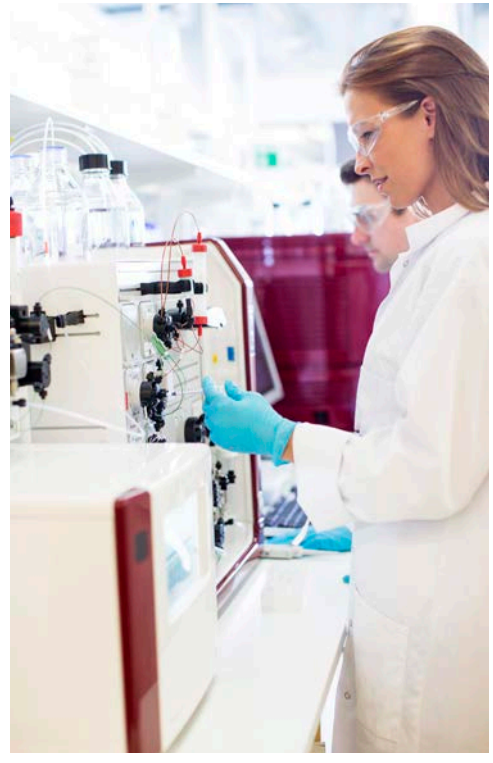
cytiva

Good **ÄKTA** system practice

Cue Cards

1. Good ÄKTA system practice

- Keep the system and Fraction collector clean, both on the outside and the inside.
- Use inlet filters and filtered buffers, preferably degassed buffers.
- For optimal results, use buffers, system and columns at ambient temperature during a purification run.
- Centrifuge or filter the samples prior sample loading, unless columns that can handle crude samples are used.
- Use the Comprehensive System Wash and cleaning regularly for optimal performance of the whole instrument, including Fraction collector. See page 5.
- To prevent cross-contamination and bacterial growth in the instrument, perform a System cleaning in place (System CIP) after each run. See page 5.
- Limit system exposure to harsh cleaning solutions to maximum 2 hours.
- Fill the system with 20% ethanol to minimize bacterial growth prior storage.
- Set up UNICORN™ system notifications to let the system remind you when your action is needed. For example, let a message appear once a week stating: “Time to change pump rinsing solution”. See page 6.



For further details see *ÄKTA™ avant User Manual and ÄKTA pure User Manual.*

2. Run-Essentials

Before

1. Check the 20% ethanol pump rinsing solution. Change if it appears opaque or if the solvent level in the container has decreased.
2. Check all tubing to make sure no tubing is nicked.
3. Make sure all buffers have the same temperature as the system to minimize air bubble issues.
4. Prime inlets, i.e. fill all used buffer inlets with liquid.
5. Purge pumps to remove air from the pump heads.
6. Start a flow and tighten connectors if leakage is observed.
7. Calibrate the pH electrode (if used).
8. Perform a System Preparation run to fill the entire flowpath with the right buffer into the system (including the Fraction collector).
9. Start a flow to check if signals from monitors look stable and realistic If not, see p. 7-12.
10. Wipe off the Fraction collector sensors (code reader & drop sync sensor) with a tissue and fill the Fraction collector with the tubes/plates needed.
11. Attach needed column(s) and set correct column pressure – if running manually.

During

Watch out for:

- Overpressure, see page 8
- Fluctuating pressure, see page 9
- Fluctuating UV signal, see page 10
- Leakage → tighten connectors
- **Last tube filled** (upon fractionation) → change tubes, see page 4

Sources of sound during operation:

- Air ventilation
- UV monitor – when using 2-3 wavelengths
- Pump movement
- Mixer
- Changing valve position
- Fraction collector movements

After

For short term storage:

1. Empty Fraction collector
2. Perform a Comprehensive System Wash and/or cleaning
3. Clean used columns in order to increase their lifetime and prevent:

- sample contamination
- cross contamination
- protein precipitation
- column clogging

For long term storage:

If the instrument is not going to be used for a couple of days or longer, clean as above and then fill

- instrument
- columns
- pH flow cell with storage solution

For further details see *ÄKTA avant User Manual and ÄKTA pure User Manual*.

