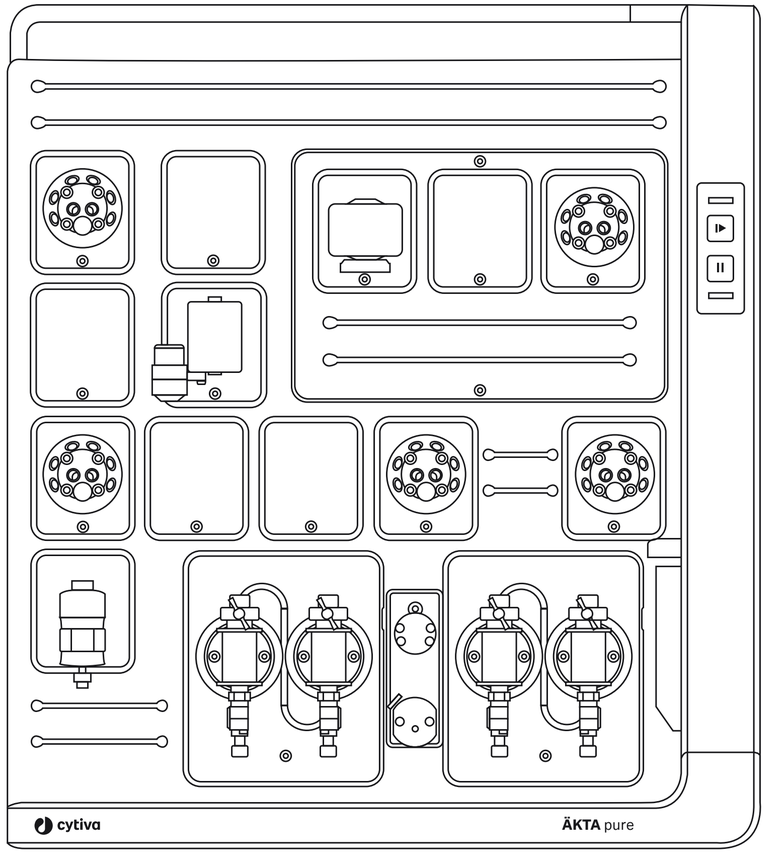
Instrument management handbook

ÄKTA Laboratory-scale Chromatography Systems



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01

Introduction

Introduction

This handbook, *ÄKTA™ Laboratory-scale Chromatography Systems*, is focused on liquid chromatography systems used for protein purification at research laboratory scale. Beginners can use the handbook to obtain an overview of how purification systems work and to learn about important considerations for achieving successful results. Experienced system users will also find valuable and detailed information on different hardware modules.

A chromatography system should be used when reproducible results are important and when manual purification becomes too time-consuming and inefficient. Systems provide more control than manual purification because of the ability to automatically control the flow rate and monitor the progress of the purification as well as to make controlled gradients and automatically collect fractions. Systems can perform automatic, simple, step-gradient elution as well as high-resolution separations using accurately controlled linear-gradient elution.

This handbook addresses different aspects of ÄKTA chromatography systems, such as the effect of system peak broadening on resolution, choosing sample injection technique, and selecting an appropriate mixer. It also gives straightforward advice on how to avoid problems such as air bubbles in the pump, how to troubleshoot problems such as high back pressure, and how to perform cleaning of system components.

The appendices include a general introduction to the different ÄKTA laboratory-scale systems and columns as well as information on how to determine exact delay volumes for a specific system.

Symbols

This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations

This symbol indicates where special care should be taken

Common acronyms and abbreviations

A_{280}	Absorbance of light at specified wavelength, in this example, 280 nanometers
AC	Affinity chromatography
CF	Chromatofocusing
CIP	Cleaning-in-place
CV	Column volume
DS	Desalting (group separation by gel filtration; buffer exchange)
FPLC	Fast protein liquid chromatography
GF	Gel filtration (sometimes referred to as SEC; size exclusion chromatography)
HIC	Hydrophobic interaction chromatography
i.d.	Inner diameter
IMAC	Immobilized metal affinity chromatography
IEX	Ion exchange chromatography (also seen as IEC in the literature)
mAU	Milli absorbance unit
MPa	MegaPascal; unit of pressure
mPas	Unit for viscosity (1 mPas = 1 cP, i.e., 1 centiPoise)
o.d.	Outer diameter
PM	Preventive maintenance
RPC	Reversed phase chromatography
R_s	Resolution, the degree of separation between peaks
s	Second(s)
SEC	Size exclusion chromatography (same as gel filtration)
UV/Vis	Ultraviolet/visible light

Chromatography terminology

Back pressure

The pressure caused by column or system components in the system flow path.

Chromatogram

A graphical presentation of detector response(s).

Chromatography

From Greek *chroma*, color, and *graphein*, to write.

Chromatography medium/media

The stationary phase, also called resin. The chromatography medium is often composed of a porous matrix (base matrix). The matrix is usually functionalized by coupling it with ligands that can bind molecules to be separated.

CIP (cleaning-in-place)

Common term for cleaning chromatography columns and/or systems with the purpose of removing unwanted/nonspecifically bound material.

Column

Usually column hardware packed with chromatography medium.

Column hardware

The column tube and adapters. All pieces of the column except the chromatography medium/the packed bed.

Column hardware pressure

The pressure inside the column during chromatography. Column hardware pressure that is too high can break the column.

Degassing

Removal of dissolved air from buffers/solutions.

Delay volume

The volume corresponding to a part of the system. Fractionation delay volume is the volume of tubing and system components between a monitor and the fraction collector. Gradient delay volume (also called dwell volume) relates to the volume between the point where two solutions are mixed and the column.

Efficiency

Measured as number of theoretical plates. High efficiency means that sharp peaks will be obtained.

Flow rate

Flow through a column and/or chromatography system. Expressed in ml/min.

Flow velocity

Flow rate divided by the cross-sectional area of a column. Expressed in cm/h.

Inline

A component that is part of the flow path.

Medium/media

Same as chromatography medium/media.

Peak broadening

The widening of a zone of solute (e.g., a protein) when passing through a column or a chromatography system. Gives rise to dilution of the solute and reduces resolution. Also termed band broadening or zone broadening.

Pressure over the packed bed

The pressure drop across the packed bed upon passage of solution through the column. Caused by flow resistance in the packed bed.

Resolution

Measurement of the ability of a packed column to separate two solutes (peaks).

Selectivity

Measure of the relative retention of two solutes in a column. Related to the distance between two peaks.

System volume

The total volume of all tubing and system components outside the packed chromatography bed. (Sometimes referred to as system dead volume.)

02

Liquid chromatography systems and important considerations

Liquid chromatography systems and important considerations

A number of benefits can be derived from using an automated protein purification system. Such a system:

- Ensures more controlled conditions and reproducible results
- Purifies proteins automatically without the need for user interactions during the run
- Allows sensitive samples to be purified more efficiently
- Allows use of high-resolution media
- Provides inline detection that helps in making decisions, for example, when the column has become equilibrated, when to collect fractions, etc.
- Allows automated collection of purified protein in small or large volumes
- Uses software that makes it easy to create methods, monitor runs, and evaluate results

Protein separation takes place in a column. Buffers and other liquids are delivered via the system pump, and sample can be applied in different ways (e.g., using a syringe to fill a sample loop or by using a sample pump). Detectors (e.g., UV/Vis absorbance, conductivity, pH) are placed after the column to monitor the separation process. The purified proteins are collected in the fraction collector. Figure 2.1 shows a typical system's flow path.

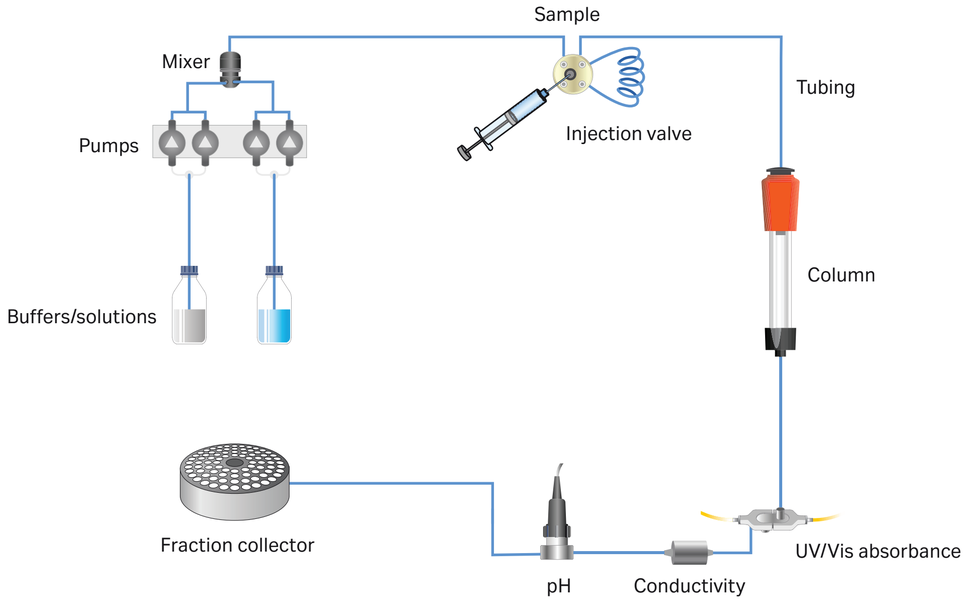


Fig 2.1. Typical flow path for a chromatography system.

Overview of chapters

This section provides a short description of Chapters 3 to 10.

Peak broadening and resolution

To obtain a pure product it is important to optimize the system's flow path, assuring that it matches the column performance. A poor match where system volume is too large can result in diluted peaks with decreased resolution and less pure protein (Fig 2.2). Tubing that is too narrow might result in back pressure that is too high for the column hardware. Learn more about how to avoid these problems in Chapter 3, *System volume effects on resolution and fraction collection.*

Sample loading

The sample is typically applied to the column using either a prefilled sample loop or a pump. Learn more about the different techniques and when to use them in Chapter 4, *How to choose sample injection technique*.

Liquid delivery

The performance of the pump is important for ensuring reliable and reproducible results. One common cause of unsuccessful chromatography is air bubbles in the pump. This can cause pulsations in the flow delivery, resulting in an inaccurate flow rate. This effect can be observed as disturbances in the pressure curve. Learn how to condition the pump in a proper manner, as described in Chapter 5, *Liquid delivery and pumps*. See also Figure 2.3.

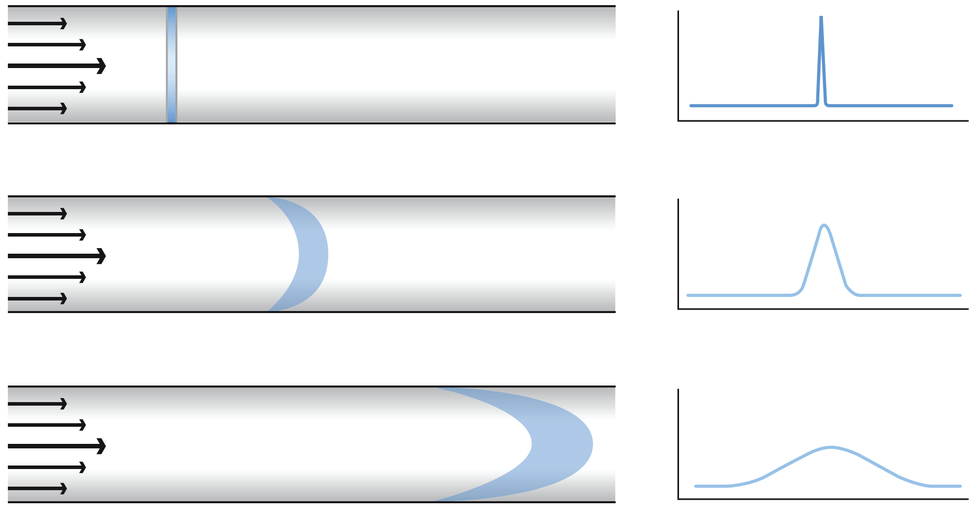


Fig 2.2. Peak broadening in tubing. Liquid flows faster in the middle of a tube as compared with closer to the walls. The farther a protein peak passes through a tube, the broader it becomes, as depicted in the chromatograms shown on the right.

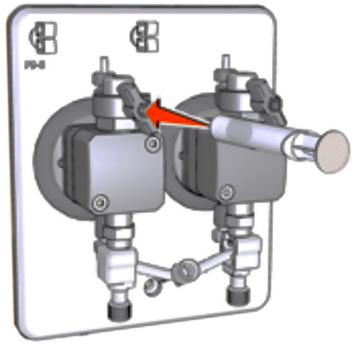


Fig 2.3. Remove air bubbles by using a syringe to draw liquid from the purge valves. The figure shows a pump head from ÄKTA avant 25.

Buffer mixing

The mixer has two important functions in the system. The first is to provide a homogeneous mix during gradient formation where two or more liquids are used to create the gradient. The second is to even out pulsation effects from the pump. The mixer that is delivered with the system will cover most of the applications within the flow rate range of the system, but in some cases it might be necessary to switch to a mixer of a different volume to obtain optimal results.
See Chapter 6, *Gradient formation and mixers*, for more information on this topic. See also Figure 2.4.

Pressure

High-performance media used to achieve high resolution require a high-performance pump that can operate under high pressure. Generally, such pumps can generate a higher pressure than the column hardware and media can withstand. It is therefore important to monitor the system pressure so that it does not exceed the limits of the column. Read more about this in Chapter 7, *System pressure*.

Detectors

Different detectors are used to follow the progress of the purification. For protein detection, multiple wavelength absorbance detectors are often used. The majority of proteins can be detected by measuring UV absorbance at 280 nm, but other wavelengths can also be used to gather additional information (see example in Fig 2.5). The conductivity monitor is used to follow column equilibration and salt gradient formation. For some applications it is important to also monitor pH. For more information see Chapter 8, *Sample monitoring and detectors*.

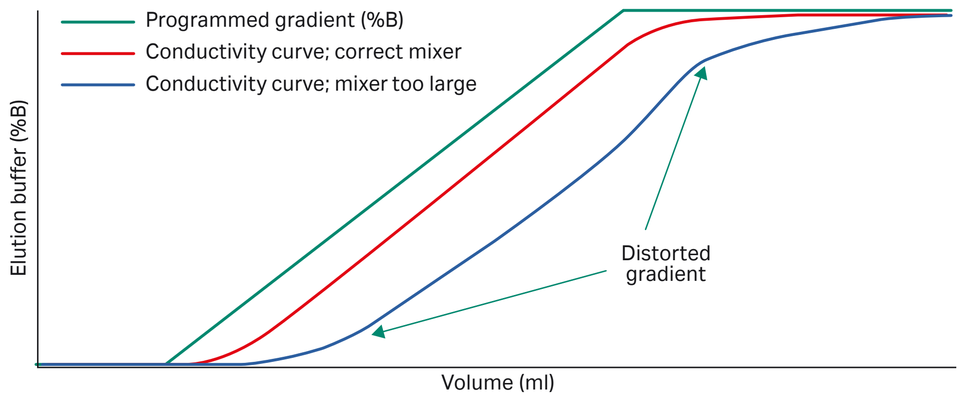


Fig 2.4. The actual gradient will differ from the programmed gradient column in a system with too large a mixer.

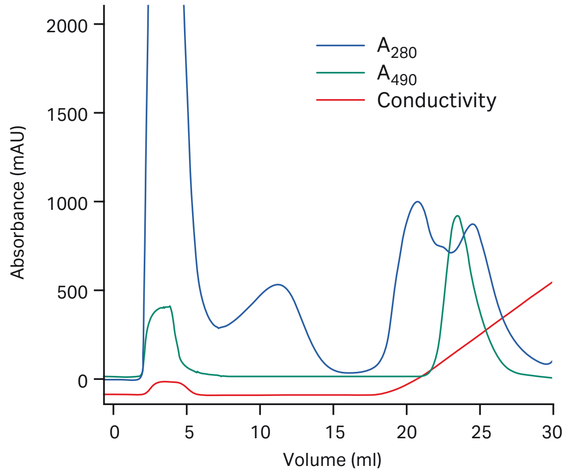


Fig 2.5. Specific detection of green fluorescent protein (GFP) at 490 nm.

Fraction collection

Preparative purification requires that the purified protein can be collected and fractionated. The eluted materials are collected in fractions using a fraction collector or an outlet valve. See Chapter 9, *Fraction collection*, for information about different ways of controlling protein peak fractionation and what the important parameters are for successful protein collection. See also Figure 2.6.

System cleaning

To ensure the long-term performance of the system, regular maintenance is important. When not using the system for some time, it is important to store it properly. Chapter 10, *Cleaning and storage of system components,* describes how to properly clean the different components of the system. To minimize the risk of salt precipitation that can damage the seals, avoid long-term exposure of system components to high salt concentrations.

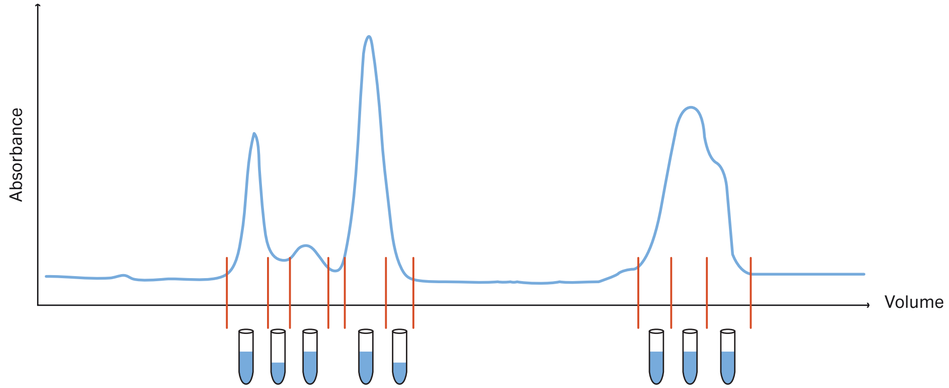


Fig 2.6. During this run, "peak fractionation" was used to collect the eluted proteins.

03

System volume effects on resolution and fraction collection

System volume effects on resolution and fraction collection

This chapter describes how the internal volume of the system affects liquid transportation and protein purification results.

The main applications for protein chromatography are either to analyze a protein sample or to prepare pure protein, sometimes referred to as preparative purification. For a successful result in both of these applications, high resolution is often important. High resolution is obtained by the use of chromatography media with combined high selectivity and efficiency. High selectivity ensures that the protein is bound to the media. High efficiency means that the protein peaks obtained are narrow and that good separation can be achieved between them.

Analytical chromatography systems generally handle small sample volumes. To minimize sample dilution and loss, components in an analytical system should have small internal volumes and allow usage of high-resolution media.

In preparative chromatography, it is important to use a chromatography medium and column that generate an appropriate resolution. It is important to keep the distance between the column and the fraction collector short to avoid dilution of the separated proteins. This is also important when purifying small amounts of protein to avoid protein losses due to dilution effects.

Tubing dimensions affect resolution

All components in the system (flow cells, valves, etc.) must in some way be connected to each other with tubing. Excess tubing will give unnecessary peak broadening, that is, the separated proteins will be diluted, and resolution (purity obtained) will be decreased. Peak broadening is due to the flow rate in the tubing being higher toward the middle compared with close to the walls of the tubing. The result is that a protein peak passing through the system will become broader as it moves through the tubing, as illustrated in Figure 3.1.

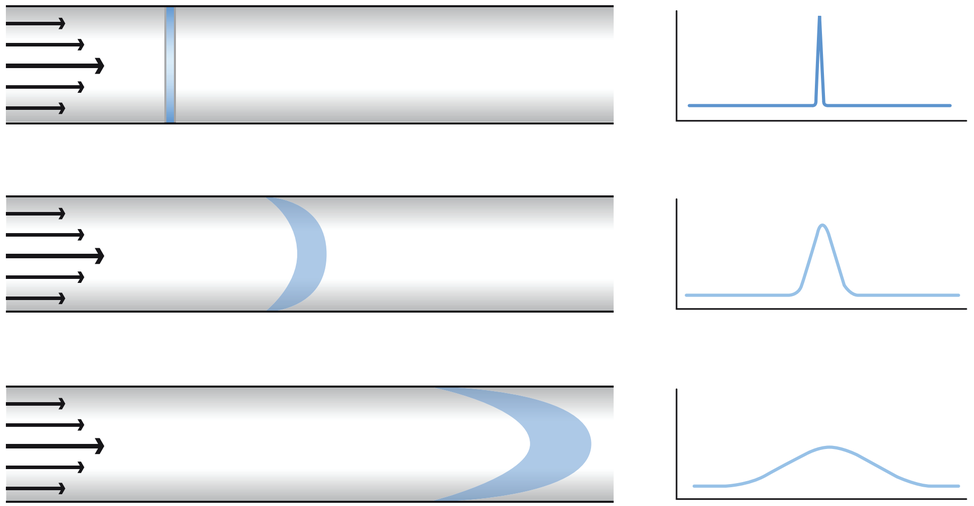


Fig 3.1. Schematic description of protein peak broadening in tubing. Liquid flows faster in the middle of a tube as compared with closer to the walls. The farther a protein peak passes through a tube, the broader it becomes, as depicted in the chromatograms shown on the right.

To achieve the best purification result, it is important to find the optimal tubing parameters for the purification setup. Figure 3.2 shows an example in which a sample was analyzed using tubing of different inner diameters (i.d.). Here, the resolution is most affected when going from 0.75 mm to 0.25 mm i.d. tubing. Decreasing the tubing diameter further will not have a large effect on the resolution. At the same time, the back pressure in the system will increase as tubing diameter is decreased. This must also be taken into consideration.

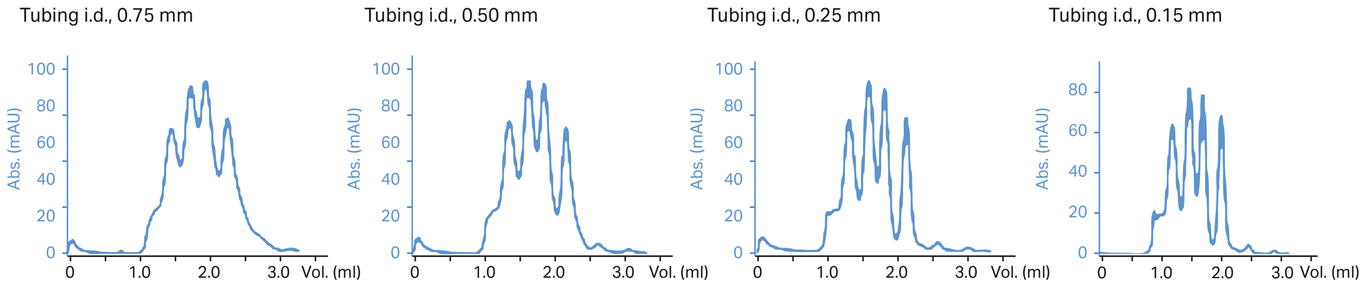


Fig 3.2. The resolution increases as the tubing diameter decreases. Column: Superdex™ 200 5/150 GL (CV_t 3 ml). Flow rate: 0.3 ml/min.