3. Fraction collector F9 and F9-C operation

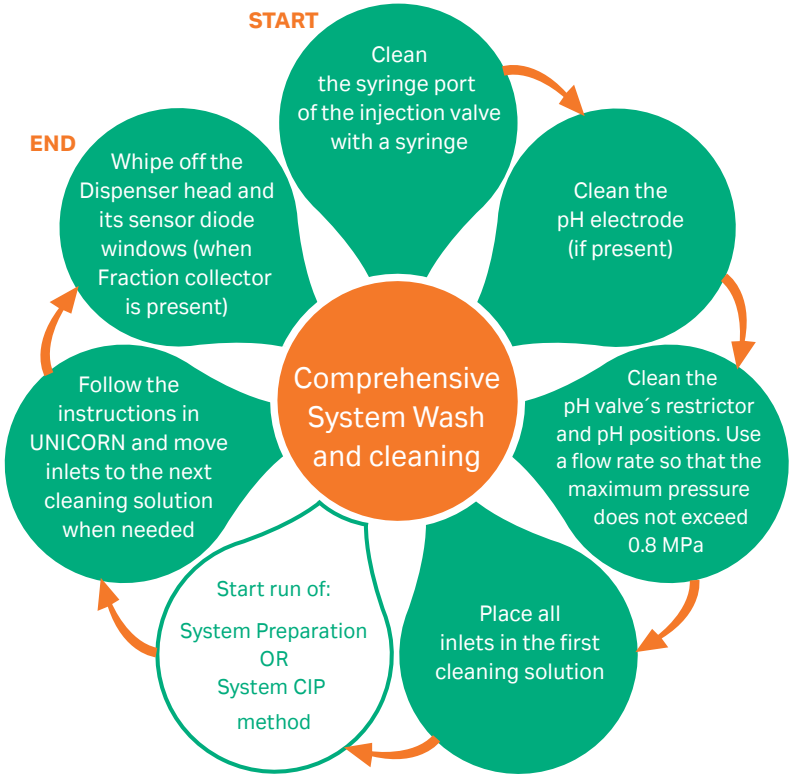
How to...	Steps	Outcome
Set Fraction collector content	1. Make sure there is no run ongoing and the instrument is in state ***READY***. 2. Open and close the Fraction collector door. 3. The Fraction collector performs a **Full scan**. **NOTE:** The Fraction collector content does not reset after the method has ended unless the door has been opened and closed.	The Fraction collector content is updated and all cassettes are ready to be used. To view the content of the Fraction collector, in the ***System control module*** select ***View:Fraction Collector Content***.
Take out fractions during a run or in the middle of scouting or method queue runs	1. Pause the run. 2. Open the Fraction collector door. 3. Remove the desired tubes. 4. Close the the Fraction collector door. 5. The Fraction collector performs a ***Quick scan***. 6. Press ***Continue***. **NOTE**: If deep well plates have been removed these need to be replaced with deep well plates of the same type before continuing the run.	Fractionation continues in the next tube/ well.
Take out cassettes during a run or in the middle of scouting or method queue runs	1. Pause the run. 2. Open the Fraction collector door. 3. Remove the desired cassette(s). 4. Replace any removed cassette(s) with the same type (filled or empty). 5. Close the Fraction collector door. 6. The Fraction collector performs a ***Quick scan***. 7. Press ***Continue***.	Fractionation continues into the next tube/well.
Change tubes/ plates after ***Last tube filled*** *message*	1. Open the Fraction collector door. 2. Remove all cassette(s) that has been fully utilized. 3. Replace any removed cassette(s) with the same type. 4. Close the Fraction collector door. 5. The Fraction collector performs a ***Quick scan***. 6. Press ***Continue***.	Fractionation continues in the first tube/well of the replaced cassette. Fractionation always starts in the lowest cassette position number (1-6).

NOTE: The fraction collector is specially designed to automatically detect the cassettes present in the fraction collector but also to maximize the usage of the tubes/ wells . i.e. every tube in the fraction collector should be available for fractionation. The fraction collector keeps track of which tubes/wells have been used during the runs and it is therefore not possible to manually start fractionation in a specific cassette position or in a specific row. It is however possible to select next tube/well, skip two tubes/wells, next line, or next cassette.