Peak broadening after the UV/Vis detector

In a given chromatogram, the UV/Vis absorbance curve shows the purification result as it was while the proteins passed the absorbance detector. What happens between the UV/Vis absorbance detector and the fraction collector is not visible in the chromatogram. This "hidden" effect can sometimes be dramatic, especially for high-resolution columns. Figure 3.3 shows the effect of using larger i.d. and/or longer tubing and thereby increasing the system volume. The consequence of increasing the system volume is that the high resolution obtained in the column might be spoiled as the protein peaks progress to the fraction collector.

Use tubing that is as short as possible between the absorbance detector and the fraction collector.

In a system designed for high-performance separations, the recommendation is to use narrow and short tubing to keep the peak broadening low. The drawback is that narrow tubing will increase the back pressure. Read more about this in Chapter 7, *System pressure*. An optimal combination of tubing length and i.d. is required to achieve the resolution needed and at the same time keep the back pressure within the pressure limit of the column used.

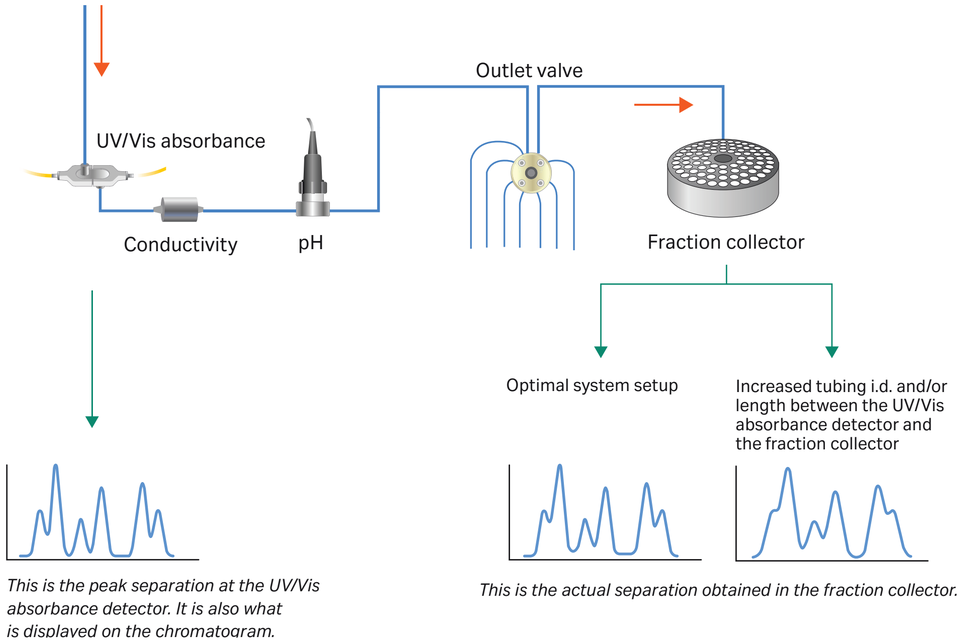


Fig 3.3. Consequence of the "hidden" system contribution if using tubing that is too long or that has a large i.d. between the UV/Vis absorbance detector and the fraction collector.

System volume considerations

For a given chromatography system, the relative system contribution to peak broadening will depend on both the bead size of the chromatography medium and column dimensions. Small beads and narrow columns result in narrow peaks (a high-performance column), whereas large beads and wide columns result in wide peaks.

The system volume can contribute significantly to the peak broadening of a narrow peak, but will contribute almost nothing to a wide peak. As illustrated in Figure 3.4, the system effect on resolution will be much larger for smaller peaks.

It is important to know that the effect of the system contribution on the peak width is nonlinear, as can be seen in Figure 3.5. This graph shows the contribution from a typical laboratory-scale system with 0.5 mm i.d. tubing. In this example, the system contribution has little effect on peaks that are larger (broader) than 3 to 4 ml. On the other hand, if the peaks are less than 1 ml, the system contribution becomes significant.

Besides tubing diameter, peak broadening is also affected by tubing length and the dimensions of valves and flow cells. It is therefore important to determine if the column to be used is suitable for the system. Do not run smaller columns than recommended for the system (see the selection guide in *Related literature*). If a smaller column is needed, consider minimizing the system volume by modifying the system, for example, by changing tubing to a smaller i.d. and/or excluding components from the flow path to minimize the system volume.

If making hardware changes that will affect the system volume, remember to update relevant delay volumes in the software. (For more information refer to Chapter 9 and Appendix 3).

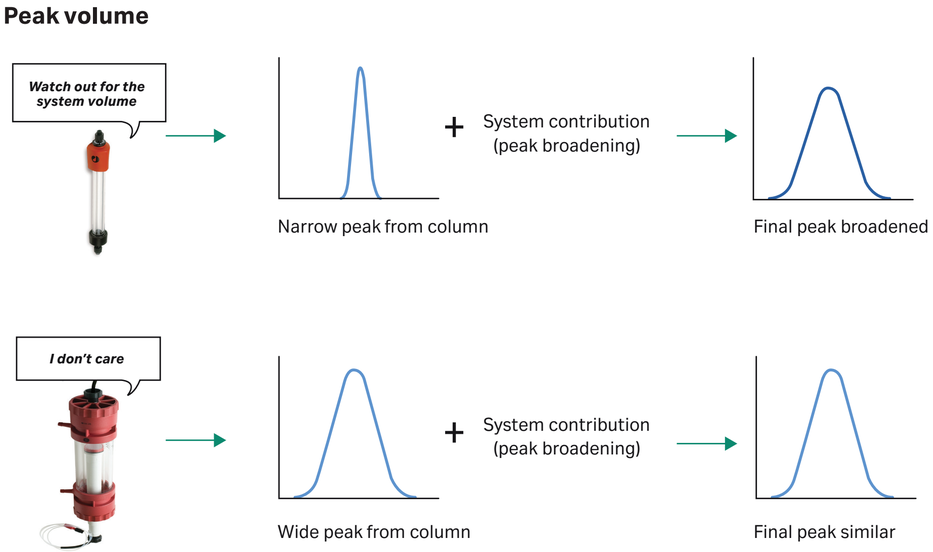


Fig 3.4. Smaller peaks are more affected than larger peaks (same system in both cases).

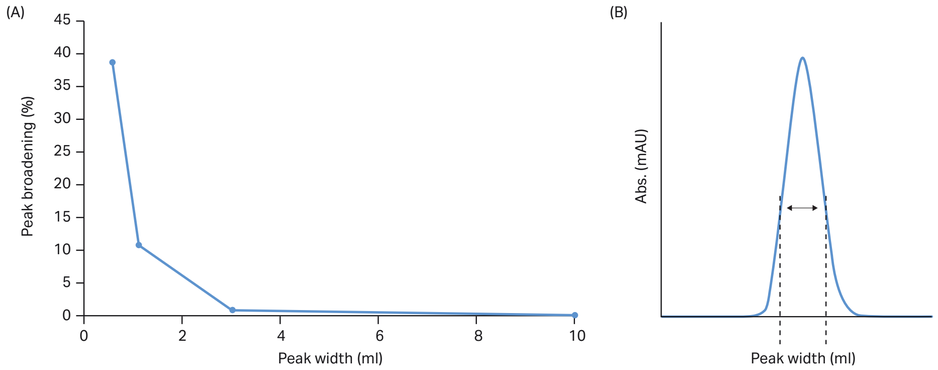


Fig 3.5. (A) The relative system contribution depends on the peak width. (B) Peak width is in this example determined at the half height of the peak.

Effect of sample volume on resolution

Sample volume does not affect resolution in chromatography techniques involving adsorption of the target protein onto the column. Examples of binding techniques are affinity chromatography (AC), ion exchange chromatography (IEX), and hydrophobic interaction chromatography (HIC). Size exclusion chromatography (SEC), however, is a nonbinding chromatography technique, and a sample zone is therefore broadened during passage through the SEC column. As a result, the sample gets diluted, and the resolution will decrease with increasing sample volume.

Figure 3.6 shows an SEC example in which different volumes of a sample were applied to a Superdex 200 10/300 GL column. In the first case, 250 µl of sample was applied, which correspond to 1% of the column volume. In the second case, 1000 µl of sample was applied, which corresponds to 4% of the column volume. As can be seen, the resolution was higher when a smaller sample volume was used.

The loaded sample volume should be kept small when using a nonbinding chromatography technique. To achieve the highest resolution in SEC, a sample volume of less than 2% of the total column volume is recommended.

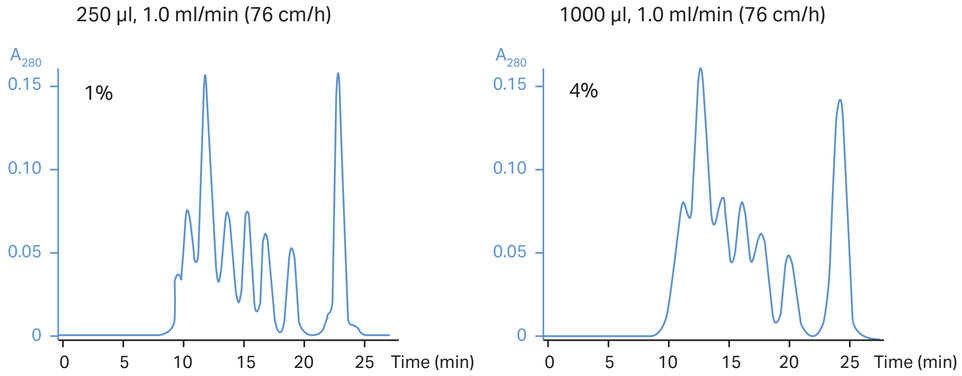


Fig 3.6. Effect of sample volume on resolution in SEC. Sample volume as percentage of media volume. Column: Superdex 200 10/300 GL.

04

How to choose sample injection technique

How to choose sample injection technique

There are three common ways of applying the sample to the column:

1. From a prefilled sample loop
2. Direct injection via the sample pump
3. Direct injection via the system pump

Table 4.1. Sample application techniques

Technique	Sample volume	Important	Benefit
Tubing loop	Small 10 µl to 10 ml	Filling and emptying the loop in a correct manner	Handles small volumes
			High reproducibility
			Minimizes sample loss if partially filled
			Can be used at high pressure
Superloop™	Intermediate 100 µl to 150 ml	Filling and cleaning the loop	Minimizes sample loss
			Allows repeated injections without manual interactions in-between
Sample or system pump	Large 5 ml* to several liters	Removing air bubbles from pump	Is convenient for large volumes
		Priming the tubing with buffer/sample before start	
		Cleaning the pump afterward	

* Sample volume in the lower range requires tubing with small i.d. to minimize sample loss.

Tubing loop

Tubing loops are used for smaller sample volumes. The loop must be filled and emptied in a correct manner. Reproducibility when using a loop will be high because the sample application is independent of any variation in flow rate. Sample loops of different volumes are available, ranging from a few microliters up to 10 ml.

When filling the loop it is important to consider the fluid dynamics, as explained in Figures 4.1 and 4.2.

The flow rate of the sample entering the loop will be higher toward the middle compared with close to the walls of the tubing; this creates a parabolic flow profile in the loop (Fig 4.1). Thus, in order to fill the loop completely, a larger volume needs to be loaded, which is explained in the next section and illustrated in Figure 4.2.

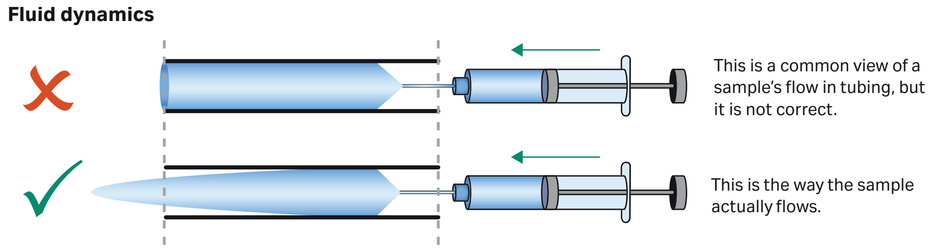


Fig 4.1. Fluid dynamics through tubing.

Filling a tubing loop

There are two ways to fill a tubing loop: partial filling and overfilling; see Figure 4.2. With partial filling, there is no sample loss but reproducibility is lower if the same procedure is repeated. With overfilling, a better volume accuracy is obtained. For a complete fill, load three to five times the loop volume to obtain high accuracy. The needed volume depends on the loop dimensions (length and i.d.). Generally, the larger the loop volume the less overfill is needed.

For a partially filled sample loop, do not fill more than half of the total loop volume. If more is applied, a portion of the sample might pass through and out of the loop, as shown in Figure 4.2.

Emptying a tubing loop

To avoid dilution when emptying a tubing loop, empty it in the opposite direction from which it was filled.

The volume to achieve complete recovery will vary with the flow rate, loop dimensions, and the properties of the sample, but usually three to five times the loop volume is sufficient.

Figure 4.3 shows an example of the recovery achieved at different volumes when emptying a 100 µl loop at 0.5 ml/min. To empty the loop completely, in this example a buffer volume corresponding to three times the loop volume was needed.

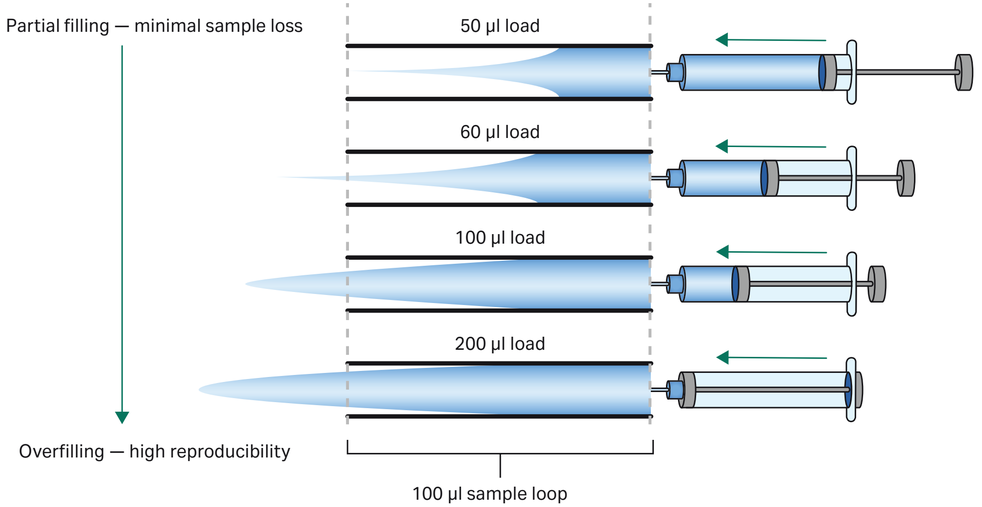


Fig 4.2. Filling a sample loop (here i.d. was 0.50 mm).

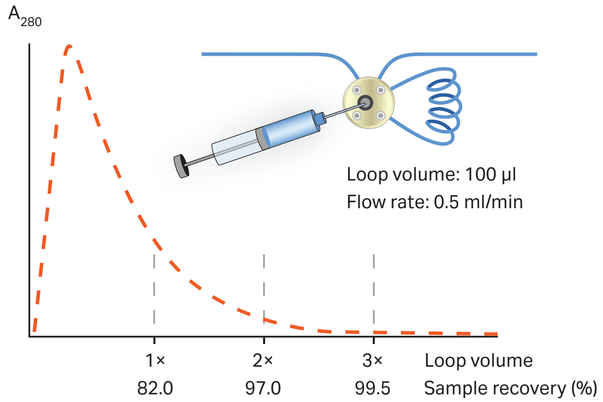


Fig 4.3. The elution profile and recovery when emptying the contents of a completely filled sample loop. In this setup a tubing loop with i.d. 0.50 mm was used.

To achieve high sample recovery, use a large volume to empty the loop. For nonbinding techniques (e.g., desalting and SEC), there are sample volume limitations due to the size of the column used.

Figure 4.4 shows an example of how resolution can be improved by decreasing the volumes used to empty the loop during sample injection. This is a common way of working when analytical studies are performed.

Before starting, decide whether the most important aim is high recovery or high resolution.

Superloop

Superloop is available in three different sizes, 10, 50, and 150 ml, and can be used for sample volumes in the range of 100 µl to 150 ml. They can be used to inject the complete sample volume onto the column or to make repeated injections of a sample without manual interactions in between. Figure 4.5 depicts a 10 ml Superloop.

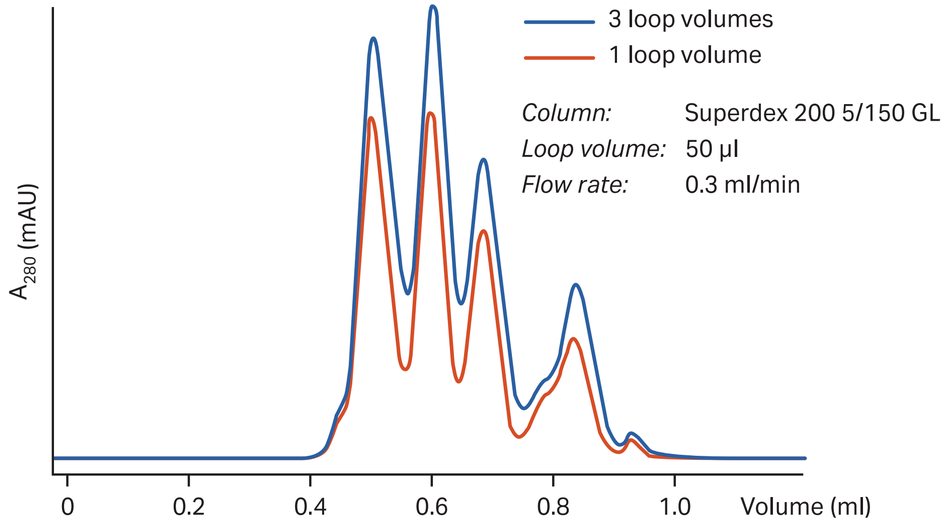


Fig 4.4. The chromatogram shows how the separation in SEC is affected by the different volumes used to empty the loop during sample injection.

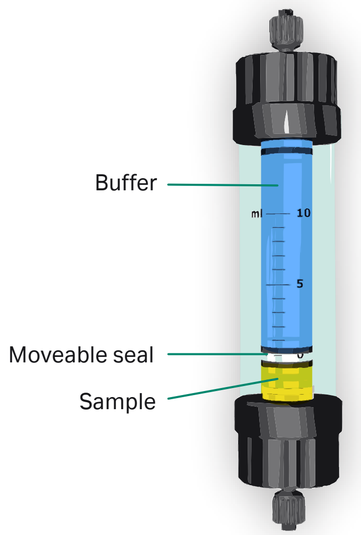


Fig 4.5. Schematic drawing of a 10 ml Superloop.

How to fill and empty a Superloop

A Superloop is connected to the injection valve and is initially filled with buffer. Sample is loaded from the bottom, either manually using a syringe or by using a sample pump (Fig 4.6A). The sample is injected onto the column by pushing buffer into the top of the Superloop so that the seal moves downward, pushing the sample out of the Superloop. The seal hinders mixing of sample and buffer (Fig 4.6B). When the moveable seal reaches the bottom position, the buffer will automatically bypass the seal to the column, following the sample (Fig 4.6C).

Considerations when using a Superloop

The flow rate delivered from a Superloop is determined by the system flow rate. In situations where it is more important to inject the entire sample, run the pump for slightly more than the estimated sample volume to make sure that the Superloop and tubing are completely emptied (Fig 4.6C).

Superloop has a limited pressure range: 4 MPa (40 bar, 580 psi) for the 10 and 50 ml loops and 2 MPa (20 bar, 290 psi) for the 150 ml loop. Always make sure that the system pressure alarm limit does not exceed these values when the Superloop is connected inline.

If using a column with a higher pressure tolerance than that of the Superloop being used, remember to lower the pressure limit during sample application. Also bypass the Superloop before increasing the flow rate to normal.

The moveable seal in Superloop 10 ml and 50 ml has an O-ring made of fluorocarbon rubber that has limited chemical resistance. It can be used in aqueous buffer solutions and alcohols while other solvents should be used with caution.

Solvent-resistant O-rings for Superloop 10 ml and 50 ml are available as accessories.

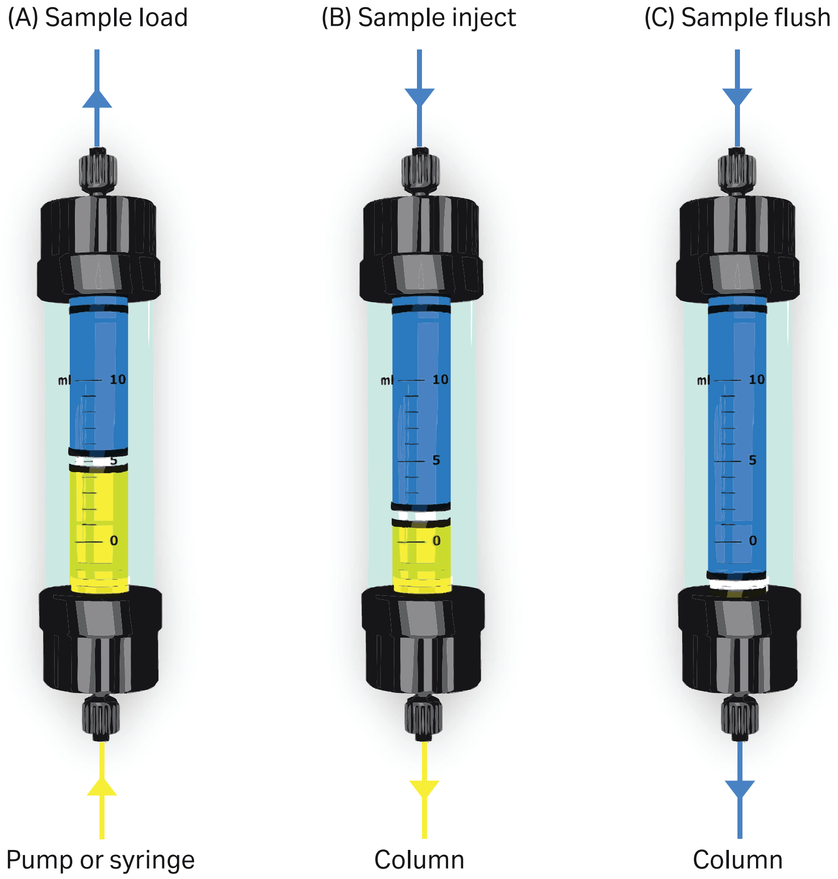


Fig 4.6. Filling and emptying a Superloop. In these images, sample is colored yellow and buffer blue.

How to prepare a Superloop

Before connecting a Superloop to the system, remove the upper end piece, as shown in Figure 4.7.

Position the moveable seal in the bottom of the Superloop and fill it by pouring buffer into the glass cylinder as shown in Figure 4.8. Reassemble the top piece and make sure that no air bubbles are trapped inside, as shown in Figure 4.9.

To apply sample at a preferred temperature, allow water of the desired temperature to circulate in the outer shield of the Superloop.

Fig 4.7. Upper end piece removed from a Superloop.

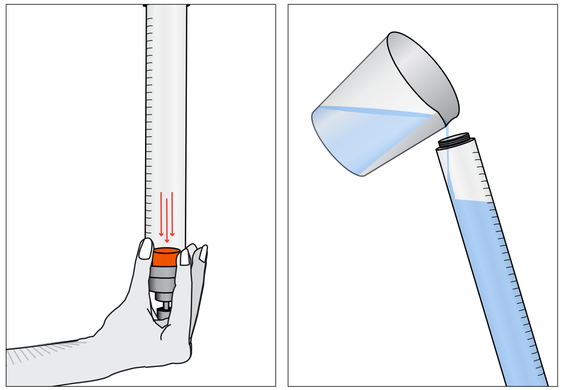


Fig 4.8. Moveable seal should be in bottom position (left). Buffer is poured into the cylinder (right).