NOTE: There is a possibility to try out other deep-well plates then the ones approved by Cytiva, provided that the deep-well plate specifications are fulfilled. In some cases, the automatic cassette scanning may need to be disabled in ***System Control/System Settings/Fraction collector/Cassette configuration*** to make them work. Make sure to manually update the Fraction collector content in ***System settings*** every time the content is changed. For further details see ÄKTA avant User Manual and ÄKTA pure User Manual ("References" on page 14).

4. Comprehensive System Wash and cleaning

... contains both **manual** (green) and **automated** (white) procedures. It is used to wash/clean the system flowpath, i.e. not any column.



For further details see *ÄKTA avant User Manual and ÄKTA pure User Manual*.

4.1 Create UNICORN method(s)

In all relevant method phases select modules and parts to cover the entire flowpath, for example:

- All A&B inlets
- All sample inlets
- All column positions to be washed

  NOTE: Make sure no columns are attached.
- All outlets (including outlet to Fraction collector)
- Injection valve
- Fraction collector* (if present)

4.2 Method outline forSystem Wash

(Use the predefined method for System Preparation)

Method settings
System Preparation – Water
System Preparation – Buffer

4.3 Method outline forSystem Cleaning

(Use the predefined method for System CIP)

Method settings
System CIP – Water
System CIP – 0.1-0.5 M NaOH
System CIP – Buffer**
System CIP – 20% ethanol

* Wash of Fraction collector accumulator is included.

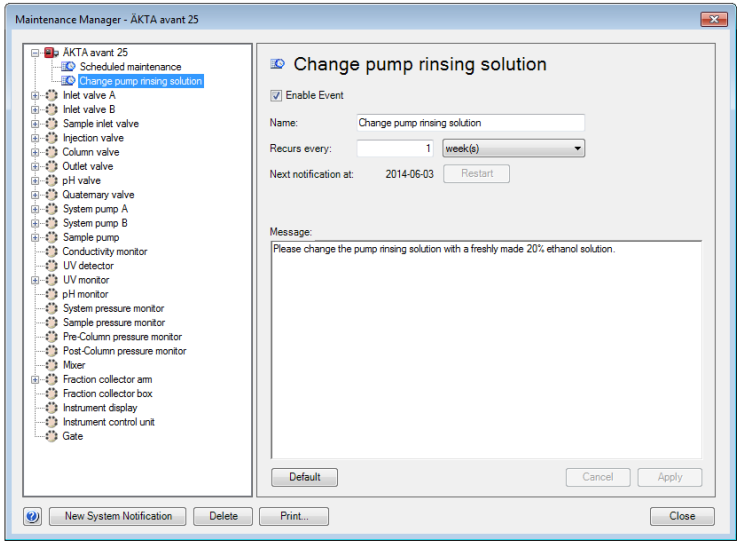
** If water is used instead of buffer, this step will take longer time.

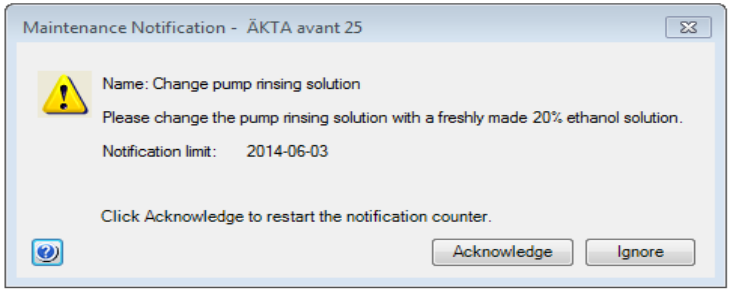
5. UNICORN system notifications

Automated maintenance notifications for the system can remind you when your action is needed. Follow the steps below in order to set a new notification. In this example an automatic notification for changing the pump rinsing solution is set.

Step	Action
1.	In the ***System Control*** module, select ***System:Maintenance Manager*** to open the ***Maintenance Manager*** dialog.
2.	In the ***Maintenance Manager*** dialog, click the ***New System Notification***. *Result*: The ***NewNotification*** field appears.
3.	In the ***NewNotification*** field: a. Enter a name for the notification, e.g. "Change pump rinsing solution." b. Select a time interval after which the notification will be issued, e.g. one week. c. Write a message in the message input field that will be shown for the maintenance notification, e.g. "Please change the pump rinsing solution with a freshly made 20% ethanol solution." d. Click **Apply** to save the changes and apply the notification settings.
4.	Handle the maintenance notification when the set time interval has been reached: • Click ***Acknowledge*** to reset the counter for a new maintenance notification period. • Click ***Ignore*** to close the dialog without action.

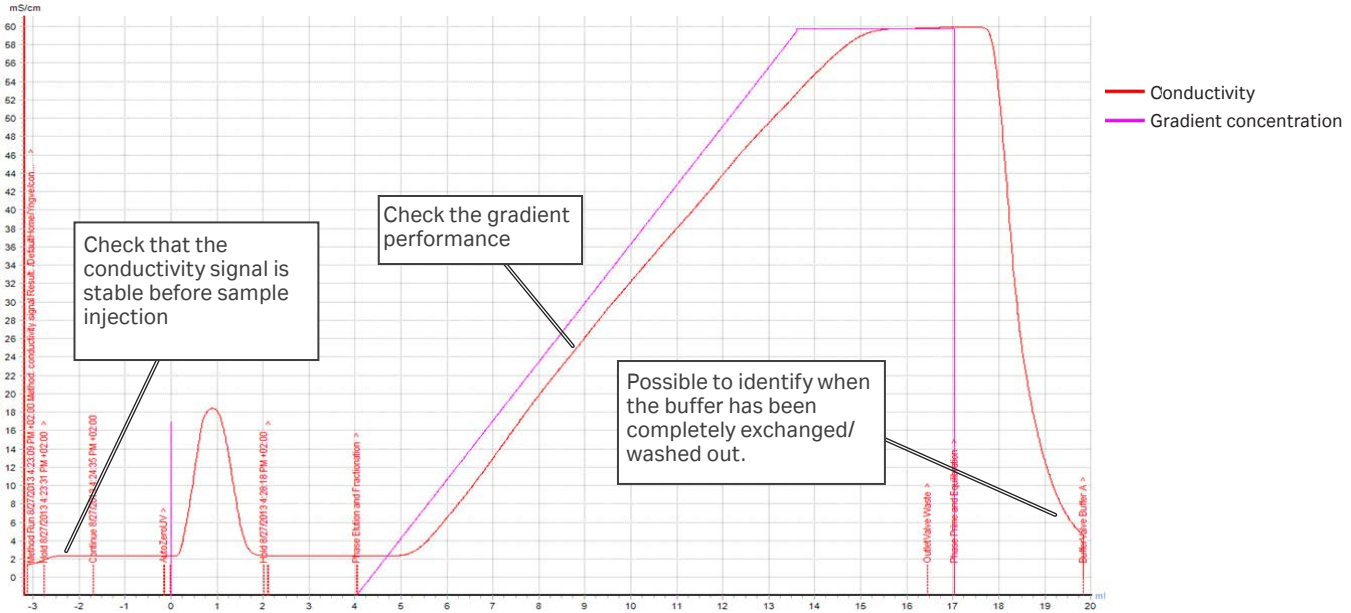
NOTE: The Maintenance Notification will be displayed each time the System Control module is opened until the notification is acknowledged.





6. Troubleshooting

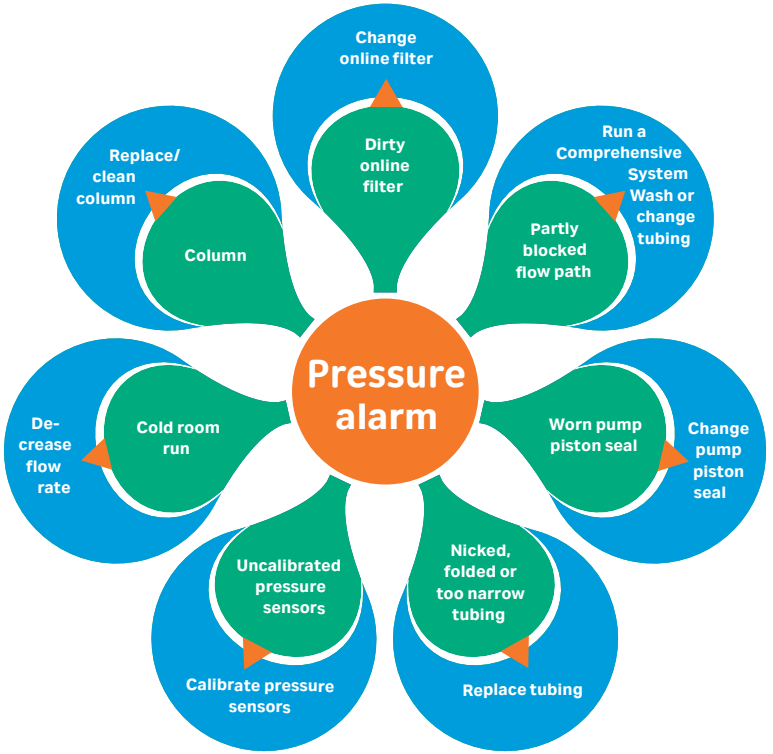
6.1 Conductivity signal – a useful troubleshooting tool