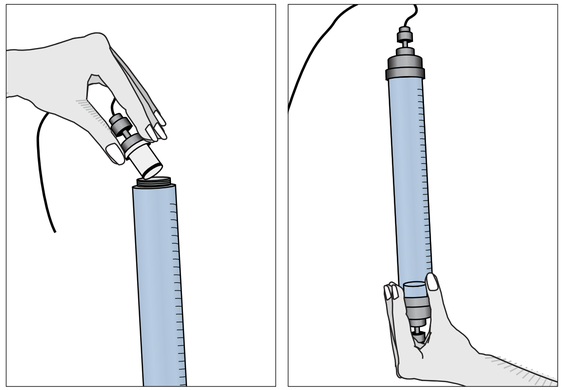


Fig 4.9. How to mount the top piece (left) to reassemble the Superloop (right).

How to connect a Superloop to the injection valve

A Superloop is connected to the same ports as a tubing loop. To find out which port should be connected to the bottom of the Superloop, connect a syringe to the injection valve, and turn the valve to position “load.” Inject liquid, and check where it comes out, as shown in Figure 4.10. Connect the tubing from the bottom of the Superloop to this port. Be careful not to introduce air bubbles in the sample compartment.

The top of the Superloop should be connected to the port where liquid from the pump is directed to during injection mode. If unsure, start a flow rate and change to position “inject.” Where the liquid comes out is the port to which the Superloop top should be attached.

How to clean a Superloop

A Superloop can be cleaned while connected to the system. This is achieved by pumping a cleaning or sanitizing agent through the Superloop. The standard recommendation is to pump 0.5 M NaOH for 30 min. Make sure to rinse the loop properly after using NaOH; for example, wash with water followed by buffer until a neutral pH is achieved.

To avoid carryover when changing sample, it is recommended to disassemble the Superloop and clean all parts separately.

Wear gloves and safety glasses when using hazardous/corrosive chemicals.

Autosampler

By using an autosampler, several small sample volumes can be injected automatically, which is convenient in, for example, protein analysis or micro purification work. The autosampler ensures that one sample at a time is used to fill the sample loop Table 4.2 lists different autosamplers and their capabilities.

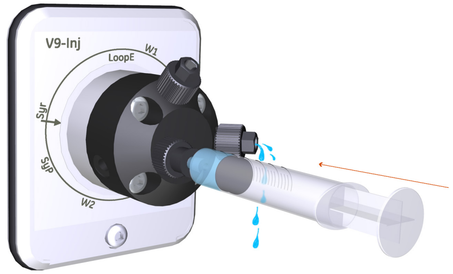


Fig 4.10. Check where to connect the bottom of the Superloop by injecting buffer into the injection valve.

Table 4.2. Autosampler options

Autosampler	Capacity	Cooling	Use with
A-900	1 × 96-well plate, 1.5 ml vials or 160 × 0.5 ml vials	Yes	ÄKTAexplorer/purifier
A-905	1 × 96-well plate, a 384-well plate, or 48 × 1.5 ml vials	Yes	ÄKTAexplorer/purifier/micro
Alias™ autosampler (Sparks Holland)	2 × microplates according to recommended microplate (SBS) standards. 96-well high- and low-well in addition to 384-well, 2 × 48 (1.5 ml) vials, or 2 × 12 (10 ml) vials	Yes	ÄKTA avant, ÄKTA pure and I/O box

[1] A-900 and A-905 have been discontinued.
[2] Alias refers to the Alias autosampler supplied by Spark Holland. To connect Alias autosampler to ÄKTA pure, refer to Instructions 29040427.

Sample loading using a pump

A sample or system pump can be used to apply sample directly onto the column. Figure 4.11 shows an example of a flow path including a sample pump. When using a pump, a desired, predetermined volume can be chosen, or an air sensor can be used to allow loading of the entire sample (undefined volume) onto the column. When the sample container is emptied, air will trigger the air sensor and the sample valve will turn to another port. This also prevents air being injected into the column. For serial purification runs, the pump can be used together with an inlet valve to serially load different samples.

Before applying sample onto the column, the following preparation is important:

1. To ensure correct volume delivery, air bubbles must be removed from the pump(s) as described in Chapter 5.
2. The flow path from the sample bottle to the injection valve must be filled with sample (primed) before starting the sample application.

Preparing sample inlet

When the pump is started, the volume from the sample container to the injection valve will be directed to the column. If the flow path has not been prefilled (i.e., primed) with sample, the actual sample volume applied to the column will be smaller than anticipated.

Sample inlet preparation volume

The volume needed to fill the sample inlet depends on the tubing and components included in the flow path. The easiest way to determine this is to calculate the volume theoretically. To do this, all tubing and components from the sample vessel to the injection valve should be included. See Appendix 3 for details.

It is also possible to determine the volume experimentally. Disconnect the column from the flow path. Fill the system with buffer and use buffer containing 1% acetone as sample. Use the pump to apply the acetone solution. Note the volume it takes until the UV/Vis absorbance detector (using A_{280}) detects acetone. NaCl can be used instead of acetone. In this case, measure the volume it takes for the conductivity monitor to detect the salt. Note: the volume obtained from experimental determination is slightly higher than when using the calculation method, because the path from the injection valve to the detector is added.

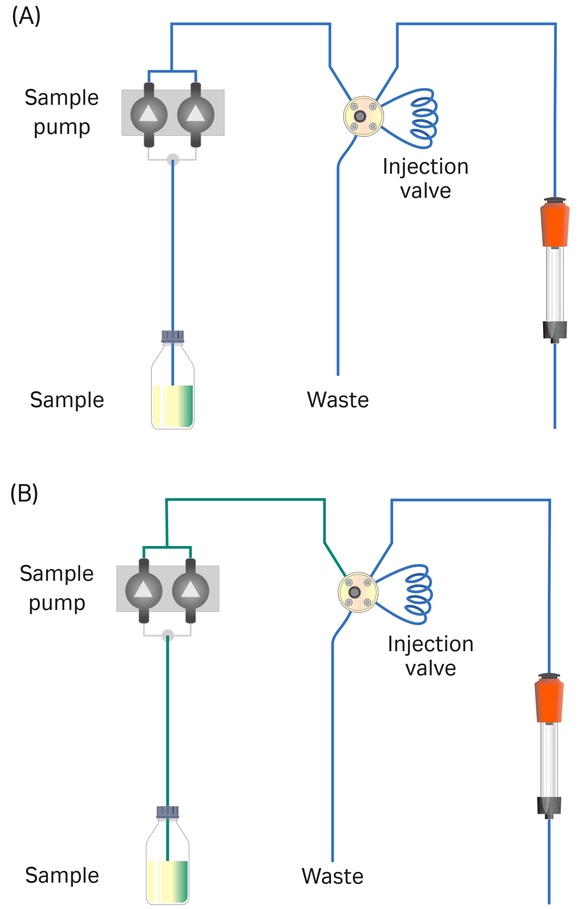


Fig 4.11. From the start, the flow path (including the sample inlet) is filled with buffer (A). Primed tubing, that is, the tubing between inlet and injection valve, is filled with sample (B), ensuring that the correct volume is injected onto the column during sample application. In the figure, sample is colored green and buffer blue.

Applying a fixed sample volume

To apply a fixed sample volume using a pump, first determine the volume needed to prime the flow path with sample as described above.

Place the sample inlet in buffer and remove air bubbles from all pumps that will be used by purging as described in Chapter 5. Immerse the sample inlet in the sample container and start the priming. After priming, the system is ready for sample application.

Applying all of the sample using an air sensor

To apply all the sample, use a pump and an air sensor. Prime the sample inlet to be used with buffer, and remove air bubbles from the pumps as described in Chapter 5. Then immerse the sample inlet in the sample container and use the pump to apply the sample (Fig 4.12A). Apply the sample to the column until the air sensor detects air bubbles (Fig 4.12B). After air has been detected, the sample valve switches to a buffer inlet allowing the remaining sample from the sample valve to the injection valve to be applied onto the column (Fig 4.12C).

In some systems, preprogrammed methods are available that can be used to prime the sample pump and air sensor with sample.

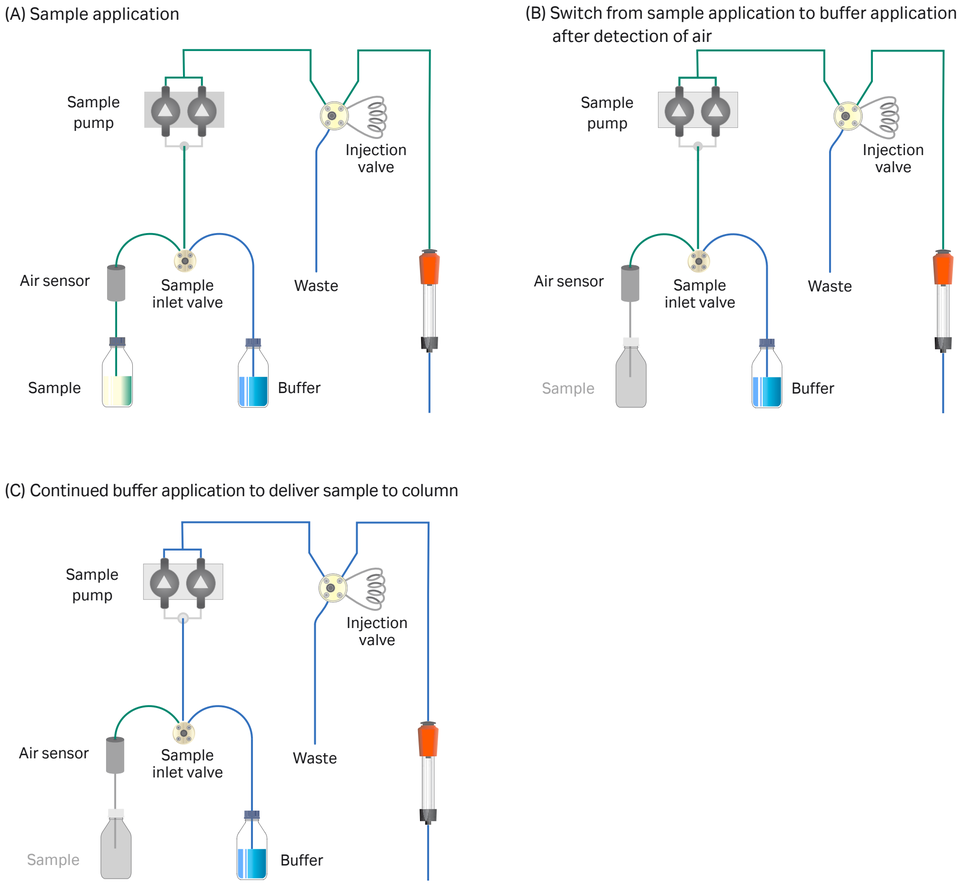


Fig 4.12. Sample application example where a sample pump and air sensor are used to apply sample. In the figure, sample is colored green and buffer blue. Refer to text in images for descriptions of (A), (B), and (C) panels.

05

Liquid delivery and pumps

Liquid delivery and pumps

This chapter describes the high-performance pumps used in laboratory-scale ÄKTA systems. Accurate flow rate, reproducible liquid delivery, and low pulsation are essential for an optimal purification result. Because the column and media used in laboratory-scale chromatography often generate varying back pressure, the pump must also function under both high and low pressure.

Note: The descriptions in this chapter are not applicable for systems with peristaltic pumps, for example, ÄKTA start.

Always make sure to remove all air bubbles in the pump before starting a run.

For optimal separation, make sure that the pump delivers the correct flow rate.

Conditioning the pump for accurate liquid delivery

Some systems have two pumps to be able to create accurate gradients. Other systems use one pump and a switch valve to form gradients. Each pump normally contains two pump heads that work in opposite mode to create a homogeneous flow rate.

How to detect air bubbles in the pump

Air bubbles present in the pump cannot be detected by visual inspection of the pump. Instead, the pressure curve can be analyzed.

When the pump runs against a back pressure above 0.2 MPa (20 bar, 290 psi), air bubbles present will be seen by disturbances in the pressure curve (Fig 5.1). To generate a back pressure above 0.2 MPa (20 bar, 290 psi), a reference tubing with smaller i.d. can be used (Table A2.1 in Appendix 2).

The accuracy of the volume delivered is affected by even very small air bubbles (a few microliters) trapped in the pump.

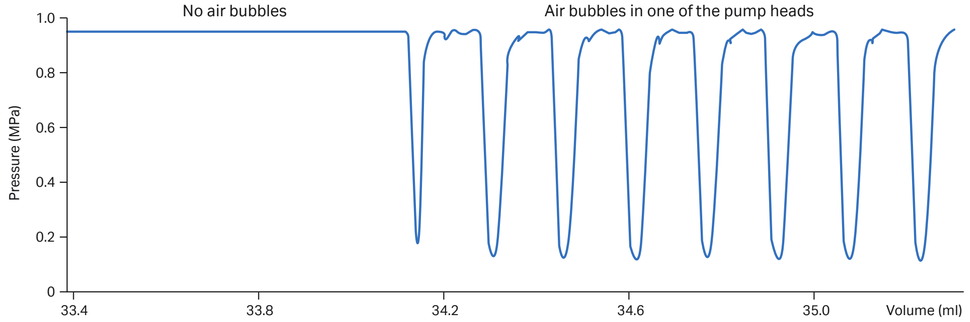


Fig 5.1. System pressure curve appearance when air bubbles are present in the pump.

How to remove air bubbles

Air bubbles are removed from the pump by using a syringe to draw liquid via the purge valve of the pump as described below. This procedure is referred to as “purging.”

To avoid air entering the pump, make sure that all inlets are prefilled with liquid. Also, check that all tubing connections at the pump and inlets are tight.

To purge the pump, connect a syringe to the purge valve (Fig 5.2). Open the purge valve and draw liquid slowly into the syringe. It is very important to draw the liquid slowly, no more than 1 ml/s, otherwise an under-pressure will be generated and more air bubbles will be released in the pump.

The purging will be more efficient if the pump is run at a flow rate around 10% of the system’s maximum flow rate. Such a flow rate will help to mechanically release any air bubbles adhering to the walls inside the pump head. Normally, pumps designed for higher flow rates are more easily purged because of the larger volume of the pump head.

For optimal results, purge all pump heads of the pump.

After purging, check that all air bubbles have been removed by analyzing the pressure curve (Fig 5.1). Start a flow and run the pump at a pressure above 0.2 MPa (2 bar, 29 psi). If the pressure curve indicates that there are still air bubbles present, repeat the purging process and check the pressure curve again.

If air bubbles remain after purging using buffer, use 100% methanol instead. Make sure the pump contains water, then use a syringe to draw 100% methanol into the pump and let it run at 10% of the system’s maximum flow rate until the pressure curve disturbances (Fig 5.1) disappear. To remove the methanol, stop the pump and switch to water. Make sure that no air bubbles are introduced. Run the pump at a flow rate of 1 ml/min for 5 min to wash away the methanol. Then purge the pump again using a syringe.

If the pump gives an inaccurate flow rate even after removal of air bubbles, contact your Cytiva business Service representative.

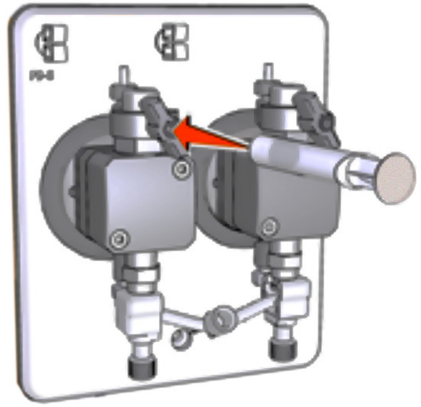


Fig 5.2. Remove air bubbles by using a syringe to draw liquid from the purge valves.

Air bubble origin

Air bubbles may be released from the liquid when the pressure drops. When the pump is running, the pressure inside the pump will be higher than the atmospheric pressure during liquid delivery. When the pump is in the suction phase, the pressure will be below atmospheric pressure, and air bubbles might be released. To avoid this situation, place the bottles above (or at least at the same height as) the pumps.

NEVER place the liquids below the pump unless the user manual states that it is possible (some systems have a pump design that allows such placement).

Because solutions are always in contact with air, it is recommended to degas them prior to use.

Pay special attention to liquids stored at low temperature that will be used at room temperature. More air is dissolved at lower temperature, therefore allow time for the liquids to adjust to room temperature before use.

Air bubbles might also be generated when switching between aqueous and organic solvents in the pump. Due to different capacities to dissolve air, bubbles can be released when two liquids are mixed. To avoid this situation, when switching between different liquids, direct the flow path to the waste position of the injection valve and pump at a fairly high flow rate (> 50% of the system's maximum flow rate) for some time.

Description of the pump and rinsing system

Functionality of the pump

Due to the design of the ÄKTA pumps, they are virtually pulse-free and do not introduce sheer forces that disrupt or break down proteins mechanically. The pumps can also operate at both high and low pressure, which makes them convenient for various conditions encountered in protein purification.

To generate the set flow rate during operation, the pumps use an algorithm to control how the pistons move. As long as there are no air bubbles in the pump, the flow rate accuracy will be high, with an error rate typically ≤ 2%.

Most ÄKTA pumps consist of two pump heads (Fig 5.3). The individual heads are identical but operate in opposite phase to each other, using individual stepper motors. The two pistons and pump heads work alternately to provide continuous low-pulsation liquid delivery.

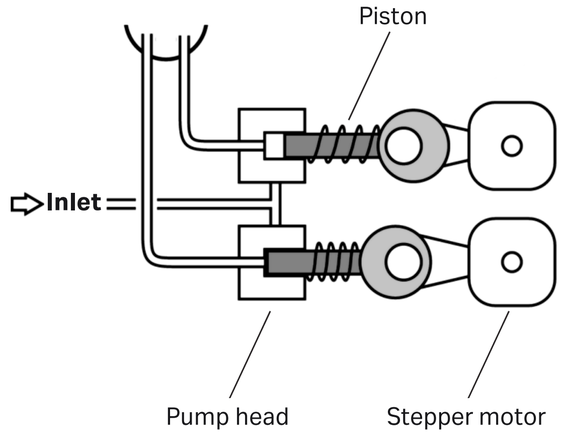


Fig 5.3. Schematic view of the P-901, P-903, P-905, P9, P9-S, and P9H pumps.

Figure 5.4 shows the pump head design. As the piston moves out of the chamber during the suction phase, the inlet check valve will open and the outlet check valve will close, allowing the chamber to fill up with liquid. During the delivery phase, the outlet check valve will open while the inlet check valve will close. During this phase, the piston will move into the chamber, pushing the liquid out of the pump

Piston seal rinsing system

The piston seal rinsing system has two functions:

1. It protects the piston seals and pump heads by preventing a buildup of deposits consisting of components from solutions used, for example, salt crystals.
2. It prolongs the lifetime of the seal by preventing it from drying.

The inlet and outlet tubing of the rinsing system are most often placed in the same container. The rinsing system should always be filled with 20% ethanol, which then circulates on the back side of the pump head as shown in Figures 5.5 and 10.2. In this process, deposits will be flushed out, and the ethanol prevents microbial growth.

Check the 20% ethanol solution frequently. Change it once a week or if the solution appears opaque or the ethanol level in the container has decreased.

To be reminded about the change, place the rinsing solution where it is visible, for example, on top of the system or mounted on the instrument wet side.

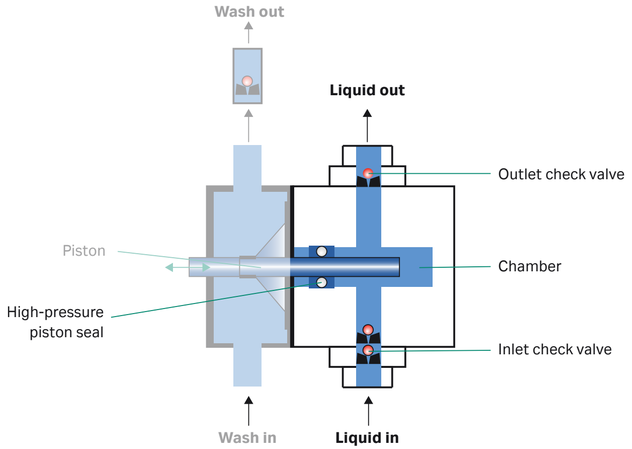


Fig 5.4. Schematic view of one pump head from P-901, P-903, P-905, P9, P9-S, and P9H.

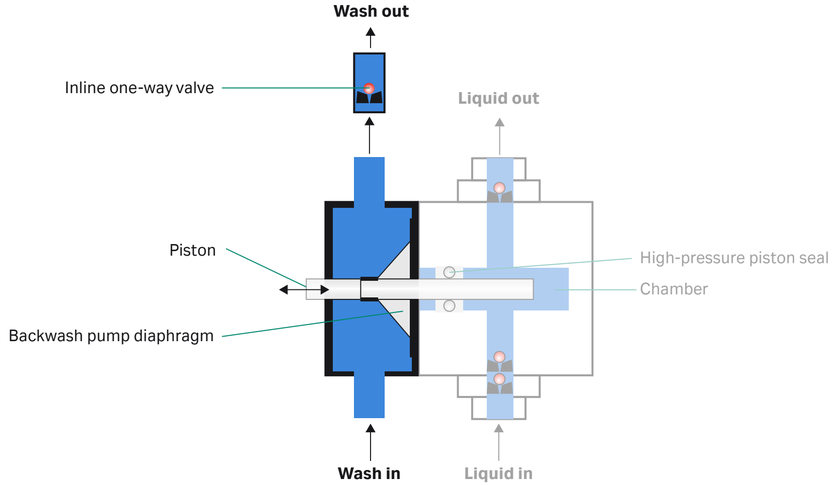


Fig 5.5. Schematic view of the piston seal rinsing system of the P-901, P-903, P-905, P9, P9-S. and P9H pump.

Air sensor to protect column

An air sensor used at the buffer inlet prevents introduction of air into the column and system. Once the air sensor alarm is triggered, the system will stop.

☞ To avoid air bubbles becoming trapped within the air sensor and subsequently triggering the alarm, always mount the air sensor's inlet and outlet vertically and in an upflow direction (i.e., opposite to gravity flow).

Different levels of sensitivity of air detection can be set; see Table 5.1 for general advice.

Table 5.1. Setting the level of sensitivity of air detection

Level	Detects	Usage
Low	Large volume of air	Provides protection against running out of buffer/solution
Medium/Normal	Air bubbles of medium size (e.g., 30 to 100 µl)	Aborts sample application when using the pump to apply complete sample
High[1]	Small air bubbles (e.g., tenths of microliters)	When the air sensor is placed between the injection valve and column

[1] Use high sensitivity with care because it can catch stray air bubbles that are not detrimental to the process and this might unnecessarily activate the alarm, pause the run, and stop the flow.