Note: ***The conductivity signal becomes distorted at higher salt concentration since the ion activity decreases when the concentration increases, i.e. a different curvature than the theoretical one is therefore normal at high salt concentrations.***

For further details see *ÄKTA avant User Manual and ÄKTA pure User Manual*.

6.2 Pressure alarms

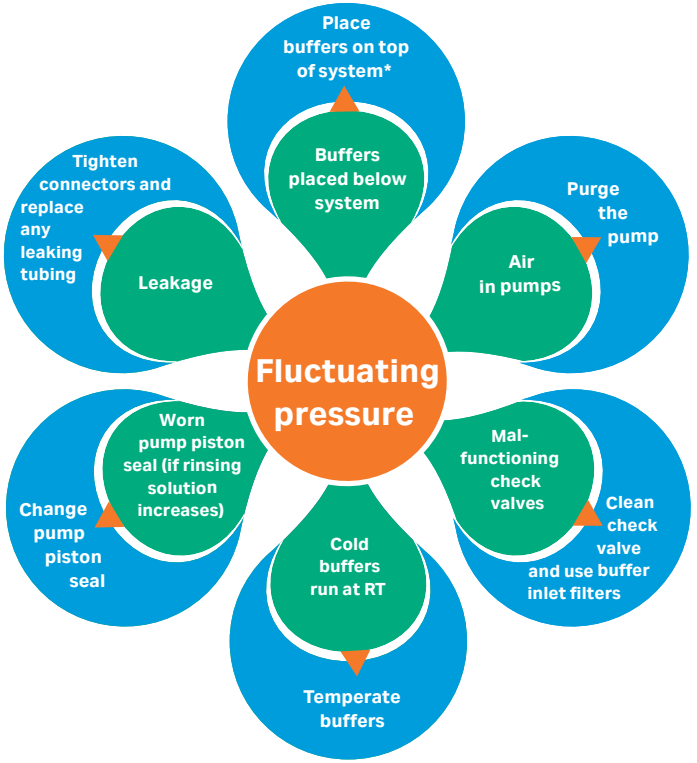


For further details see *ÄKTA avant User Manual and ÄKTA pure User Manual*.

6.3 Backpressure contributions

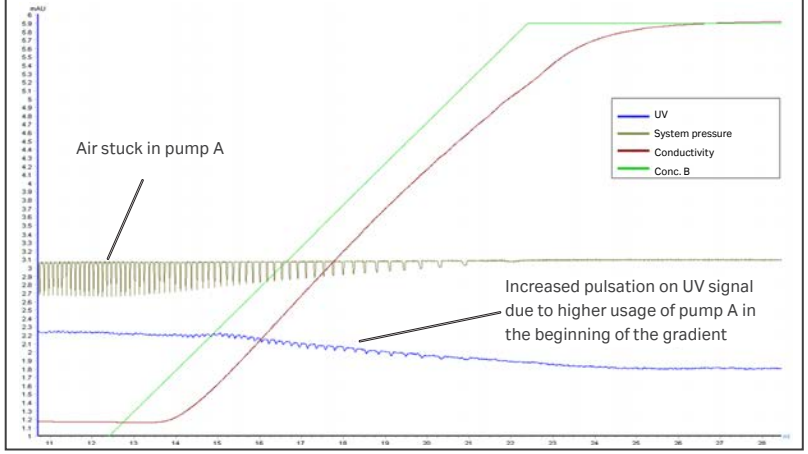
Source	How to minimize the contribution	Note
Tubing	Keep the tubing as short as possible and optimize the i.d	A larger i.d. will decrease the back pressure but will have a negative effect on resolution. See page 13
Inline filter	Change the filter regularly.	The inline filter will prevent particles in the solutions from entering the flow path and column. With time, the filter will start to clog and the pressure will increase.
Buffer/solution	Decrease the flow rate when running high-viscosity buffers/ solutions.	Mixing different liquids, e.g., in a gradient, can increase the viscosity and result in higher back pressure.
Temperature	Decrease the flow rate when running at low temperature.	Viscosity increases at lower temperature.
Sample	Dilute viscous samples or decrease the flow rate during sample application. Remove the sample inlet filter and if using the system pump to apply sample, bypass the mixer.	To avoid over-pressure, ÄKTA pure and ÄKA avant systems have optional pressure-controlled sample application, where the flow rate is decreased as the pressure increases and vice versa.
Column	Clean the column.	See column instructions for cleaning.
	Do not use smaller beads or column diameter than the application requires.	Smaller beads will give higher resolution but also higher back pressure.