06

Gradient formation and mixers

Gradient formation and mixers

Gradients are used during elution of absorbed proteins from the column. High accuracy in flow rate delivery is key in generating an optimal gradient. How to ensure an accurate flow rate is described in Chapter 5. For proper gradient formation, it is important to minimize the effect of pump pulsation and to make sure that the liquids used to form the gradient are mixed to a homogeneous solution before entering the column. A mixer will accomplish these functions. Different approaches may be taken, and both dynamic and static mixers are used in chromatography systems. Some systems have two pumps to be able to create accurate gradients. Other systems use one pump and a switch valve to form gradients.

Choosing mixer size

The delivered volume and type of solutions will determine the mixer size needed (see Table 6.1).

Usually the mixer supplied with the system will cover a broad range, but there are occasions when changing to a different mixer size should be considered. Check the system user manual to find out which mixer to use.

A larger mixer might be needed when creating gradients with aqueous and organic solvents. Improper mixing of aqueous and organic solvent can be seen as disturbances in the absorbance baseline. Change to a larger mixer and perform a test run without a column, to make sure that the absorbance baseline is stable.

Table 6.1. Recommendations for mixer size

When running	What to do
Small columns at a flow rate in the lower range and with small gradient volume	Changing to a smaller mixer will reduce the effects of the system volume
High flow rates and/or using solutions that are hard to mix, e.g., high salt concentration or mixing aqueous with organic solvents	Change to a larger mixer for proper mixing

Conductivity disturbances

Perturbations to the shape of the conductivity curve suggest improper mixing. If the internal volume of the mixer is too large, the shape and slope of the gradient will be affected, which can be observed on the conductivity curve as disturbances to the slope. This effect is most pronounced at low and high conductivity, as shown in Figure 6.1. It is especially important to be aware of this effect when scaling up to larger columns.

When changing to a different mixer size, the slope of the actual gradient can be compared with the programmed gradient by performing a test run without a column.

Gradient delay volume

When planning a gradient run, it is important to consider the system's delay volume prior to the column. This is called the gradient delay volume. In the chromatogram, the actual gradient will be delayed compared with the programmed gradient (%B curve), as seen in Figure 6.2. The shape of the gradient is also affected by mixer effects. Make sure that the conductivity reaches the programmed gradient value by the end of the run, by continuing to run at the final elution conditions until the target value is reached. The volume to add needs to be determined experimentally.

In ÄKTA systems, the default mixer effect has been included in the so-called "gradient delay volume" of the system/UNICORN™ software.

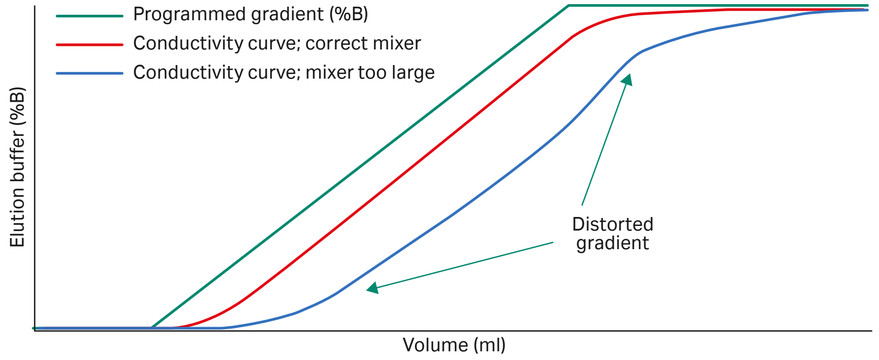


Fig 6.1. The actual gradient will differ from the programmed gradient in a system with too large a mixer.

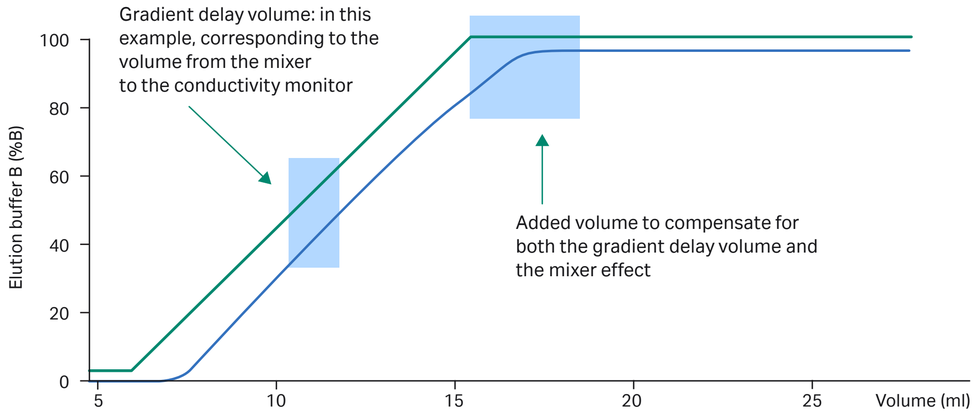


Fig 6.2. Actual gradient (blue) compared with programmed gradient (green).

Maintaining a constant gradient slope when changing column size

Gradient length is often defined in terms of X column volumes (CV). Maintaining a constant gradient will ensure that the slope of the gradient will not change when scaling up or down. For example, if the gradient length is 10 CV, for a 1 ml column this corresponds to 10 ml and for a 10 ml column this corresponds to 100 ml.

The gradient delay volume is independent of the column used; it will be the same as long as the columns are run in the same system and with the same mixer. For example, if the gradient delay volume is 5 ml, add 5 ml when running the columns in the example above. Thus, for the 1 ml column, the total volume added would correspond to 10 ml + 5 ml, and for the 10 ml column it would correspond to 100 ml + 5 ml.

If the system comes with preprogrammed methods, the gradient delay volume is included in the method (e.g., in the system volume compensation block).

Appendix 3 describes how to calculate delay volumes.

During scale-up or scale-down, make sure that the optimal mixer size is used (see previous page). If the new scale requires a mixer change, remember to also update the “gradient delay volume” in the system/UNICORN software.

07

System pressure

System pressure

A back pressure will be generated when running liquid through the system. If the back pressure exceeds any of the set pressure limits, an alarm will be triggered and the system will stop. This is a common problem in chromatography. It is therefore important to understand the cause of high pressure to be able to avoid it.

Back pressure effects

It is important to keep the back pressure as low as possible because columns and system components are often sensitive to high pressure. Table 7.1 highlights contributions to high back pressure and includes suggestions on how to avoid it.

Table 7.1. Factors contributing to increased back pressure

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 Chapter 3
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 inline filter if the system pump is used to apply the sample	To avoid over-pressure, some systems have pressure-controlled sample application, where the flow rate is decreased as the pressure increases
Column	Clean the column Do not use smaller beads or column diameter than the application requires	See column instructions for cleaning procedures Smaller beads will give higher resolution but also higher back pressure
Flow restricttor	When using chromatography media that generate low pressure at high flow rate, consider removing the flow restrictor. Note, however, that there is a risk of air bubbles entering the UV/Vis absorbance cell.	The reason the flow restrictor is present is to prevent air bubbles in the UV/Vis absorbance cell. This is important when running columns that generate high back pressure

Tubing contribution to back pressure

To keep peak broadening low, the tubing should have a small i.d. and be short (see Chapter 3). The drawback is that narrow tubing increases the back pressure in the system. If the system is equipped with tubing that is too narrow, the pressure generated can be too high for the column being used.

Figure 7.1 shows the pressure generated by tubing of different i.d.'s. In the example, a tubing i.d. of at least 0.35 mm is required to run a column with a pressure limit of 0.5 MPa (5 bar, 72.5 psi). In practice, the recommendation is to not run close to the column pressure limit, because the pressure alarm will stop the system. In this example, the recommendation would be to use 0.5 mm tubing.

Pressure alarms

To protect column hardware and packed bed of chromatography medium against pressure that is too high, it is important to use correct settings for the pressure alarms. To find out the pressure limits, check the column and medium instructions, and set the alarm(s) as described below.

For systems that measure the pressure only at the system pump:

- The pressure alarm limit should be set to the lowest limit of either the column hardware or the packed medium bed

For a system with three pressure sensors:

- The pressure alarm for pre-column pressure should be set to the column hardware limit. This is affected by the pressure generated by the column plus the system flow path located after the column
- The pressure alarm for Δp should be set to the limit for the packed bed, if available

The relationship between the pressures is:

Δp = p1 (pressure generated by the column and the flow path after the column) – p2 (pressure generated by the flow path after the column), see Table 7.2 and Figure 7.2.

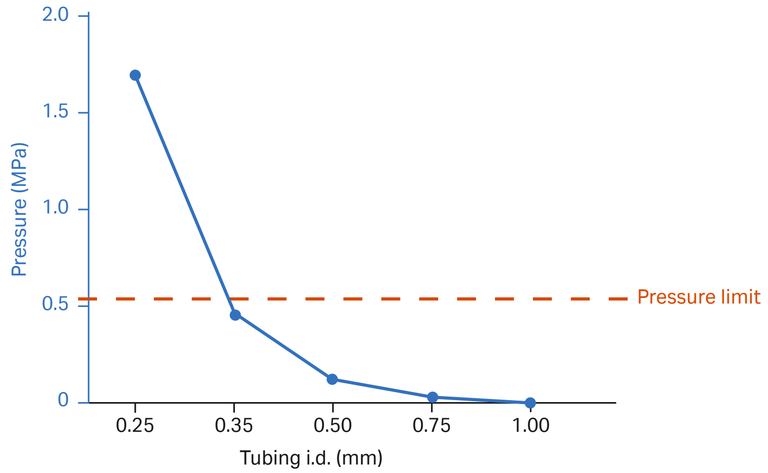


Fig 7.1. Effect of tubing i.d. on back pressure. Length of tubing: 200 cm. Flow rate: 10 ml/min. Solution used: water at room temperature.

Table 7.2. Pressure monitoring

Monitor	Measured pressure	Includes	Pressure limit protects
P_{System}	System pressure	Complete flow path including column	System*
p1†	Pre-column pressure	Column + flow path after column	Column hardware
p2†	Post-column pressure	Flow path after column	N/A
Δp = p1 – p2	Delta-column pressure	Column	Packed bed‡

* For systems with one pressure monitor at the pump, the column can still be protected by setting the system pressure limit to whichever is the lowest limit of either the column hardware or the packed medium bed.

† Available in ÄKTA avant and ÄKTA pure (Column Valve V9-C for five columns). For ÄKTA pure (without column valve or with Column Valve V9-Cs [for one column]), p1 is calculated based on system pressure (P_{system}), flow rate, temperature, and tubing dimensions.

‡ To fully utilize the potential of columns (e.g., Superdex Increase and Superose Increase columns) the individual delta-column pressure limit need to be determined. A detailed description on the procedure is available in the Instruction for use included with the columns.

Use Δp alarms for:

- Quality check of self-packed column
  A Δp that is too high for a newly packed column indicates that packing can be improved
- Notification for increased delta-pressure
  After repeated use the column collects impurities and the pressure will increase. To regain optimal column conditions, perform cleaning-in-place (CIP), and/or change the column top filter
- Flow regulation
  Adapt flow to avoid pressure alarm using ‘Pressure controlled sample application’ as described in the section *Pressure-controlled sample application*

Pressure monitoring

The total system pressure is generated by the complete system flow path. All ÄKTA chromatography systems measure this pressure at the system pump (Fig 7.2), while some systems also have additional sensors located before and after the column (p1 and p2). This allows calculation of the pressure drop (Δp) over the column, which gives useful information about the condition of the packed bed. In ÄKTA pure systems using no column valve or including a single column valve, p1 is calculated and displayed as pre-column pressure.

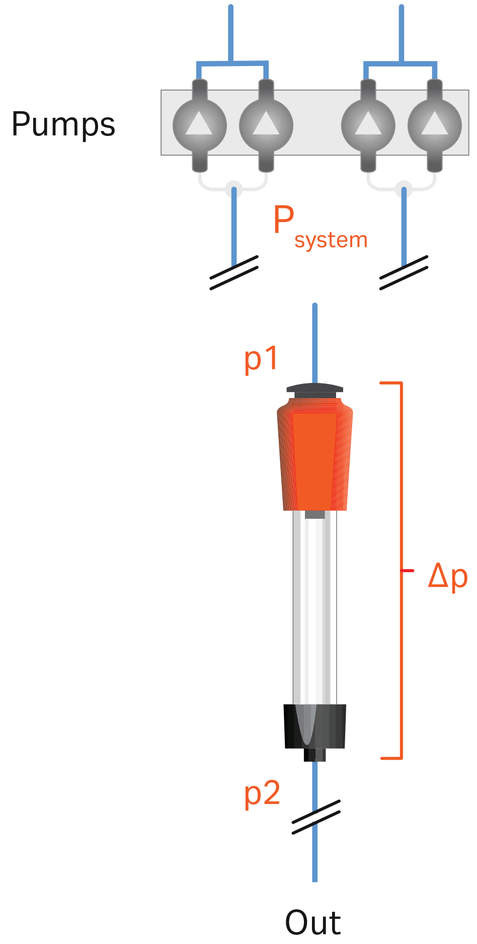


Fig 7.2. Pressure is measured in ÄKTA chromatography systems at the system pump pre-column (p1) and post-column (p2).

Different alarm limits can be set for the different pressure monitors as explained in Table 7.2.

The measured values include the tubing used to connect the column to the instrument.

Tubing dimension (inner diameter, length) could affect the pressure value.

The effect of back pressure on column and packed bed

The pressure is generated by the flow through the system. For optimal chromatography performance it is important to understand the principle of the pressure drop over the different parts of a chromatography system.

The column hardware and the packed bed have different pressure tolerance as described in Figure 7.3 and 7.4.

To protect the column hardware:

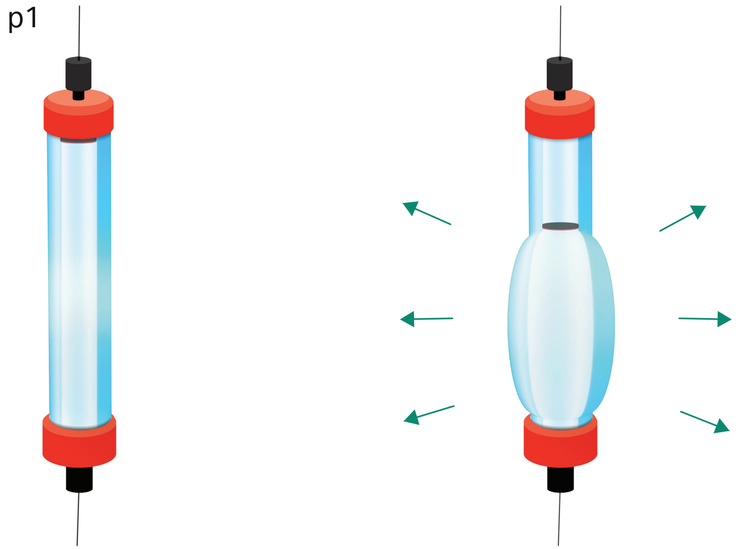
The pressure affecting the column hardware (p1) depends on the sum of back pressure generated by the column itself and the back pressure generated by the system after the column.

Column hardware pressure limit is the maximum pressure the hardware can withstand without being damaged. This value is fixed for each column type.

The column hardware pressure limit is included in User Instructions and in UNICORN column list for each column type, respectively.

To protect the packed bed:

The maximum flow rate is the maximum flow rate that the packed bed of chromatography medium can withstand without risking gap formation or bed collapse to occur. The maximum flow rate is found in the column instructions and UNICORN column type list and is determined at room temperature and with water. When changing running conditions such as temperature and viscosity, the maximum flow rate is affected (Table 7.3). It is therefore important to adjust the flow rate according to running conditions used.



For illustration only,
This is not how an actual column will look.

Fig 7.3. The pre-column pressure (p1) affects the column hardware. The column will break or start leaking when the column hardware pressure limit is reached.

Table 7.3. How to set maximum flow rate at commonly used running conditions; rule of thumb.

Liquid	Flow at room temperature	Flow in cold room	Typical application
Water	Max flow rate*	Max flow rate/2	†
20% ethanol	Max flow rate/2	Max flow rate/4	Storage
10% glycerol	Max flow rate/2	Max flow rate/4	Stabilization
2 M ammonium sulfate	Max flow rate/2	Max flow rate/4	HIC
0.5 to 1 M sodium hydroxide	Max flow rate*	Max flow rate/2	Cleaning

* Determined at room temperature with water
† Most commonly used buffers such as PBS and Tris have approximately the same viscosity as water

The maximum flow rate is fixed for a specific column type (column dimension and chromatography medium) at the defined viscosity and temperature while the maximum Δp generated at the maximum flow rate vary between individual columns.

Δp or maximum pressure drop over the packed bed is provided for each column type in column instructions and UNICORN column list and gives a starting value.

An adapted flow rate according to running conditions (Table 7.3) is a good way to protect the packed bed.

Function of a flow restrictor

A flow restrictor creates a steady back pressure. It therefore prevents air bubbles, which might interfere with detector signals, from forming after the column due to the column pressure drop. In addition, a flow restrictor can be used to prevent siphoning if, for example, solutions are placed above the pump. A flow restrictor can be compared to a cork on a bottle of champagne (Fig 7.5). The pressure generated by the restrictor will help to keep the air dissolved in the solution.

Fig 7.4. Δp is the pressure that affects the chromatography medium within the column. Maximum delta column pressure is defined as the pressure drop over the packed bed at maximum flow rate. Exceeding these limits can cause gap formation (indicated by arrow) or collapse of the packed bed.

Fig 7.5. The flow restrictor can be compared to the cork on a champagne bottle.

A hypothetical example of how a flow restrictor affects the packed column at a flow rate of 1 ml/min is shown in Figure 7.6. With no flow restrictor (Fig 7.6A), the flow rate generates a pump pressure reading of 0.3 MPa (3 bar, 44 psi). This pressure equals the pressure drop over both the chromatography medium and the column hardware. For simplification, the back pressure generated by tubing after the column is excluded in this example. When a flow restrictor generating a back pressure of 0.2 MPa (2 bar, 29 psi) is added after the column (Fig 7.6B), the pressure over the column hardware (p1) is affected and will be 0.5 MPa (5 bar, 72 psi). Hence, the system pressure reading at the system pump will be 0.5 MPa (5 bar, 72.5 psi). However, the pressure drop over the packed bed is still 0.3 MPa (3 bar, 44 psi), because Δp = p1 – p2.

The flow restrictor only affects the column hardware pressure whereas the pressure on the packed bed is unaffected.

Removal of flow restrictor

Our recommendation is to keep the flow restrictor inline because there is a risk of getting detector disturbances from air bubbles that are formed in the solution.

When using HiTrap™ and HiPrep™ columns with a system that monitors the pressure only at the pump, consider the following modification: Instead of removing the flow restrictor to avoid triggering the high pressure alarm, increase the pressure limit to include the pressure contribution from the flow restrictor (e.g., 0.2 MPa [2 bar, 29 psi]). Do not set the pressure limit to more than 0.5 MPa (5 bar, 73 psi), however, because this is the column hardware pressure limit for HiTrap and HiPrep columns. (Note: This has already been implemented in the UNICORN column list for supported columns).

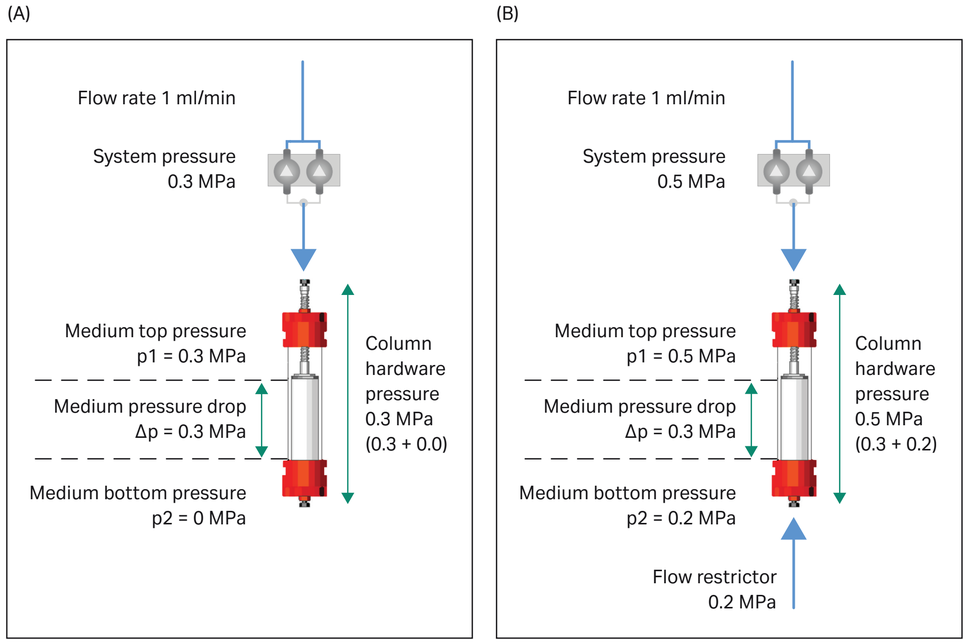


Fig 7.6. Flow restrictor effect on different pressure readings. (A) without flow restrictor, (B) with flow restrictor.

Troubleshooting high back pressure

A number of reasons could explain high back pressure (Table 7.1). A logical approach to identify the problem is presented in Figure 7.7. First bypass or disconnect the column. If the high pressure is released in bypass mode, the column needs to be checked and cleaned. See Appendix 4 for a workflow suggestion. If the high pressure is not related to the column, locate the system blockage as described below.

☞ For information about how to clean the column, please see the column instructions.

To find the flow path blockage, start the pump at a flow rate that will keep the pressure low enough so that the alarm is not triggered. Take note of the measured system pressure. Then, starting from the fraction collector, loosen the first connector. If there is no change in pressure, tighten it again and move to the next connector (toward the pump). Loosen this connector, check for any change in pressure, tighten, etc. until the one that releases the pressure has been located. This is where the flow path blockage is. Very often the blockage is caused by obstructed tubing. If this is the case, replace as needed. In less severe cases, perform cleaning in place of the system flow path.

In some cases, a system calibration is needed to reset the pressure sensors. This should be performed at zero pressure. For details, see the specific system user manual.

Viscous samples and solutions

The system back pressure is affected by the viscosity of a liquid. Some salts, high salt concentrations, and low temperatures increase viscosity of the liquids, as well as mixtures between organic and aqueous solutions. Crude samples (e.g., cell lysates) are often highly viscous.

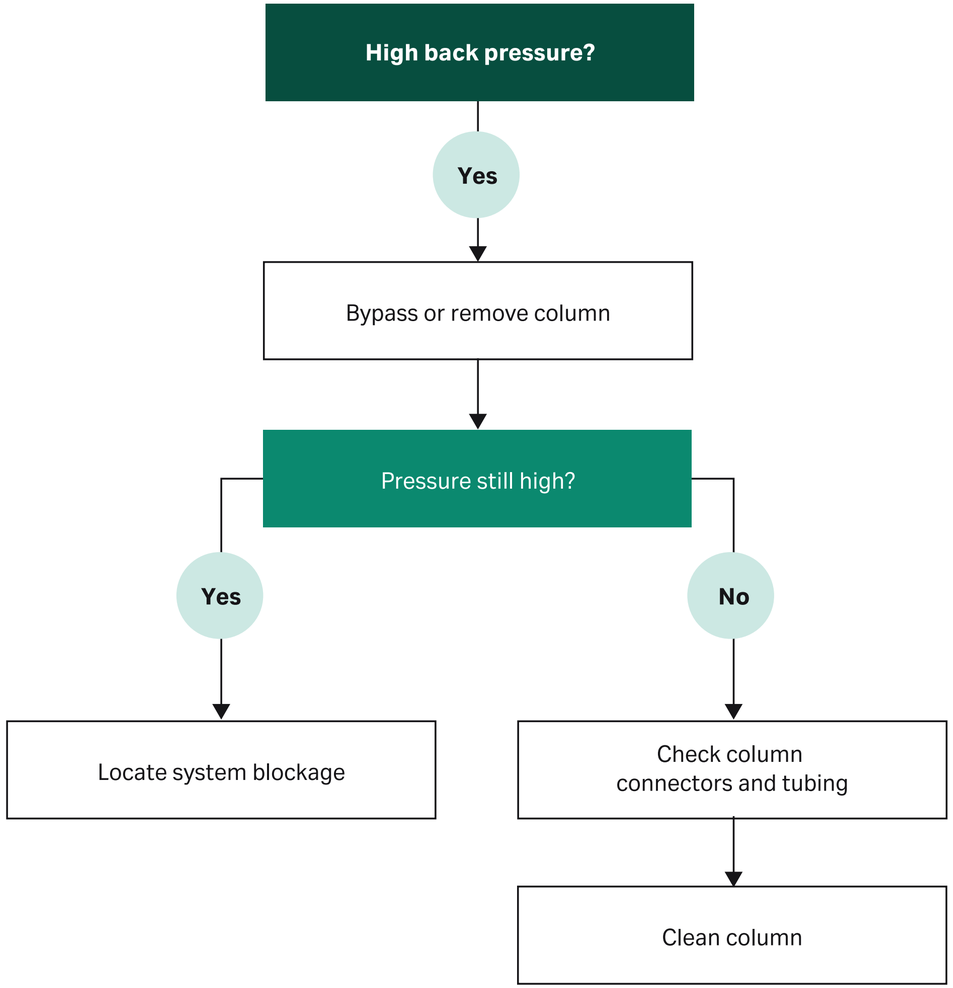


Fig 7.7. Troubleshooting high back pressure.

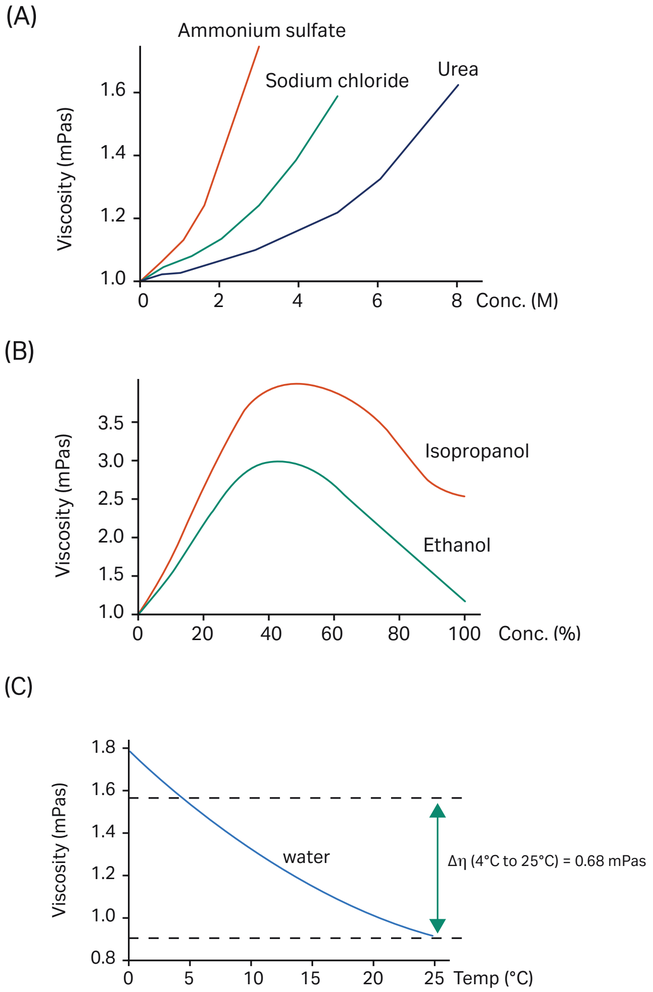


Fig 7.8. Effect of type of salt, salt concentration (A), content of organic solvents in water (B), and temperature (C) on viscosity. 1 mPas = 1 cP.

Figure 7.8 shows some examples of how the viscosity varies for commonly used liquids and temperatures in chromatography.

The viscosity of common buffers and solutions, even including 1 M NaCl and 1 M NaOH, will only be slightly higher than water and will therefore normally not become an issue during chromatographic runs (Fig 7.8A). When mixing water with organic solvents (e.g., 20% ethanol), the viscosity will be significantly higher (Fig 7.8B) and the generated back pressure substantially increased. This phenomenon is noticed when washing ethanol from a column. To keep within the pressure limit, the flow rate needs to be reduced.

The pressure increases with decreasing temperature because viscosity is temperature dependent. At cold-room temperature (approximately 4°C), the pressure generated will be nearly twice as high compared with a room-temperature (approximately 25°C) run (Fig 7.8C). Due to the column pressure limit, a decreased flow rate is needed to avoid high pressure.

Pressure-controlled sample application

When applying sample, the buildup of material on the column can be significant, leading to the pressure limit being reached and the alarm triggered. The buildup consists of contaminants such as denatured proteins, nucleic acids, and lipids. This buildup of material can occur even if the sample has been clarified before the run.

In some ÄKTA systems, pressure-controlled sample application can be used. During the run, the system will then monitor the pressure, and if it approaches the set pressure limit, the flow rate will gradually decrease to avoid triggering the alarm.

Figure 7.9 shows an example in which 150 ml of a sample was applied onto a column. After approximately 110 ml (i.e., 44 min), the pressure became too high and the flow rate was automatically down-regulated so that the pressure stayed at an acceptable level. When the pressure decreased (during the wash phase), the flow rate was automatically up-regulated.

Column:	HiScreen™ Capto™ adhere
Sample:	Elution pool from MabSelect SuRe™; pH 6.75, conditions adjusted to 15 mS/cm conductivity with NaCl
Load:	Flowthrough mode, 150 ml of 200 mg MAb/ml
Flow rate:	2.5 ml/min
System:	ÄKTA avant 25

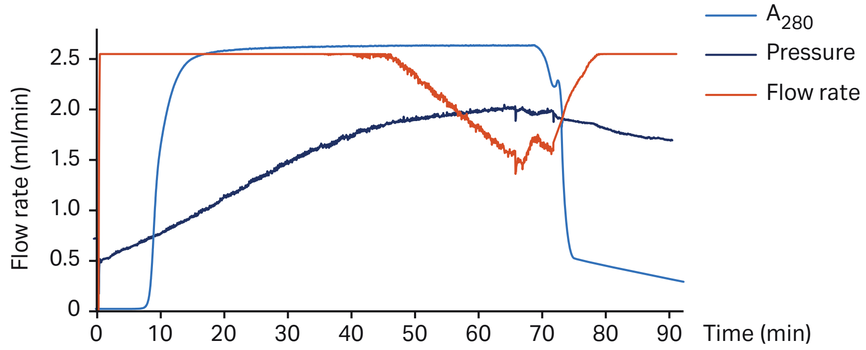


Fig 7.9. Pressure-controlled sample application.

08

Sample monitoring and detectors

Sample monitoring and detectors

Inline detectors are used in protein purification. To monitor the purification process, it is common to use a UV/Vis absorbance detector, because a majority of proteins will absorb light at 280 nm. The area under the absorbance curve corresponds to the protein concentration and gives an indication of the amount of protein.

Other types of detectors can be used to gather more information about the purification process, for example, conductivity and pH monitors.

Monitoring UV/Vis absorbance

Wavelength to use

Measuring UV absorbance at 280 nm will provide information about eluted proteins and the total protein content. The ability of proteins to absorb UV light is predominantly due to the presence of tryptophan, tyrosine, and phenylalanine, which strongly absorb at 280 nm. However, some proteins have only a few or nonexposed aromatic amino acid residues and therefore show weak absorbance at 280 nm.

Apart from proteins, other biomolecules also have the ability to absorb light. For a purification scheme it is sometimes useful to check these. Table 8.1 shows some examples of wavelengths that can be used to detect different biomolecules.

If obtaining a low absorption reading at 280 nm, try detection at 214 nm where peptide bonds absorb light.

ÄKTA UV/Vis absorbance detectors are linear up to 2000 mAU. Signals higher than this are not proportional to the protein concentration.

Some chromatography systems have multiwavelength detectors that view target protein and critical impurities simultaneously. Some proteins absorb at multiple wavelengths, for example, GFP, which also has an absorbance maximum at 490 nm. Measuring at both 280 and 490 nm will in this case help to identify which peak contains the target protein (Fig 8.1).

Table 8.1. Wavelength to detect different biomolecules

Wavelength (nm)	Absorption
214	Peptide bonds, part of peptides and proteins
230	Organic compounds or chaotropic salts
260	DNA/RNA
280	Aromatic amino acids residues (tryptophan, tyrosine, and phenylalanine)
390/420	Coenzymes (e.g., in hemoproteins)
490	Green fluorescent protein (GFP)
600	Protein aggregates

The possibility of gaining information about contaminants in the sample can be useful during purification. Strong absorbance around 230 nm indicates that organic compounds or chaotropic salts are present. A high reading at 260 nm indicates the presence of nucleic acids.

The ratio A_{260}/A_{280} is a measure of DNA and/or RNA purity, and is thus a useful analysis method when purifying DNA or RNA. If the ratio is close to 2 it indicates highly pure DNA/RNA.

How to calculate protein concentration and amount

The software for ÄKTA chromatography systems includes functionality for calculating concentrations and amounts. Simply enter the extinction coefficient for the protein and the path length of the UV/Vis flow cell used, and the software will calculate concentration and amount based on the UV absorbance data at 280 nm.

To obtain highly accurate results, two criteria are very important:

1. The UV/Vis absorbance signal must be within the linear range of the UV/Vis detector.
2. The exact UV/Vis flow cell path length should be used in the calculation.

For ÄKTA avant, the exact path length has been predetermined and is included in the software calculations. For other systems, the exact path length of each individual system needs to be determined experimentally by measuring the absorbance of one or several solutions with known absorbance (see *Ordering information* to determine which UV/Vis flow cell calibration kit to use). When using a UV/Vis flow cell calibration kit, the exact path length is determined according to Lambert-Beer's law (see below). The exact path length must then be manually entered into the system setting of the software.

Which UV/Vis flow cell to use

According to Lambert-Beer's law, the relationship between absorbance and concentration can be described as:

$A = \varepsilon \times b \times c$

where

A = absorbance, ε = extinction coefficient, b = cell path length, and c = concentration.

To get the absorbance signal within the linear absorbance range, different cell lengths can be chosen (described in Table 8.2).

The amino acid sequence of a protein can be used to calculate its theoretical absorbance coefficient. Web-based calculators are available to assist in determining this number. See, for example, *http://www.biomol.net/en/tools/proteinextinction.htm* for one such calculator.

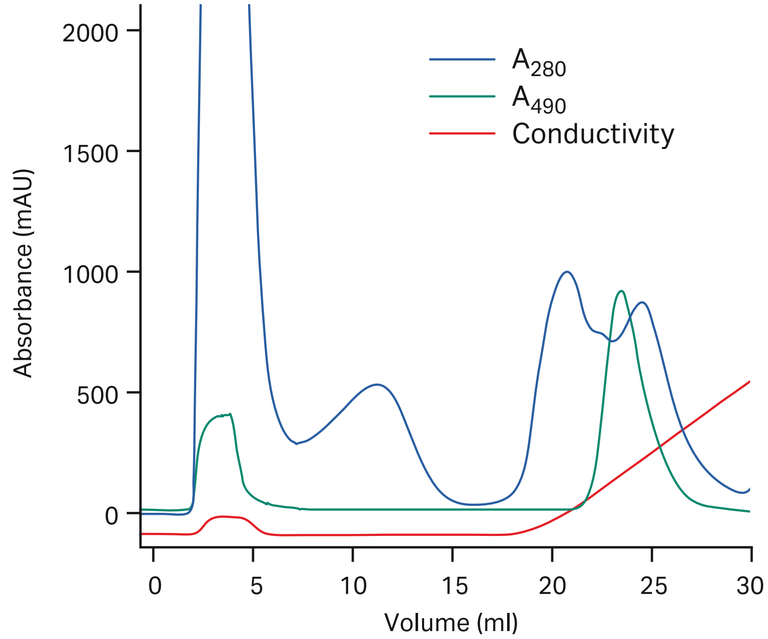


Fig 8.1. Specific detection of GFP by absorbance measurements at 490 nm.

Table 8.2. Effect of UV/Vis flow cell path length

Absorbance signal	Effect of UV/Vis flow cell path length
Low	Switching to a longer cell will increase the signal
High	If the absorbance is outside of the linear range, switching to a shorter cell will decrease the signal

Liquids and compounds

During purification runs, different solutions and compounds can cause deviations in the UV/Vis absorbance curve. Table 8.3 includes a list of some common examples and how to address them.

Noisy and insensitive UV/Vis absorbance measurements

The most common cause of noisy and insensitive UV/Vis absorbance curves is a dirty flow cell. Clean the flow cell as described in Chapter 10.

The problem can also be due to air bubbles within the flow cell. See the discussion on flow restrictors in Chapter 7 for more details.

An aging UV lamp

With time, the light intensity of the UV/Vis absorbance detector lamp will decrease. When a low intensity warning is given, it is time to replace the lamp.

Note: The displayed UV/Vis absorbance signal will be the correct value as long as no intensity warning is issued. This is possible because the detector uses a reference signal against which the measured UV/Vis absorbance is normalized.

Table 8.3. Dealing with deviations in the UV/Vis absorbance curve

Effect	Cause	What to do
Unexpected drift in the UV/Vis absorbance curve or False negative or positive peaks	Difference in refractive index, e.g., when switching from: • Water to organic solvent during RPC • High salt to no salt during HIC	Switch to a solvent with a different refractive index if possible When evaluating results and performing peak integration, adjust the baseline
High UV/Vis absorbance baseline	The solution is absorbing UV light, e.g.,: • Citrate buffers at 214 nm • Impure imidazole at 280 nm • Oxidized form of DTE at 280 nm	Use another buffer system instead of citrate if possible Use imidazole of high purity DTE, a reducing agent, oxidizes over time. Use only freshly made solutions.

Monitoring conductivity

The conductivity monitor is used to detect changes in salt concentration and other charged molecules during a chromatographic run. It can be used to gather a variety of information as described in Table 8.4.

A current is applied across the conductivity cell, and the electrical resistance between the electrodes is measured and used to calculate the conductivity in the eluent.

The conductivity is linear only to a salt concentration of approximately 0.3 M. It is therefore important to measure the conductivity of the solution that is used rather than calculating it.

In a salt gradient, a decrease in linearity will be seen with increasing salt concentration (Figure 8.2).

Conductivity measurements are temperature dependent

The conductivity signal will increase with temperature according to:

$C_t = C_{t\,cal}\,(1 + \alpha)^{\Delta t}$

where C_t = the measured conductivity; $C_{t\,cal}$ = conductivity at reference temperature; and Δt = the difference between reference temperature and actual temperature.

The constant α is concentration- and salt-dependent, but 0.02 is a good mean value for many salts.

In all ÄKTA systems, a temperature sensor is mounted within the conductivity cell to allow temperature compensation. The compensated conductivity value is displayed, which means that conductivity curves generated at different temperatures can be compared.

Table 8.4. Examples of information gathered from conductivity monitoring

Used during	Used for
Equilibration	Stable signal indicates that column is equilibrated
Sample application and wash	Detection of salt peaks
Gradient elution	Monitoring gradient formation
Desalting	Detection of salt peaks
System troubleshooting	Erroneous flow rate seen as disturbances in conductivity curve

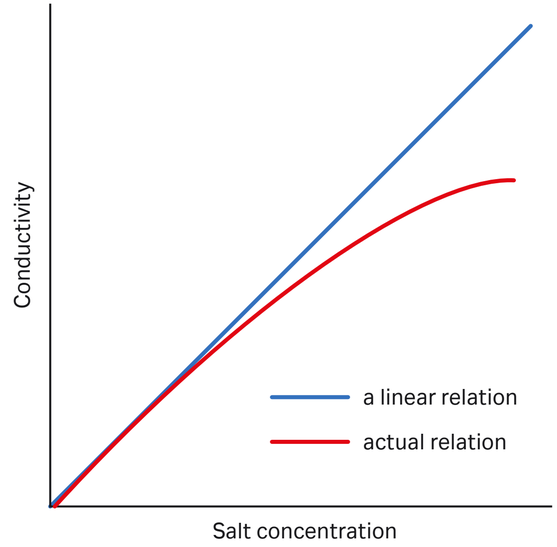


Fig 8.2. Illustrated relationship between conductivity and salt concentration.

Monitoring pH

For most ÄKTA systems, a pH electrode can be connected at the low pressure side (i.e., after the UV/Vis absorbance detector). To receive accurate measurements, it is important to calibrate the pH electrode. pH electrodes are sensitive to, for example, 20% ethanol, and it is therefore important to store them in appropriate storage solutions (see the pH detector's user manual).

If a FR-904 flow restrictor is used, make sure that the pH sensor is placed after the flow restrictor because it cannot withstand the back pressure generated.

Other detectors

With some systems, it is possible to incorporate signals from an external detector, that is, from a non ÄKTA detector. This can be useful for applications where, for example, highly sensitive detectors or more qualitative information is needed.

Common detectors used in combination with ÄKTA systems includes fluorescence, light scattering, and refractive index.

09

Fraction collection

Fraction collection

Preparative chromatography requires that material eluted from the column is collected. Two common methods, employing either a fraction collector or a multiport outlet valve, are used to direct the eluent to different containers (tubes or bottles). Table 9.1 compares these methods.

The volume of the collected fractions is often different during different steps in a chromatographic run. During sample application, larger fraction volumes are collected as a safety measure in case the target protein were to pass straight through the column. The flowthrough is collected in one or a few fractions corresponding to the volume of the sample applied and the subsequent wash. During elution, smaller fraction volumes are usually collected, and an eluting peak is normally divided into a number of fractions in order to obtain pure protein from overlapping peaks.

Different fractionation modes can often be chosen for fraction collectors that have tubes or wells positioned in rows. Collection can then often be performed from left to right for each row or in serpentine mode, where every other row goes in the opposite direction. When serpentine mode is chosen, the risk of spillage is minimized.

Straight fractionation and peak fractionation

To be able to analyze different parts of the peak, the fraction size during elution is usually set to a value smaller than the expected peak volume. When straight (sometimes called fixed) fractionation is used, the fraction collector will continuously switch tubes according to the set volume throughout the entire fractionation, as shown in Figure 9.1A. To further increase the purity of the collected protein peaks, “peak fractionation” can be used. The UV/Vis detector is then used to determine when to start and stop peak fractionation, as shown in Figure 9.1B. Straight fractionation and peak fractionation can also be combined during a run.

Table 9.1. Two methods for collecting purified sample

Fraction collector	Outlet fractionation
Fraction size 100 µl to 250 ml	Fraction size > 5 ml
Possible to collect many fractions (typically 20 to 200)	Number of fractions limited to number of outlet valve ports (typically 8 to 10/valve)
Used for complex samples where several peaks are expected	Used when a few, defined peaks are expected

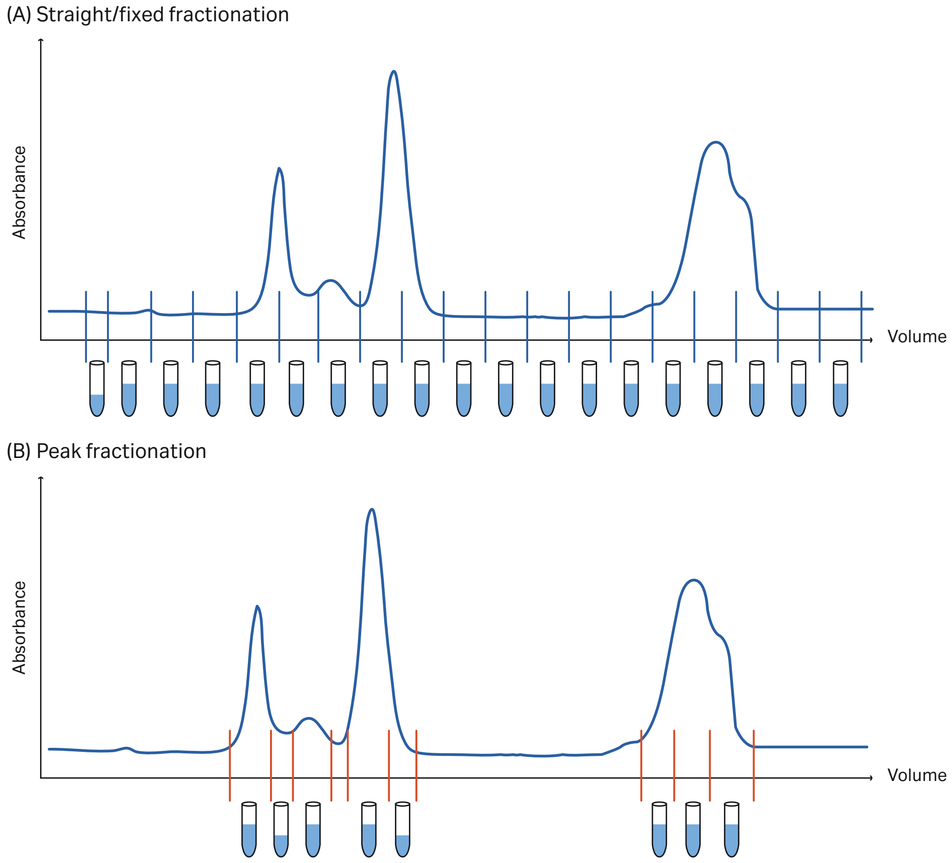


Fig 9.1. Straight fractionation (A), Peak fractionation (B).

Fractionation delay volume

The fractionation delay volume is the volume between the UV/Vis detector's flow cell and the fraction collector. It is important that the correct delay volume is entered in the software. The defined delay volume will be used by the system to calculate the time T_1, which is when the peak reaches the fraction collector. T_1 is used to synchronize the fractionation marks in the chromatogram with the tube switch of the fraction collector (Fig 9.2). At the start of the fraction collection, the delay volume is directed to waste or the first fractionation tube depending on which system is used.

The delay volume depends on the tubing and components included in the flow path. Determine the delay volume theoretically or experimentally by including the volume from all tubing and components between the absorbance detector and the fractionation tip. Appendix 3 provides a detailed description of how to determine the delay volume.

Remember to include the tubing to and from the fraction collector's accumulator if that is used.

Spillage-free fractionation

To minimize spillage, a drop synch function is often included in ÄKTA fraction collectors. A sensor at the fraction collector outlet detects the presence of droplets and synchronizes tube change. The maximum flow rate for drop sync depends on the surface tension of the liquid and the i.d. and shape of the fractionation tubing tip. When the liquid starts to flow continuously, it cannot be used. The maximum flow rate is also limited by how fast droplets can be detected. Typically, drop sync can be used for lower flow rates (i.e., below 2 to 3 ml/min).