6.4 Fluctuating pressure



* For 150 ml/min systems, buffers can also be placed next to the system if preferred.

Air in pump, example

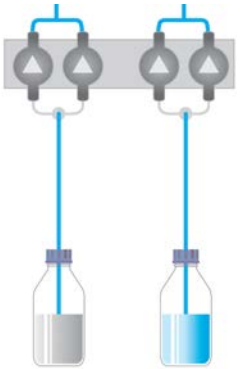


Diagnose malfunctioning check valves

Inlet check valve diagnostic test, "air bubble test":

- Run the pump at 0.5 ml/min.
- Lift the inlet tubing a few seconds to let a small air bubble in.
- Normal function: air bubble moves forward and then stops, and so on, no backward movement.
- Malfunction in inlet check valve: air bubble moves backwards before stopping.

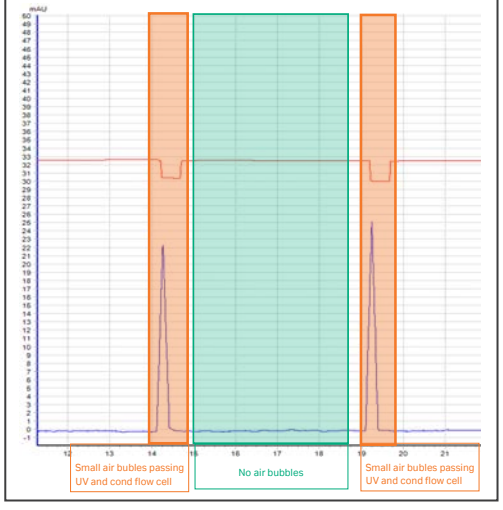
After test: Purge the pump and make sure that the air bubble is not stuck in the pump.



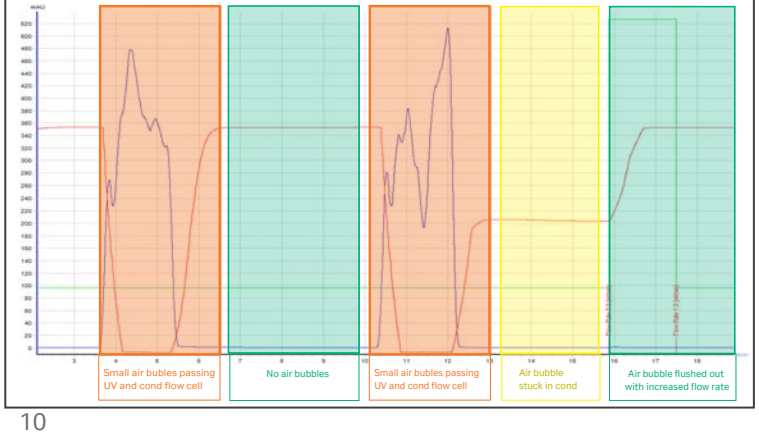
For further details see *ÄKTA avant User Manual and ÄKTA pure User Manual*.