Another way to avoid spillage between fractionation tubes is to include an accumulator. During tube change, the accumulator stores liquid, which is then pushed out rapidly when a new tube is in position for collection. The accumulator can be used for higher flow rates and is included in Frac-950 and the fraction collector of ÄKTA avant. See Appendix 5 for how to troubleshoot fraction collection issues.

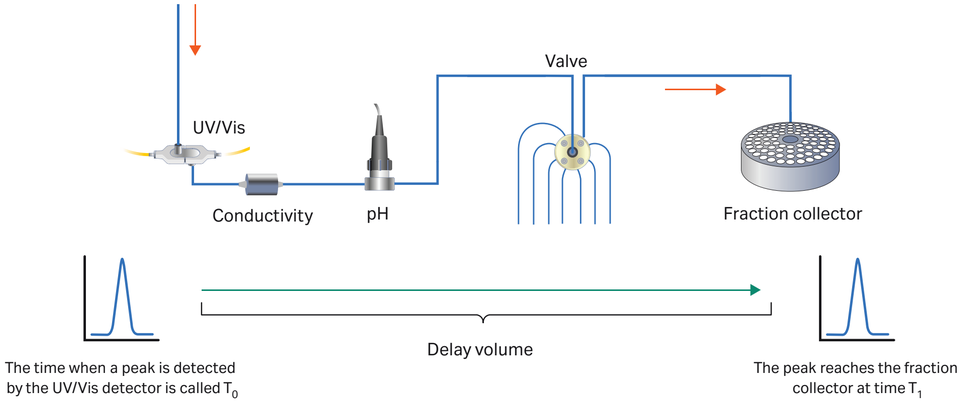


Fig 9.2. T_1 is the time when the fraction collector moves in order to collect the fractions to match what was detected in the UV/Vis detector. $T_1 = T_0$ + Delay volume /flow rate.

10

Cleaning and storage of system components

Cleaning and storage of system components

System lifetime and performance will be maximized if proper cleaning and storage routines are followed. This chapter describes how to maintain the system. Table 10.1 provides maintenance tips to help keep the system running problem-free for a long period of time.

Table 10.1. Tips on preventive maintenance

Purpose	What to do
Keep back pressure low by preventing particles entering the flow path	Use filtered solutions. A filter pore size of 0.45 µm is recommended. Use inlet filters on all inlet tubing (Fig 10.1A) Replace the inline filter regularly (Fig 10.1B)
Clean system to prevent carryover between runs and contamination of the flow path	Clean the system flow path regularly with 0.5 to 1 M NaOH Create appropriate cleaning procedures Replace pump rinse solution (20% ethanol) once a week (only applicable for pumps with a rinsing system)
Keep system clean to prevent microbial growth in the flow path	Use 20% ethanol as storage solution when system will not be used for 2 days or more
Avoid condensation in system components	Leave the power ON if the system is in cold room (the UV/Vis absorbance detector lamp can be turned off to save lamp run time) When the system is moved to a new temperature, allow some time (usually hours) for it to adjust to ambient temperature
Protect exterior of the instrument	Wipe off spillage to avoid corrosion of metal parts
Prolong lifetime of the UV/Vis absorbance detector lamp	Turn off the UV/Vis absorbance detector lamp on UPC-900 when not in use
Avoid software connection problems	If system is controlled by a computer, reboot the system and PC at least every 14 days. Remove temporary files regularly

(A)

(B)

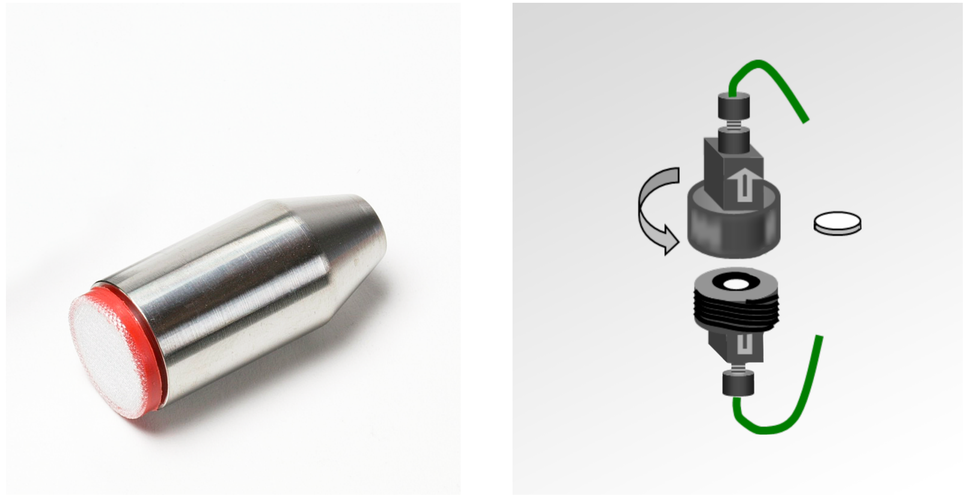


Fig 10.1. Inlet filter (A) is mounted on the inlet tubing. Inline filter (B) is connected in the flow path after or in the mixer. Note: An inline filter is also referred to as an online filter in some literature.

Cleaning the system

Minimal cleaning

Because salt from buffer solutions might precipitate and clog valves and tubing, wash the entire system flow path with buffer or water after every run. It is also important to remove any sample from the inlet tubing as soon as possible after each run.

Thorough cleaning

A general cleaning recommendation is to flush the system once a week when it is in use, with 0.5 to 1 M NaOH. Start with flushing the pumps at a moderate flow rate.

Prepare a system cleaning method — and use it! Perform cleaning without a column attached or by bypassing the column(s). Make sure that the entire flow path is cleaned, and change valve position while washing the system flow path with cleaning solution. Clean all tubing including the fraction collector tubing, accumulator, and the manual injection port.

See Table 10.2 for suggested cleaning solutions.

By generating a back pressure, for example, by placing flow restrictors on used waste tubing, the cleaning solution will, during the cleaning method, more easily reach “dead” spaces, for example, within valves.

Wear gloves and safety glasses when using hazardous/corrosive chemicals.

Make sure that valve ports without tubing connected are plugged during cleaning and that all waste tubing is inserted in waste containers.

System storage

Store the system in 20% ethanol to prevent microbial growth when not in use for more than 2 days. When preparing the system for storage, prevent precipitation of buffer components upon mixing with ethanol by rinsing the system with water. Then fill the system with a 20% ethanol solution. Make sure that the entire flow path is filled — including all inlet and outlet tubing.

For some ÄKTA systems, premade methods for preparing the system for storage are included in the software.

Prior to using the system after storage, remove the ethanol using water.

Table 10.2. Cleaning solution suggestions to use for system components, excluding the pH electrode

To remove	Use
Buffers and salts	Water
Proteins, lipids, cell debris	0.5 to 1 M NaOH
Proteins, lipids, and cell debris not removed by NaOH	1 to 10 M acetic acid
Lipids and other hydrophobic components not removed by NaOH or acetic acid	Organic solvent, for example, 100% isopropanol

Cleaning recommendations

System pump

Because precipitated salts can clog valves and shorten seal lifetime, it is important to rinse the pump with buffer or water as soon as possible after a run.

Most ÄKTA pumps have a rinsing system with a circulating 20% ethanol solution. Figure 10.2 shows an overview of the rinsing system. The rinsing solution is in contact with the back side of the pump heads at all times and prevents microbial growth. Change the rinsing solution once a week. Note that the solution evaporates over time. If the optional path (without recirculation) is used, the rinsing solution needs to be filled more frequently.

Sample pump

Rinse the sample pump after each run, with water, buffers, or cleaning agents that remove any sample traces. For a sample pump with a rinsing system, for example, ÄKTA avant, follow the same procedure as described above.

UV/Vis flow cell

The cleaning requirement of the UV/Vis flow cell will vary. For general cleaning, use 10% Decon™ 90 as described below. If use of a detergent is not desired or if the cell is not sufficiently clean after use of 10% Decon 90, test one of the solutions listed in Table 10.3.

Use a syringe to inject a small amount of 10% Decon 90 detergent directly into the flow cell and leave it for at least 20 min before rinsing with water. For a rigorous cleaning, use either warm Decon 90 solution (approximately 60°C) or leave the flow cell with the detergent overnight.

Table 10.3 lists other solutions recommended for cleaning the UV/Vis flow cell.

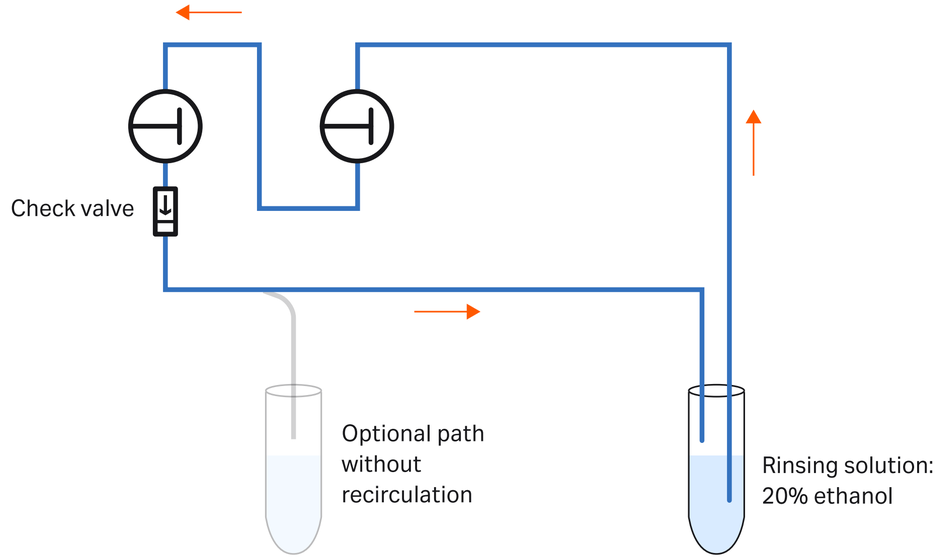


Fig 10.2. Schematic view of the pump rinsing system.

Table 10.3. Solutions for cleaning absorbance detector flow cell

To remove	Use
Buffers, salts, and detergents	Water
Proteins	0.5 to 1 M NaOH for 15 min, then flush with water
Lipids and other hydrophobic components	30% to 100% isopropanol, then flush with water

pH electrode

The pH electrode is one of the most sensitive components in the system. Recommended solutions for cleaning the pH electrode are listed in Table 10.4.

After the electrode has been cleaned, it needs to be restored and calibrated. See the user manual for guidance.

Fraction collector

It is important to keep both the exterior of the fraction collector and the drop synch photo cell clean. Wipe off spillage immediately and use a cloth and water or mild cleaning agent to clean the exterior. The drop sync photo cell should be wiped carefully with a damp cloth. Usually the tube rack can be disassembled for cleaning.

After cleaning, check that the fractionation tubing is positioned correctly and that it does not block the light path of the drop sensor. If the light path is blocked, an error message will be received during fractionation.

For fraction collectors that have an accumulator for spillage-free fractionation, remember to also add an accumulator wash in the system cleaning method.

Table 10.4. Solutions for cleaning pH electrode

To remove	Use
Salt deposits	Alternating 100 mM HCl and 100 mM NaOH
Lipid deposits	Detergent or organic solvent
Protein deposits	1% pepsin in 100 mM HCl. Remove thoroughly afterwards!

Appendix

Appendix 1
System components in laboratory-scale ÄKTA systems

Table A1.1 highlights some system components and the ÄKTA system they relate to. For each component, information such as pressure limit, flow rate range, internal volume, etc., is noted.

Table A1.1. Parameters for ÄKTA system components

Pumps	Flow rate range	Stroke volume[1]/ total internal volume	Max. pressure	Used with[2]
ÄKTA start peristaltic pump	0.5 to 5 ml/min (for wash up to 10 ml/min)	N/A/Internal volume: 126 µl	0.5 MPa (5 bar, 73 psi)	ÄKTA start
P-901	0.01 to 100 ml/min	286 µl/1.4 ml	10 MPa (100 bar, 1450 psi)	ÄKTApurifier UPC 100, ÄKTApurifier 100, ÄKTAexplorer 100
P-903	0.001 to 10 ml/min	36 µl/0.6 ml	25 MPa (250 bar, 3625 psi)	ÄKTApurifier UPC 10, ÄKTApurifier 10, ÄKTAexplorer 10
P-905	0.001 to 2 ml/min	36 µl/0.6 ml	35 MPa (350 bar, 5075 psi)	ÄKTAmicro
P-920	0.01 to 20 ml/min	10 ml/10 ml	5 MPa (50 bar, 725 psi)	ÄKTA$_{FPLC}$
P-960	0.1 to 50 ml/min	200 µl/1 ml	2 MPa (20 bar, 290 psi)	Sample pump to ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer
P9	0.001 to 25 ml/min	54 µl/0.55 ml	20 MPa (200 bar, 2900 psi)	ÄKTA avant 25, ÄKTA pure 25
P9-S	0.01 to 25 ml/min (for wash up to 65 ml/min)	286 µl/1.4 ml	10 MPa (100 bar, 1450 psi)	Sample pump to ÄKTA avant 25 and ÄKTA pure 25
P9H	0.01 to 150 ml/min	429 µl/1.8 ml	5 MPa (50 bar, 725 psi)	System and sample pump to ÄKTA avant 150 and ÄKTA pure 150

[1] Stroke volume is the amount of liquid that is pushed out from the pump per piston.

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

Mixers	Internal volume	Max. pressure	Used with[2]
M-925 (magnetic stirrer)	90 µl	35 MPa (350 bar, 5075 psi)	ÄKTAmicro
M-925 (magnetic stirrer)	0.2 ml	35 MPa (350 bar, 5075 psi)	ÄKTAmicro
M-925 (magnetic stirrer)	0.6 ml	25 MPa (250 bar, 3625 psi)	ÄKTA$_{FPLC}$, ÄKTApurifier UPC 10, ÄKTApurifier 10, ÄKTAexplorer 10
M-925 (magnetic stirrer)	2 ml	25 MPa (250 bar, 3625 psi)	ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer
M-925 (magnetic stirrer)	5 ml	25 MPa (250 bar, 3625 psi)	ÄKTApurifier UPC 100, ÄKTApurifier 100, ÄKTAexplorer 100
M-925 (magnetic stirrer)	12 ml	10 MPa (100 bar, 1450 psi)	ÄKTApurifier UPC 100, ÄKTApurifier 100, ÄKTAexplorer 100
Static mixer	0.37 ml	3 MPa (30 bar, 435 psi)	ÄKTA start
M9	0.6 ml	20 MPa (200 bar, 2900 psi)	ÄKTA avant 25, ÄKTA pure 25
M9	1.4 ml	20 MPa (200 bar, 2900 psi)	ÄKTA avant, ÄKTA pure
M9	5 ml	20 MPa (200 bar, 2900 psi)	ÄKTA avant, ÄKTA pure
M9	15 ml	5 MPa (50 bar, 725 psi)	ÄKTA avant 150, ÄKTA pure 150

Inline filter	Internal volume	Max. pressure	Used with[2]
Filter holder	115 µl	35 MPa (350 bar, 5075 psi)	ÄKTApurifier UPC 100, ÄKTApurifier 100, ÄKTAexplorer 100
Filter holder	20 µl	35 MPa (350 bar, 5075 psi)	ÄKTApurifier UPC 10, ÄKTApurifier 10, ÄKTAexplorer 10,ÄKTAmicro
Filter holder included in mixer M9	50 µl	20 MPa (200 bar, 2900 psi)	ÄKTA avant, ÄKTA pure

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

Absorbance detector flow cells	Cell volume/total volume	Max. pressure	Used with[2]
2 mm for UPC-900	2 µl/30 µl	4 MPa (40 bar, 580 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTA start
5 mm for UPC-900	6 µl/20 µl	4 MPa (40 bar, 580 psi)	ÄKTAFPLC, ÄKTApurifier UPC
2 mm for UV-900	2 µl/7 µl	2 MPa (20 bar, 290 psi)	ÄKTApurifier, ÄKTAexplorer
3 mm for UV-900	0.7 µl/3 µl	2 MPa (20 bar, 290 psi)	ÄKTAmicro
10 mm for UV-900	8 µl/13 µl	2 MPa (20 bar, 290 psi)	ÄKTApurifier, ÄKTAexplorer
0.5 mm for U9-M	1 µl/10 µl	2 MPa (20 bar, 290 psi)	ÄKTA avant, ÄKTA pure
2 mm for U9-M	2 µl/11 µl	2 MPa (20 bar, 290 psi)	ÄKTA avant, ÄKTA pure
10 mm for U9-M	8 µl/12 µl	2 MPa (20 bar, 290 psi)	ÄKTA avant, ÄKTA pure
2 mm for U9-L	2 µl/30 µl	2 MPa (20 bar, 290 psi)	ÄKTA avant, ÄKTA pure
5 mm for U9-L	6 µl/20 µl	2 MPa (20 bar, 290 psi)	ÄKTA avant, ÄKTA pure

Conductivity flow cells	Internal volume	Max. pressure	Used with[2]
Flow cell	24 µl	5 MPa (50 bar, 725 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer, ÄKTA start
Flow cell	22 µl	5 MPa (50 bar, 725 psi)	ÄKTA avant, ÄKTA pure
Flow cell	2 µl	35 MPa (350 bar, 5075 psi)	ÄKTAmicro

pH flow cells	Internal volume	Max. pressure	Used with[2]
Standard cell	88 µl	0.5 MPa (5 bar, 73 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer
V9-pH	76 µl	0.5 MPa (5 bar, 73 psi)	ÄKTA avant 25, ÄKTA pure 25
V9H-pH	76 µl	0.5 MPa (5 bar, 73 psi)	ÄKTA avant 150, ÄKTA pure 150

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

Flow restrictors	Internal volume	Back pressure from 10 ml/min water at 20°C	Used with[2]
FR-902	10 µl	0.2 MPa (2 bar, 29 psi)	ÄKTA$_{FPLC}$, ÄKTApurifier 100, ÄKTAexplorer 100, ÄKTA avant, ÄKTA pure
FR-904	10 µl	0.4 MPa (4 bar, 58 psi)	ÄKTApurifier UPC 10, ÄKTApurifier 10, ÄKTAexplorer 10, ÄKTAmicro

Air sensors	Internal diameter	Connector to use	Used with[2]
Air-912 N[3]	1.2 mm	Fingertight connector 1/16" M	ÄKTA$_{FPLC}$, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer, P-960
Air-925 N[3]	2.5 mm	Tubing connector for o.d. 3/16'' with blue ferrule for 3/16'' o.d. tubing or Tubing connector for o.d. 1/8'' with yellow ferrule	ÄKTA$_{FPLC}$, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer, P-960
Air-915 N[3]	1.5 mm	Tubing connector for o.d. 1/8'' with yellow ferrule	ÄKTA$_{FPLC}$, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer, P-960
L9-1.2	1.2 mm	Fingertight connector 1/16" M	ÄKTA avant, ÄKTA pure
L9-1.5	1.5 mm	Tubing connector for o.d. 3/16'' with blue ferrule for 3/16'' o.d. tubing or Tubing connector for o.d. 1/8'' with yellow ferrule	ÄKTA avant, ÄKTA pure
Built-in air sensor	1.5 mm	—	ÄKTA avant and ÄKTA pure inlet valves: V9-IA, V9H-IA, V9-IB, V9H-IB, V9-IS, and V9H-IS

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

[3] To connect to ÄKTA$_{FPLC}$, ÄKTApurifier UPC, ÄKTApurifier, and ÄKTAexplorer, use the Air-900 N control box.

Valves	Internal volume	Max. pressure	Used with[2]
FV-923	113 µl	0.2 MPa (2 bar, 29 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAmicro
SV-903	113 µl	0.2 MPa (2 bar, 29 psi)	ÄKTApurifier, ÄKTAexplorer, ÄKTAmicro, ÄKTA start
IV-908	26 µl	2 MPa (20 bar, 290 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer
PV-908	7 µl	25 MPa (250 bar, 3625 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer
PV-908H	30 µl	3.5 MPa (35 bar, 508 psi)	High Flow kit for ÄKTApurifier UPC 100, ÄKTApurifier 100, ÄKTAexplorer 100
INV-907 port 1/7 port 2/3	 9 µl 5 µl	25 MPa (250 bar, 3625 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer
INV-907H port 1/7 port 2/3	 41 µl 28 µl	3.5 MPa (35 bar, 508 psi)	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer
INV-917	1.5 µl	35 MPa (350 bar, 5075 psi)	ÄKTAmicro
V9-IA, V9-IB, V9-IS, V9-I2, V9-IX	88 µl	1 MPa (10 bar, 145 psi)	ÄKTA avant 25, ÄKTA pure 25
V9H-IA, V9H-IB, V9H-IS, V9H-I2, V9H-IX	212 µl	2 MPa (20 bar, 290 psi)	ÄKTA avant 150, ÄKTA pure 150

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

Valves continued	Internal volume	Max. pressure	Used with[2]
V9-Inj	9 µl	20 MPa (200 bar, 2900 psi)	ÄKTA avant 25
V9H-Inj	23 µl	5 MPa (50 bar, 725 psi)	ÄKTA avant 150
V9-C	110 µl	20 MPa (200 bar, 2900 psi)	ÄKTA avant 25
V9H-C	191 µl	5 MPa (50 bar, 725 psi)	ÄKTA avant 150
V9-pH	15 µl (via bypass)	10 MPa (100 bar, 1450 psi)	ÄKTA avant 25
V9H-pH	36 µl (via bypass)	2 MPa (20 bar, 290 psi)	ÄKTA avant 150
V9-O	9 µl	10 MPa (100 bar, 1450 psi)	ÄKTA avant 25
V9H-O	82 µl	2 MPa (20 bar, 290 psi)	ÄKTA avant 150
V9-IAB	95 µl	1 MPa (10 bar, 145 psi)	ÄKTA pure 25
V9H-IAB	212 µl	2 MPa (20 bar, 290 psi)	ÄKTA pure 150
V9-M	14 µl	20 MPa (200 bar, 2900 psi)	ÄKTA pure 25
V9H-M	31 µl	5 MPa (50 bar, 725 psi)	ÄKTA pure 150
V9-Cs	14 µl	20 MPa (200 bar, 2900 psi)	ÄKTA pure 25
V9H-Cs	31 µl	5 MPa (50 bar, 725 psi)	ÄKTA pure 150
V9-L	17 µl	20 MPa (200 bar, 2900 psi)	ÄKTA pure 25, ÄKTA avant 25
V9H-L	76 µl	5 MPa (50 bar, 725 psi)	ÄKTA pure 150, ÄKTA avant 150
V9-O2, V9-O3, V9-C2	11 µl	20 MPa (200 bar, 2900 psi)	ÄKTA avant 25
V9H-O2, V9H-O3, V9H-C2	82 µl	5 MPa (50 bar, 725 psi)	ÄKTA avant 150
V9-Os	9 µl	20 MPa (200 bar, 2900 psi)	ÄKTA pure 25
V9H-Os	28 µl	5 MPa (50 bar, 725 psi)	ÄKTA pure 150
V9-V	14 µl	20 MPa (200 bar, 2900 psi)	ÄKTA pure 25, ÄKTA avant 25
V9H-V	31 µl	5 MPa (50 bar, 725 psi)	ÄKTA pure 150, ÄKTA avant 150

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

Fraction collectors	Capacity	Other function	Used with[2]
Frac30	30 × 1.5 ml/2 ml microcentrifuge tubes 30 × 5 ml (12 × 75 mm) or 30 × 12 ml tubes (17 × 100 mm) or 30 × 15 ml tubes (17 × 118 mm)		ÄKTA start
F9-R	175 × 3 ml (o.d. 12 mm) or 95 × 8 or 15 ml (o.d. 12 to 18 mm) or 40 × 50 ml (o.d. 30 mm)	Drop sync	ÄKTA avant, ÄKTA pure
F9-C	6 cassettes or 55 bottles (50 ml) or18 bottles (250 ml) *Cassette options:* 6 tubes (50 ml) 15 tubes (15 ml) 24 tubes (8 ml) 40 tubes (3 ml) 1 deep-well plate (24, 48, or 96 wells)	Accumulator Cassette reader Mix up to six cassettes	ÄKTA pure
Frac-920	95 tubes (o.d. 10 to 18 mm) or 175 tubes (o.d. 12 mm) or 40 tubes (o.d. 30 mm)	Drop sync	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer, or as stand-alone
Frac-950	4 microplates (96 wells) and 8 tubes (o.d. 30 mm) or 120 tubes (o.d. 18 mm) and 8 tubes (o.d. 30 mm) or 240 tubes (o.d. 12 mm) or 45 tubes (o.d. 30 mm) *Prep mode using:* 80 tubes (o.d. 30 mm) or 20 bottles (250 ml)	Accumulator Drop sync Optional prep mode Optional micro mode	ÄKTAFPLC, ÄKTApurifier UPC, ÄKTApurifier, ÄKTAexplorer, ÄKTAmicro

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

Fraction collectors continued	Capacity	Other function	Used with[2]
Built-in with ÄKTA avant	6 cassettes or 55 bottles (50 ml) or 18 bottles (250 ml) *Cassette options:* 6 tubes (50 ml) 15 tubes (15 ml) 24 tubes (8 ml) 40 tubes (3 ml and 5 ml) 1 deep-well plate (24, 48, or 96 wells)	Cooling Accumulator Cassette reader Mix up to six cassettes	ÄKTA avant

Autosamplers	Capacity	Other function	Used with[2]
A-900	96 standard vials (1.5 ml) or 160 microvials (0.5 ml)	Cooling	ÄKTApurifier, ÄKTAexplorer
A-905	1 deep or micro plate (96 or 348 wells) or 48 vials (0.5 ml)	Cooling	ÄKTApurifier, ÄKTAexplorer, ÄKTAmicro
Alias[4]	Microplates: 2 Vials: 2 × 48 (1.5 ml) or optional 2 × 12 (10 ml)	Cooling	ÄKTA avant and ÄKTA pure via I/O box

[1] Stroke volume is the amount of liquid that is pushed out from the pump per piston.