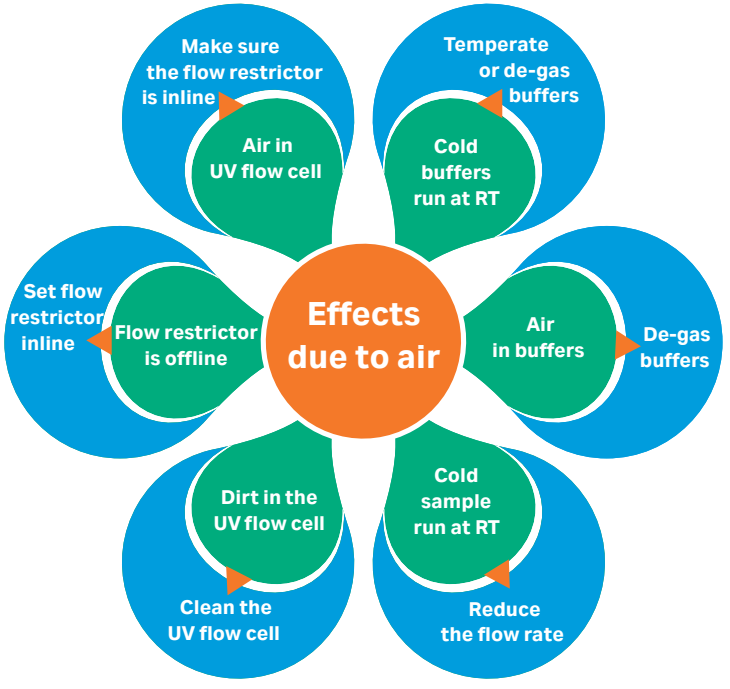
6.5 Fluctuating UV and conductivity signal, examples and solutions

Air spikes in UV signal



Large air segments passing the flow cells



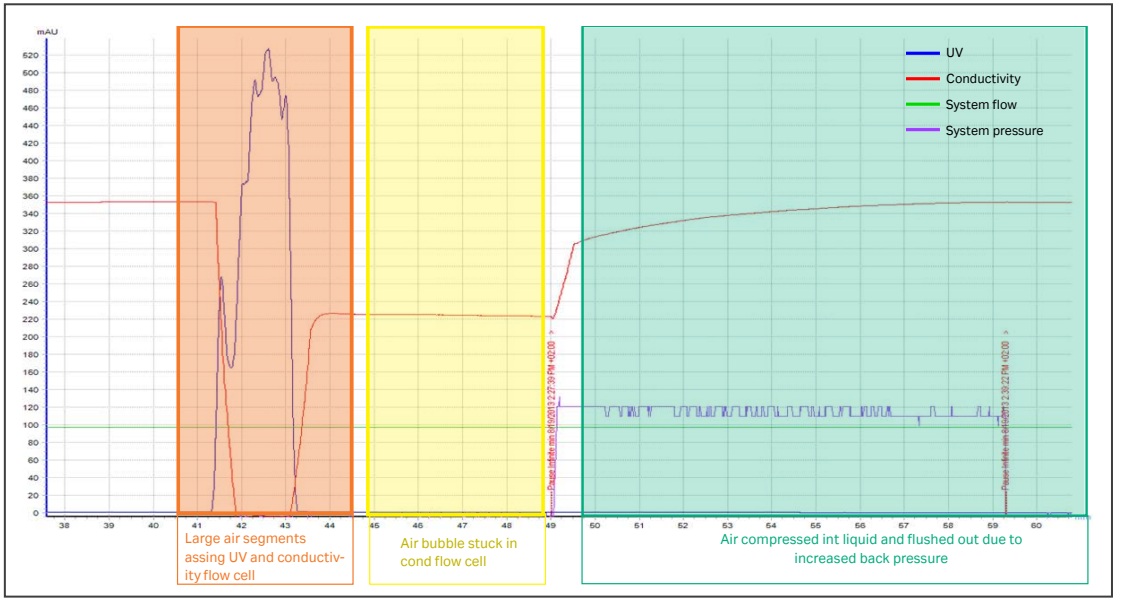


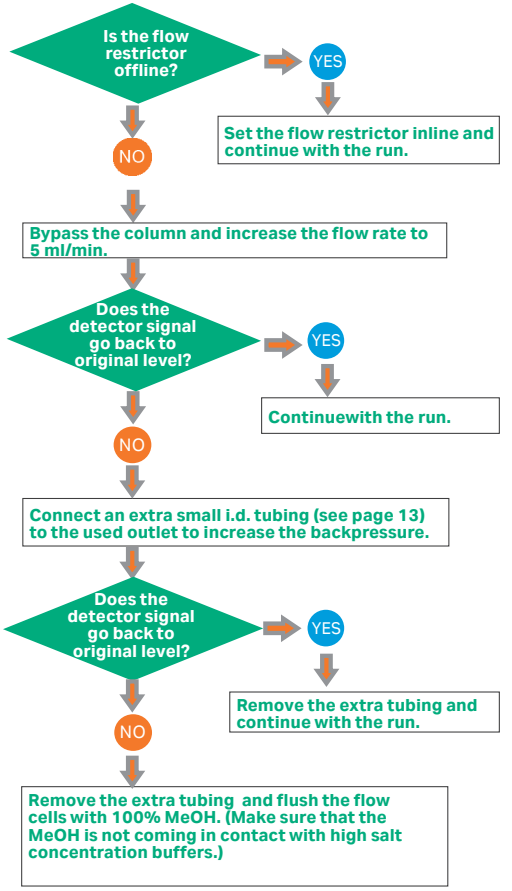
6.6 Cleaning the pH electrode and UV flow cell

Component	Contaminant	Cleaning solution
pH electrode	Salt deposits	0.1 M NaOH and 0.1 M HCl
	Lipid deposits	Detergent or organic solvent
	Protein deposits	1% pepsin in 0.1 M HCl
UV flow cell	Salt/lipid/protein deposits	Detergent, such as Deconex™

For further details see *ÄKTA avant User Manual and ÄKTA pure User Manual*.

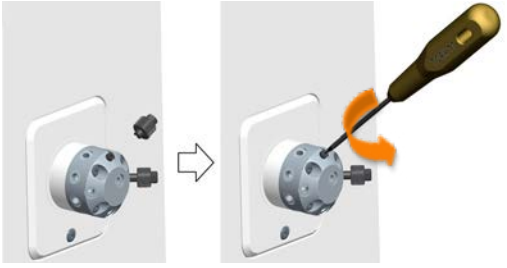
6.7 Air bubble in a flow cell





For further details see *ÄKTA avant User Manual and ÄKTA pure User Manua*l.

6.8 Broken connector tip stuck in valve



Step	Action
1.	Find a Torx™ T7 screwdriver.
2.	Firmly press the tip of the screwdriver into the broken tip.
3.	Unscrew the broken connector tip.

6.9 Tubing guide

- There are many different sizes and types of tubing that can be connected to a chromatography system.
- Tubing with a smaller i.d. holds less delay volume and will therefore generate less dilution of the protein peak. However, narrow tubing increases the system pressure, especially when running at high flow rates.
- The tubing and system used should match the application needs.

i.d.	Color of tubing	10 cm tubing corresponds to	100 cm tubing generates[1]	Standard tubing with/when
0.13 mm	Red	1.3 µl	24 MPa	Generating high back pressure, which is useful e.g. during
0.25 mm	Blue	4.9 µl	1.7 MPa	troubleshooting and when “running in” a new pump piston seal
0.50 mm	Orange	20 µl	0.11 MPa	Running at low flow rate using ÄKTA avant 25 and ÄKTA pure 25
0.75 mm	Green	44 µl	0.02 MPa	ÄKTA avant 25, ÄKTA pure 25 and ÄKTA pure 150
1.0 mm	Beige	78 µl	0.007 MPa	ÄKTA avant 150 and ÄKTA pure 150
1.0 mm	Transparent	78 µl	0.007 MPa	Outlet tubing for ÄKTA avant and ÄKTA pure
1.6 mm	Transparent	200 µl	–[2]	Inlet tubing for ÄKTA avant 25 and ÄKTA pure 25
2.9 mm	Transparent	660 µl	–[2]	Inlet tubing for ÄKTA avant 150 and ÄKTA pure 150

1 For water at 10 mL/min and room temperature
2 Negligible pressure

7. Abbreviations and References

Abbreviations

CIP Cleaning In Place

i.d. inner diameter

RT Room Temperature

References

ÄKTA Laboratory-scale Chromatography Systems Instrument Management Handbook, 29010831

ÄKTA pure User Manual, 29119969

ÄKTA avant User Manual, 29035184

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