[2] ÄKTApurifier UPC refers to ÄKTApurifier UPC 10, ÄKTApurifier UPC 100; ÄKTApurifier refers to ÄKTApurifier 10, ÄKTApurifier 10 plus, ÄKTApurifier 100, ÄKTApurifier 100 plus; ÄKTAexplorer refers to ÄKTAexplorer 10, ÄKTAexplorer 10S, ÄKTAexplorer 100, ÄKTAexplorer 100 Air; ÄKTA avant refers to ÄKTA avant 25 and ÄKTA avant 150; ÄKTApurifier 10 refers also to ÄKTApurifier 10 plus; ÄKTApurifier 100 refers also to ÄKTApurifier 100 plus; ÄKTAexplorer 10 refers also to ÄKTAexplorer 10S; ÄKTAexplorer 100 refers also to ÄKTAexplorer 100 Air.

[3] To connect to ÄKTA$_{FPLC}$, ÄKTApurifier UPC, ÄKTApurifier, and ÄKTAexplorer, use the Air-900 N control box.

[4] Alias refers to the Alias autosampler supplied by Spark Holland.

Appendix 2
Tubing guide

Many different sizes/types of tubing can be connected to a chromatography system. Tubing with a smaller inner diameter (i.d.) holds less delay volume and will therefore generate less dilution of the protein peak. Narrow tubing, however, increases the system pressure, especially when running at high flow rates. The tubing used should match the application needs.

Tubing material and dimensions

PEEK

PEEK (polyetheretherketone) is a biocompatible material that is often used for medium- to high-pressure systems. For a color description, see Table A2.1.

ETFE and PTFE

In low- or medium-pressure parts of the system (e.g., inlet and outlet tubing), ETFE (ethylene tetrafluoroethylene) or PTFE (polytetrafluoroethylene) tubing is often used. With these transparent materials, for example, air bubbles can easily be detected. ETFE and PTFE are both biocompatible materials. ETFE is the more rigid of the two.

Steel and titanium

High-pressure systems often use steel or titanium tubing. Steel is prone to corrosion, which often makes it unsuitable for purification of biomolecules.

Table A2.1. Tubing data

i.d.	Color of tubing	10 cm tubing corresponds to	100 cm tubing generates[1]	Standard tubing with
0.13 mm	Red	1.3 µl	24 MPa (240 bar, 3481 psi)	Optional for ÄKTA avant to generate high pressure
0.15 mm	Purple	1.8 µl	13 MPa (130 bar, 1885 psi)	ÄKTAmicro
0.25 mm	Blue	4.9 µl	1.7 MPa (17 bar, 247 psi)	ÄKTApurifier UPC 10, ÄKTApurifier 10, ÄKTA explorer 10, ÄKTA pure 25
0.50 mm	Orange	20 µl	0.11 MPa (1.1 bar, 16 psi)	ÄKTA$_{FPLC}$, ÄKTApurifier UPC 10, ÄKTApurifier 10, ÄKTA explorer 10, ÄKTA avant 25, ÄKTA pure 25, ÄKTA start
0.75 mm	Green	44 µl	0.02 MPa (0.2 bar, 2.9 psi)	ÄKTApurifier UPC 100, ÄKTApurifier 100, ÄKTA explorer 100, ÄKTA avant 25, ÄKTA pure 25, ÄKTA pure 150, ÄKTA start
1.0 mm	Beige	78 µl	0.007 MPa (0.07 bar, 1.02 psi)	ÄKTA avant 150, ÄKTA pure 150
1.0 mm	Transparent	78 µl	0.007 MPa (0.07 bar, 1.02 psi)	ÄKTA start
1.6 mm	Transparent	200 µl	—[2]	Inlet tubing to ÄKTA$_{FPLC}$, ÄKTApurifier UPC 10, ÄKTApurifier 10, ÄKTA explorer 10, ÄKTA avant 25, ÄKTA pure 25, ÄKTA start
2.9 mm	Transparent	660 µl	—[2]	Inlet tubing to ÄKTApurifier UPC 100, ÄKTApurifier 100, ÄKTA explorer 100, ÄKTA avant 150, ÄKTA pure 150

[1] For water at 10 ml/min and room temperature
[2] Negligible pressure

Internal volume

To calculate the internal volume (V) of specific tubing, use the formula:

$V = L \times \pi \times d^2/4$

L = length in mm
d = i.d. in mm

☞ If stating the dimensions in millimeters, the volume will be presented in microliters.

Back pressure

To calculate the back pressure (in MPa) generated over specific tubing, use the following formula, which is based on Hagen-Poiseuille's work:

$P = c \times L \times Q \times v/d^4$

c = 0.000000679

L = length in mm

Q = flow rate in ml/min

v = viscosity in mPas

d = i.d. in mm

☞ This formula also applies to the back pressure generated over a column. However, the constant c differs and is dependent on the chromatography medium.

☞ Keep in mind that the viscosity increases with lower temperatures (Fig 7.7).

☞ 1 MPa = 10 bar = 145 psi

Table A2.2. Viscosity values for common solutions at room temperature

Solution	Viscosity (in mPas) at 25°C
Water	0.89
1 M NaCl	0.97
1 M NaOH	1.11
8 M urea	1.66
6 M guanidine hydrochloride	1.61
20% ethanol	1.87
50% ethanol	2.41
100% ethanol	1.07
50% methanol	1.62
100% methanol	0.54
50% isopropanol	2.65
100% isopropanol	2.04

Appendix 3
Determination of delay volumes

A number of methods exist for determining the delay volume of a system. The easiest and recommended method is to perform a theoretical determination.

Theoretical determination (preferred method)

A theoretical determination is performed in three steps:

1. Identify all components in the system flow path that contribute to the delay volume of interest.
2. Determine the internal volumes of all parts. (See Appendix 1 with respect to hardware components and Appendix 2 with respect to tubing).
3. To obtain the total delay volume, sum up all the volumes.

Example: Determination of fractionation delay volume, that is, components between the UV/Vis absorbance detector and the fraction collector. In this example, an ÄKTApurifier UPC 10 is used. See Figure A3.1 for system parts.

1. Identify all system parts:
   In this example, the following parts were identified: UV/Vis absorbance detector with 2 mm cell, tubing, conductivity cell, tubing, pH cell, tubing, outlet valve, tubing, fraction collector (Frac-950).
2. Create a table and fill in all the internal volumes of each system component. Measure tubing lengths with a ruler and use Appendices 1 and 2 (or the system manual) to find out all the components' internal volumes.
   See Table A3.1 for this example.
3. The total volume is then used to update the delay volume in UNICORN System Control.

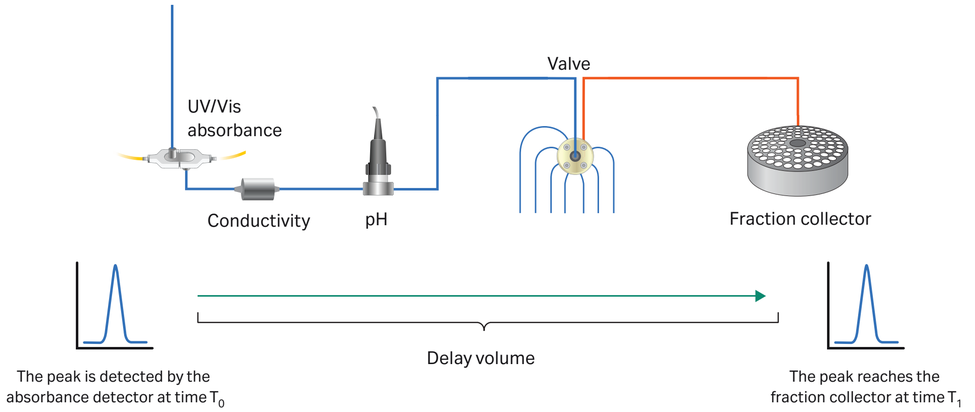


Fig A3.1. Identification of parts of the system used in example.

Table A3.1. Data for determining delay volume in example

System part	Details	Internal volume	Comment
UPC-900 UV cell	2 mm cell's total volume	30 µl/2 = 15 µl*	From Appendix 1
Blue tubing: UV/Vis absorbance detector → Cond cell	i.d. 0.25 mm, 8 cm	3.9 µl	Formula in Appendix 2
Cond cell	Standard 14 µl	14 µl	From Appendix 1
Blue tubing: Cond cell → pH cell	i.d. 0.25 mm, 10 cm	4.9 µl	Formula in Appendix 2
pH cell		88 µl	From Appendix 1
Blue tubing: pH cell → Outlet valve	i.d. 0.25 mm, 12 cm	5.9 µl	Formula in Appendix 2
Outlet valve	PV-908	7 µl	From Appendix 1
Orange tubing: Outlet valve → Fraction collector	i.d. 0.5 mm, 30 cm	58.9 µl	Formula in Appendix 2 (Accumulator was bypassed.)
Total volume		**198 µl**	

* Use half of the total internal volume of the UV/Vis absorbance cell

If a fraction collector accumulator is used, remember to also include the volume of the tubing to and from the accumulator.

Experimental methods

Experimental determination is also possible. Two methods are described below:

Measuring delay volume using the UV/Vis absorbance detector[1]

To determine the delay volume experimentally, two volumes must be measured. These are V1 and V2.

V1 = volume between injection valve and UV/Vis absorbance detector
V2 = volume between injection valve and fractionation tubing tip

1. Check that the pump is delivering the correct flow rate at 1 ml/min. If not, make sure that the pump has no air bubbles within it (see Chapter 5).
2. Fill a small sample loop (i.e., 100 µl) with a 1% to 5% acetone solution.
3. Fill the system with water. Run the pump at 1 ml/min and inject the acetone solution as a sample. The volume from point of injection to peak appearance in the chromatogram is equal to V1.
4. Reconfigure the system:
   (a) To replace the UV/Vis flow cell, disconnect the two tubing segments and connect them with a low-dead-volume connector. Use, for example, a 1/16" female–1/16" female union connector.
   (b) Mount the fractionation tubing tip into the top of the UV/Vis flow cell and connect a waste tubing from the bottom of the UV/Vis flow cell.
5. Set the Frac size to a large volume, for example, 100 ml, so that the valve is in the Frac position during the entire run. Start the pump at 1 ml/min and inject acetone solution. The volume from point of injection to peak appearance in the chromatogram is equal to V2.
6. Subtract V1 from V2 to obtain the delay volume.

[1] This method cannot be used with ÄKTA avant because the fractionation tubing cannot be disconnected by the user.

Weighing water

To determine the delay volume experimentally by weighing water, a preweighed container (e.g., a fractionation tube) is needed.

1. Make sure that the system flow path is set up so that the liquid is directed from the UV/Vis flow cell to the fraction collector.
2. Replace the inlet tubing of the UV/Vis flow cell with a Luer adapter.
3. Fill a syringe with at least 5 ml of water and inject it into the flow cell to make sure that the flow path to the fraction collector tubing tip is filled with water.
4. Fill a syringe with at least 20 ml of air (because of compression), and collect the water that is replaced while injecting the air.
5. Determine the delay volume by weighing the water.
6. Repeat at least two times for calculation of a mean value.

1 mg of water is equal to 1 µl (at 4°C; is temperature dependent).

Appendix 4
Troubleshooting column issues

If high system pressure is due to the column, the following procedure (Fig A4.1) can help to resolve the problem.

For information about how to clean the column, see the column instructions.

It is not unusual that the system back pressure increases for a short time at the start of the cleaning process.

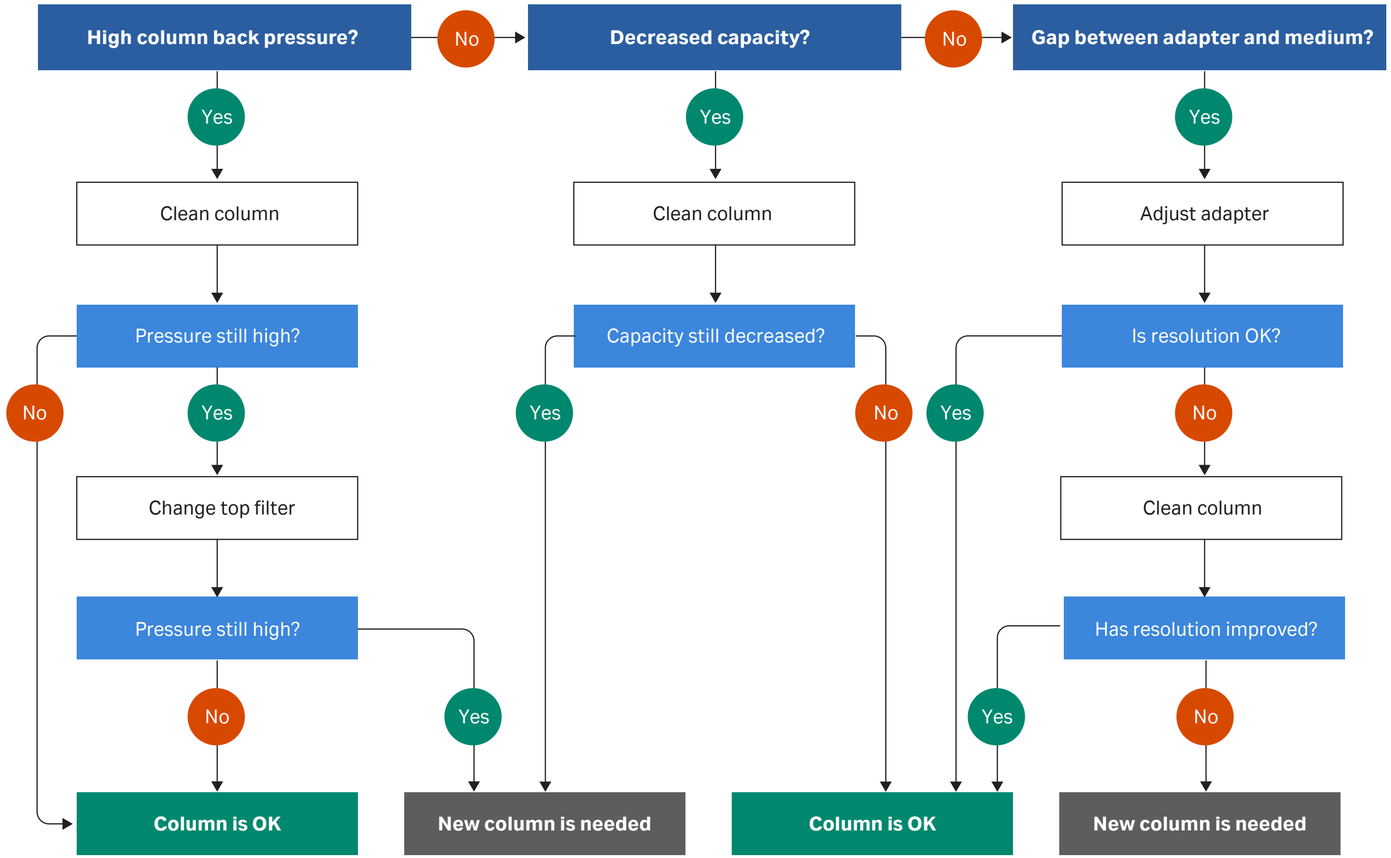


Fig A4.1. Decision tree for dealing with column issues.

Appendix 5
Troubleshooting fraction collection

Some fraction collector problems and preventive/corrective actions are listed in Table A5.1.

Table A5.1. Potential problems and solutions with fraction collectors

General problems

Issue	Cause	Preventive/corrective action
Fractionation marks and actual tube change do not match	Incorrect delay volume entered in the software	Make sure that the correct volume is entered. See Appendix 3 for a description of how to determine the delay volume.
Spillage between tubes	No synchronization of collection defined in the software	In the software, select drop sync or accumulator as appropriate, or collect in serpentine mode
	No tubes or filled tubes in fraction collector	Make sure to have the fraction collector filled with empty tubes prior to start
	Too high flow rate is used	Lower the flow rate
Error message “Sensor dirty”	Incorrect positioning of the tubing	Make sure to position the fractionation tubing tip so that it is not blocking the light path for the drop sync
	photo cell	Clean the drop sync photo cell; see Chapter 10

Frac-950 problems

Issue	Cause	Preventive/corrective action
Spillage between tubes	Incorrect selection of tube type and/or rack in the method	Make sure that the same tubes and racks used are selected in the method
	Incorrect alignment during Frac-950 initialization	Make sure that there is enough free space for the fraction collector movement If the alignment is incorrect (even after a restart), contact Service so the Frac-950 can be recalibrated
Error message “Controller Board Error 2012 Frac not Found”	Incorrect Frac-950 UniNet-1 connection	Make sure that the UniNet-1 cable is placed in the correct socket. Consult the user manual for a detailed description.

Frac-900/920 problems

Issue	Cause	Preventive/corrective action
Tubes are not fed	Drive sleeve worn out	Change drive sleeve
Tube change is not performed properly, e.g., more than one tube is fed per movement	Tube sensor worn out	Change tube sensor
Spillage between tubes	Wrong tube center position is used	Switch to the correct tube option on the fractionation arm, allowing the droplets to fall in the center of the tube
	Fractionation arm not positioned correctly	Make sure that the arm is positioned toward the tube as described in the manual

ÄKTA avant fraction collector problems

Issue	Cause	Preventive/corrective action
Failed scanning	There can be a number of reasons for a failed scanning.	Open and close fraction collector to allow system to repeat the scanning. Inspect cassettes and replace if, for example, identification bars are damaged or blocked. If problem remains, check the troubleshooting section for the fraction collector in the user manual. If problem cannot be solved, contact Service.
Liquid appears when frac door is opened	Liquid has entered the frac compartment instead of the waste container.	To avoid waste blockage, make sure that the waste tubing is not bent, curved, or in touch with the bottom of the waste container.

☞ The scanning in the ÄKTA avant fraction collector reads only number and type of racks. If no tubes/plates are present, the system will still run the method, resulting in spoiled samples.

Appendix 6
Introducing laboratory-scale ÄKTA systems

ÄKTA systems are designed for protein purification ranges from micrograms to tens of grams of target protein. All systems except ÄKTA start are controlled by UNICORN software. ÄKTA start is controlled by UNICORN start. This is a simplified version of UNICORN. UNICORN has the benefits of one common control platform and user interface for all scales of operation in chromatography and filtration.

Laboratory-scale ÄKTA systems are briefly described on the following pages. Table A6.1 lists the standard ÄKTA system configurations for currently available systems.

ÄKTA start ÄKTA pure ÄKTA avant

Fig A6.1 The standard ÄKTA system configurations.

Table A6.1. Ways of working with standard ÄKTA systems

	ÄKTA protein purification system		
Way of working	**ÄKTA start**	**ÄKTA pure**	**ÄKTA avant**
Simple, one-step desalting, buffer exchange	•	•	•
Automated and reproducible protein purification using all common techniques including support for gradient elution	•	•	•
Software compatible with regulatory requirements, e.g., good laboratory practice (GLP)		•	•
Method development and optimization using Design of Experiments (DoE)		o	•
Automatic buffer preparation			•
Automatic pH scouting		o	•
Automatic media or column scouting		o	•
Automatic multistep purification		o	o
Scale-up, process development		o	•
Software[1] for system control and data handling	UNICORN start	UNICORN 6 or later	UNICORN 6 or later

[1] A specific software version might be needed for the chosen system. See the web page for each respective system at www.cytiva.com
• = included
o = optional

Fig A6.2 ÄKTA start system.

ÄKTA start is a one step preparative chromatography system for laboratory-scale protein purification (Fig A6.2). ÄKTA start is designed as a stand-alone system, with intuitive design, simple flow path, and user-friendly touchscreen interface.

ÄKTA start makes it easy to purify a wide variety of proteins using built-in quick start methods or predefined templates, or by creating your own methods. The system can be combined with the Frac30 fraction collector, the user-friendly UNICORN start 1.0 control software, and application-focused prepacked columns.

Fig A6.3 ÄKTA pure system.

ÄKTA pure is a flexible and easily customizable protein purification system that can be tailored to meet any protein purification challenge (Fig A6.3). The system is available in two versions with maximum flow rates of 25 ml/min and 150 l/min, respectively. ÄKTA pure 25 provides fast purification of proteins, peptides, and nucleic acids from picogram to microgram levels. ÄKTA pure 150 is designed for routine large-scale preparative purification and provides smooth handling of higher sample volumes and collection of larger amounts of target protein.

ÄKTA pure replaces the discontinued systems ÄKTApurifier and ÄKTA$_{FPLC}$.

Fig A6.4. ÄKTA avant system.

ÄKTA avant (Fig A6.4) incorporates functionality for achieving fast and secure protein purification. ÄKTA avant is available in two versions, with 25 and 150 ml/min pumps. ÄKTA avant 25 is designed for screening of media and method optimization in laboratory-scale purification. ÄKTA avant 150 is designed for scale-up and robustness testing.

ÄKTA avant together with UNICORN contains several features to facilitate and automate protein purification.

A Design of Experiments (DoE) software module is integrated in UNICORN for ÄKTA avant. It allows automation of the run scheme for the experimental design and maximizes the amount of information obtained while keeping the number of experiments at a minimum during method development.

BufferPro is an advanced inline buffer preparation function that enables buffer mixing without manual interaction.

The built-in fraction collector provides security by cooling the purified samples and preventing dust from being introduced.

ÄKTA avant has a versatile valve configuration to facilitate the purification and increase reproducibility: up to eight samples can be automatically purified; the delta pressure over the column is monitored; five columns can be connected in parallel; and built-in air sensors prevent air bubbles from being introduced.

Upgraded ÄKTA system platform

ÄKTA chromatography systems have offered versatile and reliable protein purification since the 1990s. As a consequence of the renewal and improvement of the ÄKTA system platform, ÄKTAexplorer, ÄKTApurifier, ÄKTA$_{FPLC}$, and ÄKTAmicro have been discontinued. These have all been replaced by improved systems and recommended upgrades are listed in Table A6.2.

Table A6.2. Discontinued ÄKTA systems and their replacements

Product code number	Discontinued system	Upgrade to
18130000	ÄKTAexplorer 10	ÄKTA avant 25
18114505	ÄKTAexplorer 10S	
18111241	ÄKTAexplorer 100	ÄKTA avant 150
18140300	ÄKTAexplorer 100 Air	
18190026	ÄKTA$_{FPLC}$	ÄKTA pure 25 L
28406268	ÄKTApurifier UPC 10	
28406264	ÄKTApurifier 10	ÄKTA pure 25 M
28991436	ÄKTApurifier 10 plus	
28406271	ÄKTApurifier UPC 100	ÄKTA pure 150 L
28406266	ÄKTApurifier 100	ÄKTA pure 150 M
28991435	ÄKTApurifier 100 plus	
28948303	ÄKTAmicro	ÄKTA pure 25 with microgram-scale characterization flow path

Appendix 7
Principles and standard conditions for different purification techniques

The most common methods for preparative purification of proteins all involve chromatography. The methods separate according to differences between the properties of the protein to be purified (the target protein) and the properties of other substances in the sample. This appendix gives an overview of the key purification methods. Further details regarding method descriptions and practical advice, including many examples, can be found in different handbooks from Cytiva as listed under each technique.

Affinity chromatography (AC)

AC separates proteins on the basis of a reversible interaction between the target protein (or group of proteins) and a specific ligand attached to a chromatography matrix (Fig A7.1).

The interaction can be biospecific, for example, antibodies binding protein A or a receptor binding a hormone. It can also be nonbiospecific, for example, a protein binding dye substance or histidine-containing proteins binding metal ions (by immobilized metal ion affinity chromatography [IMAC], which is described in a separate section due to its importance). AC offers high selectivity, and intermediate to high capacity. Elution can often be performed under mild conditions.

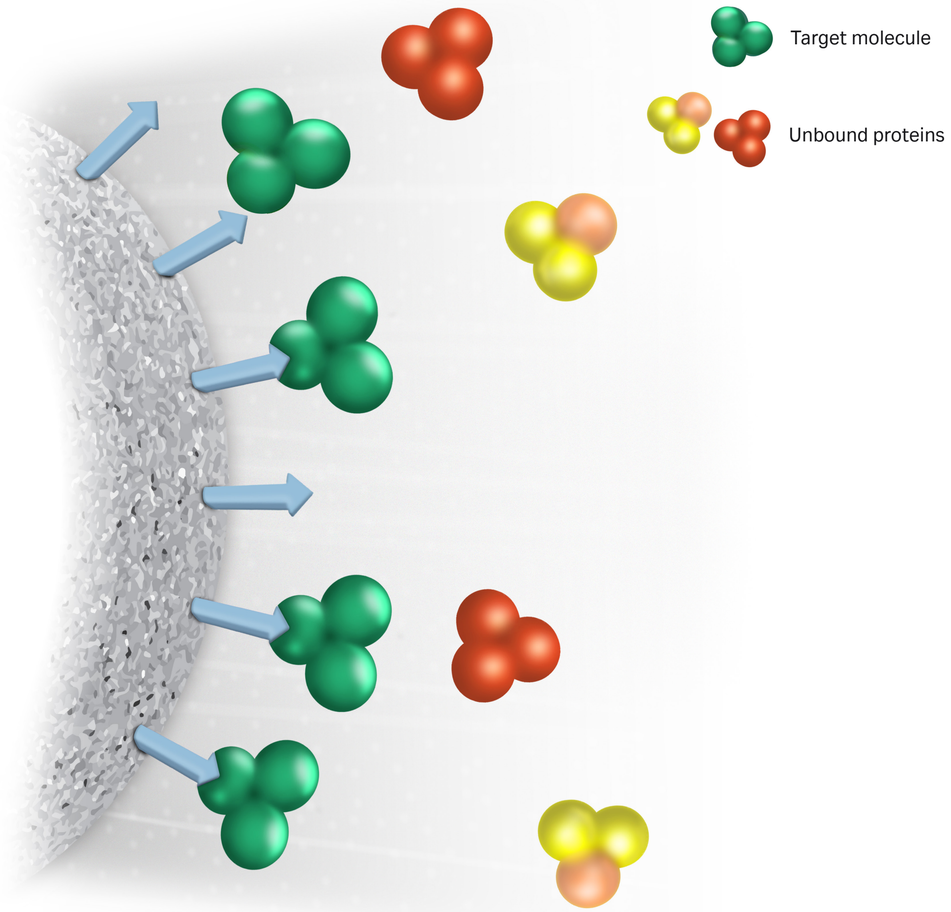


Fig A7.1. Schematic depicting AC.

Principles

In AC, the target protein is specifically and reversibly bound by a complementary binding substance (ligand). The sample is applied under conditions that favor specific binding to the ligand. Unbound material is washed out of the column, and bound target protein is recovered by changing conditions to those favoring elution. Elution is performed specifically using a competitive ligand, or nonspecifically by changing, for example, pH, ionic strength, or polarity. The target protein is eluted in a purified and concentrated form. The key stages in an AC separation are shown in Figure A7.2.

Ligands in AC are divided into two categories: monospecific and group-specific. Ligands used for monospecific AC are structurally and biologically closely related to the target molecule. This makes the selection of the ligand specific for each case. This also makes it commercially difficult to produce AC media for monospecific separations. However, preactivated media ready for a variety of coupling chemistries are commercially available for the benefit of the user who intends to run monospecific AC. Such media include CNBr-activated Sepharose™ and NHS-activated Sepharose, for immobilizing proteins via primary amino groups, or Epoxy-activated Sepharose where hydroxyl groups on the molecule are utilized for immobilization.

A group-specific ligand has an affinity for a group of related substances rather than for a single type of molecule. The same general ligand can be used to purify several substances (e.g., members of a class of enzymes) without the need to prepare a new medium for each different substance in the group. Ligands used for group-specific AC have much wider applicability and affinity media for this purpose are commercially available. Table A7.1 shows some examples of group-specific ligands and their specificities.

Due to its high selectivity, AC can sometimes be used for single-step purification or in instances when some impurities can be accepted. However, it is more common for AC to be used as the first purification step, followed by a second step to remove remaining impurities or aggregates. In cases when very high purity is required, one or more additional purification steps might be required.

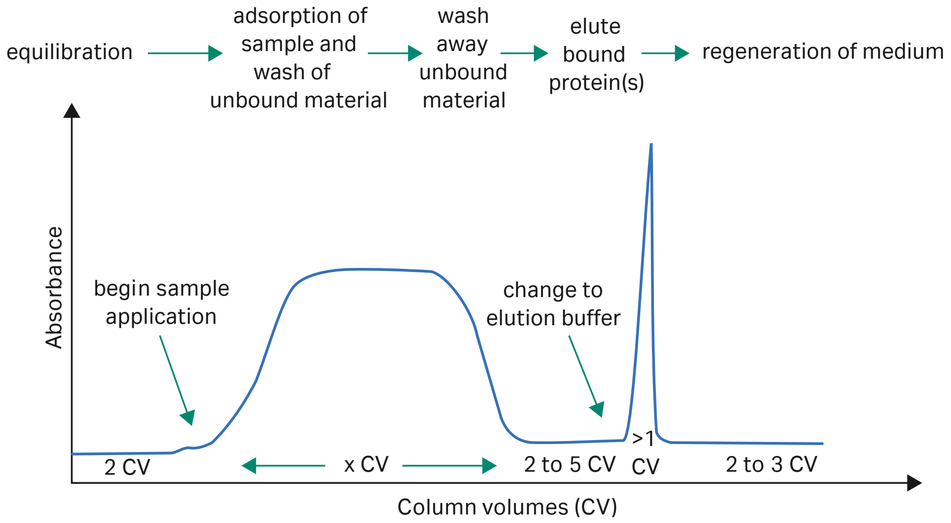


Fig A7.2. Typical affinity purification.

Table A7.1. Examples of group-specific ligands and their specificities

Ligand	Specificity
Protein A or Protein G	Fc region of IgG
Concanavalin A	Gluconpyranosyl and mannopyranosyl groups
Cibacron Blue	Broad range of enzymes, serum albumin
Lysine	Plasminogen, ribosomal RNA
Benzamidine	Serine proteases
Calmodulin	Proteins regulated by calmodulin
Heparin	Coagulation factors, lipoproteins, lipases, nucleic acid-binding enzymes
Metal ions (e.g., Ni^{2+})	Protein and peptides containing histidine

Affinity-tagged proteins for lab-scale purification

Today, most laboratory-scale purifications are performed with affinity-tagged proteins. A large number of different affinity tags as well as media and prepacked columns are available to allow selection of optimal conditions for each target protein and purification task. A common task is purification of histidine-tagged proteins using IMAC or glutathione S-transferase (GST)-tagged proteins on a medium with immobilized glutathione.

AC is also used to remove specific contaminants, for example, Benzamidine Sepharose 4 Fast Flow, which is used for removal of serine proteases. A preactivated medium can be used for covalent coupling of various ligands. For example, antibodies can be raised against the target protein and coupled on NHS-activated Sepharose for immunoaffinity purification of the desired protein.

Further information — Handbooks

Strategies for protein purification, 28983331
Purifying challenging proteins, principles and methods, 28909531
Affinity chromatography, principles and methods, 18102229
Antibody purification, principles and methods, 18103746
Recombinant protein purification handbook: principles and methods, 18114275
GST gene fusion system, 18115758

Immobilized metal ion affinity chromatography (IMAC)

IMAC is based on the interaction of proteins with certain amino acid residues on their surface and divalent metal ions (e.g., Ni^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}) immobilized via a chelating ligand. The interaction is primarily between histidine and metal ions, but also, for example, tryptophan and cysteine. Histidine-tagged proteins have exceptionally high affinity in IMAC because of the multiple (6 to 10) histidine residues of the tag. These proteins are usually the strongest binders among all the proteins in a crude sample extract (e.g., a bacterial lysate), while other cellular proteins will not bind or will bind weakly.

Principles

IMAC purification begins with equilibration of the column with a binding buffer containing a low concentration of imidazole. The concentration of imidazole depends on the selected medium (can be found in the instruction for the specific medium). Imidazole binds to the immobilized metal ion and becomes the counter ligand. The sample should be adjusted to the same imidazole concentration as the binding buffer before being loaded on the column. Proteins with histidines bind the column while displacing the imidazole counter ligands. The column is washed using the binding buffer. Elution of bound proteins is performed using a gradient of imidazole from 100 to 500 mM or by step elution.

Gradient elution (Fig A7.3) often gives two peaks, an early peak corresponding to naturally binding proteins in the lysate and a later peak corresponding to the histidine-tagged protein, which has higher affinity for the medium. Step elution (Fig A7.4) gives a single peak, with the histidine-tagged protein often of slightly lower purity. It is, however, a powerful capture step that can be combined with a second purification step such as size-exclusion chromatography to obtain higher purity.

Histidine tags are small and generally less disruptive to the properties of the protein than other tags. Because of this, tag removal after purification of a histidine-tagged protein is not always a high priority. Histidine-tagged protein expressed in *E. coli* can accumulate in two main forms; as biologically functional soluble proteins; or as large insoluble aggregates of more or less unfolded, inactive target protein.

Further information — Handbooks

Recombinant protein purification handbook: principles and methods, 18114275
Strategies for protein purification, 28983331

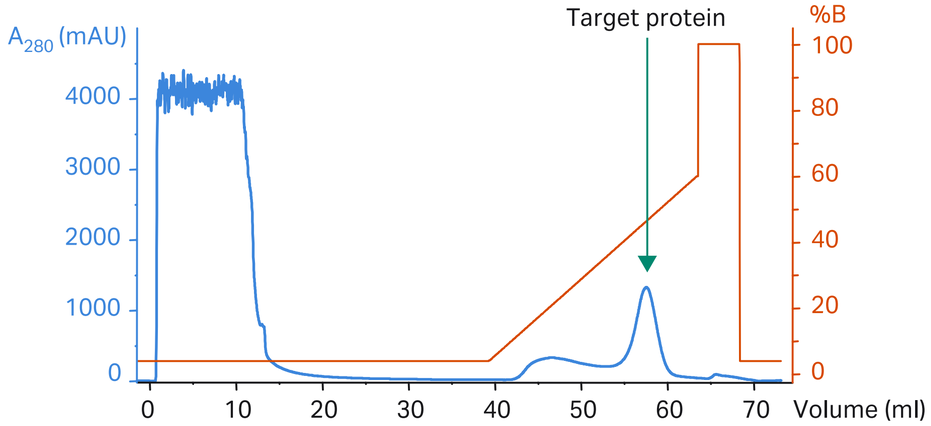


Fig A7.3. Typical IMAC purification with gradient elution.

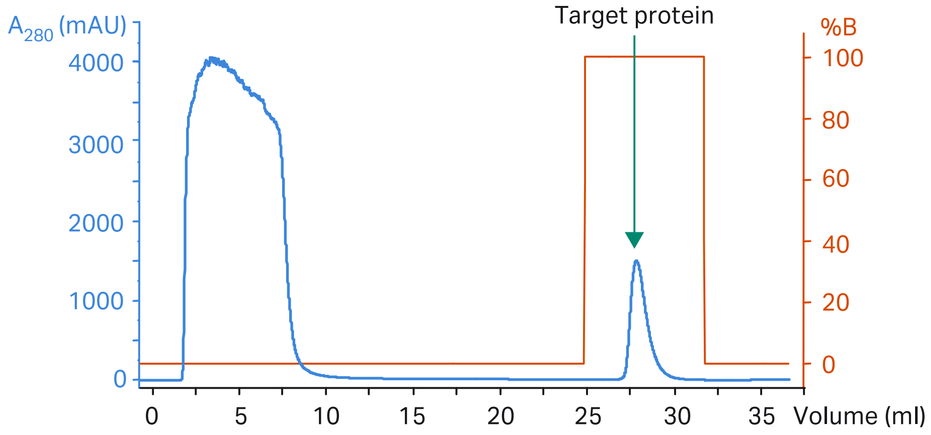


Fig A7.4. Typical IMAC purification with step elution.

Size exclusion chromatography (SEC)

SEC allows separation of substances with differences in molecular size, under mild conditions. The technique can be used for protein purification (Fig A7.5) or for group separation where the sample is separated in two major groups (Fig A7.6). Group separation is mainly used for desalting and buffer exchange of samples.

SEC is a nonbinding method (Fig A7.7), which means that no concentration of the sample components takes place. In fact, the sample zone is broadened during the passage through the column, resulting in dilution of the sample. The loaded sample volume must be kept small. In preparative SEC, maximum resolution can be obtained with sample volumes of 0.5% to 2% of the total column volume; however, up to 5% might give acceptable separation. Even larger sample volumes are appropriate if the resolution between target protein and the impurities is high. To increase capacity, the sample can be concentrated before SEC. Avoid concentrations above 70 mg/ml, because viscosity effects might cause severe band broadening (so-called viscous fingering), which reduces resolution.

Sample components are eluted isocratically (single buffer, no gradient). Separation can be performed within a broad pH, ionic strength, and temperature range. Furthermore, the medium accepts a variety of additives: cofactor, protein stabilizers, detergents, urea, and guanidine hydrochloride. The buffer composition does not usually affect resolution, although including a low concentration of salt, for example, 25 to 150 mM NaCl, is recommended to eliminate weak electrostatic interactions between proteins and the SEC matrix. Buffer conditions are selected to suit the sample type and to maintain target protein activity. A benefit of SEC is that the sample does not have to be in exactly the same buffer as that used for equilibration and running. The buffer in the sample will be exchanged into the running buffer during the separation. Equilibration buffer can thus be selected according to conditions required for further purification, analysis, storage, or use. The selection of medium is the key parameter for optimization of resolution in SEC.

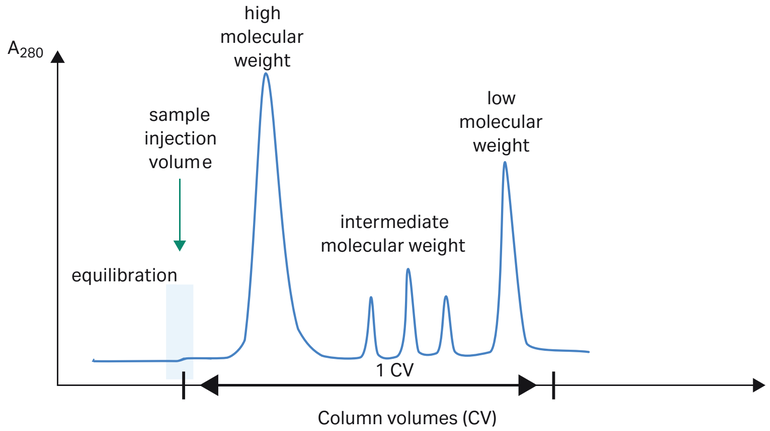


Fig A7.5. Principles of SEC purification.

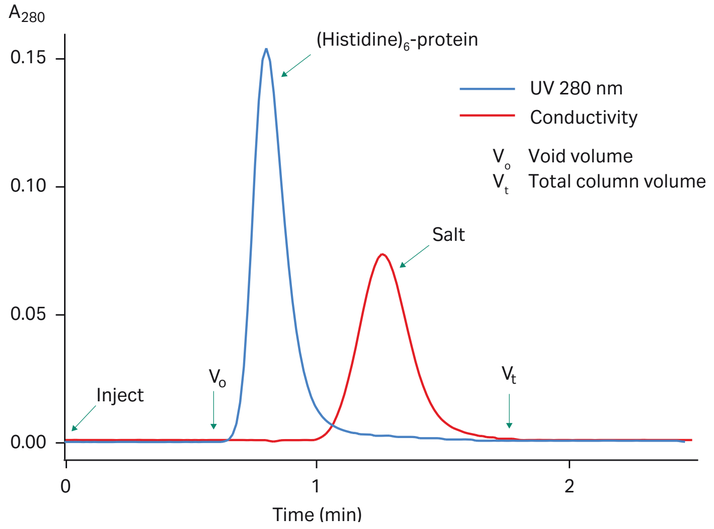


Fig A7.6. Typical example of group separation used for desalting of protein.

SEC applications

SEC is a powerful method for purification of proteins that have passed one or several initial purification steps. After those steps, the material has been concentrated and bulk impurities have been removed. SEC can be used to remove the remaining impurities. It will also remove oligomers or aggregates of the target protein. The purified target protein obtained after SEC will thus also be homogeneous in size. SEC is rarely used as a first purification step, but can be useful for small samples with moderate complexity.

Further information — Handbooks

Size exclusion chromatography, principles and methods, 18102218
Strategies for protein purification, 28983331
Purifying challenging proteins, principles and methods, 28909531

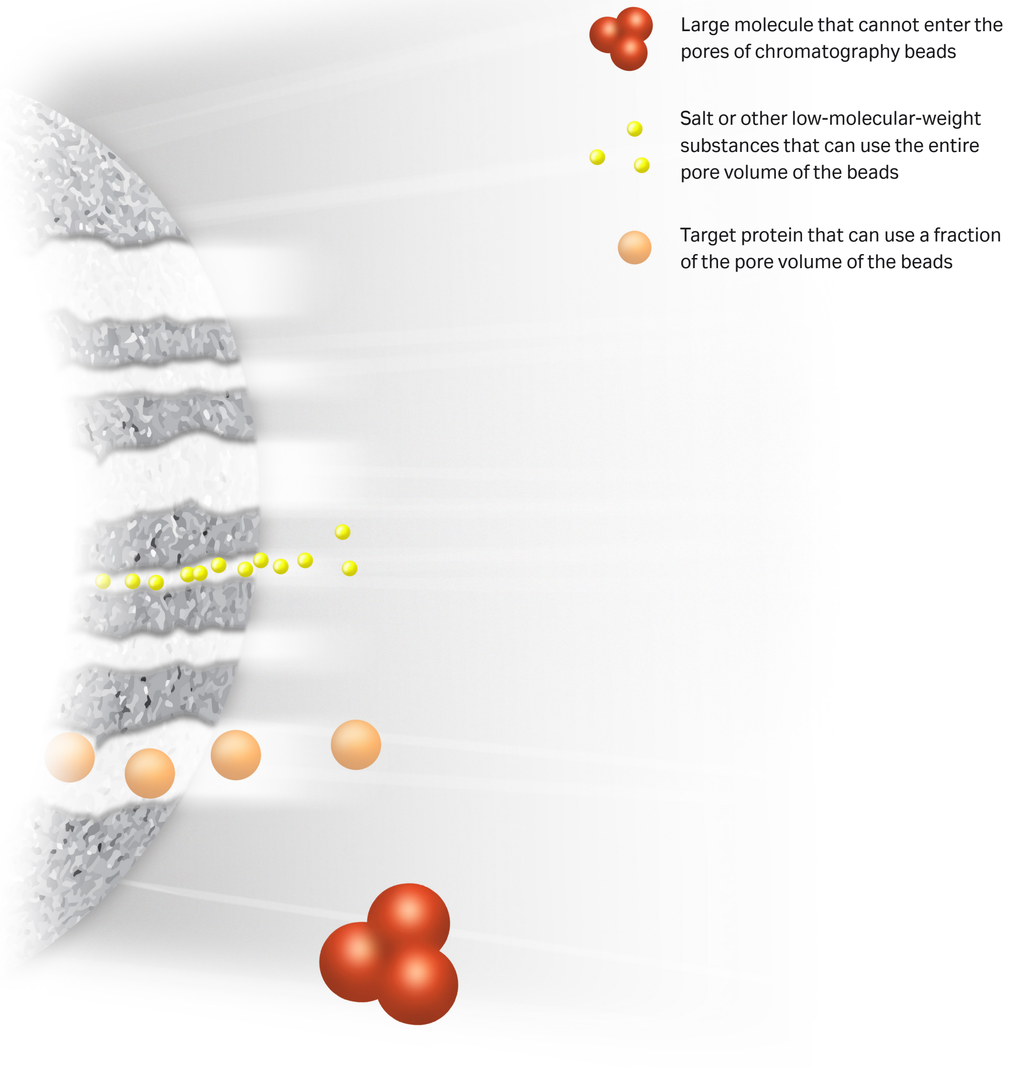


Fig A7.7. Schematic depicting SEC.

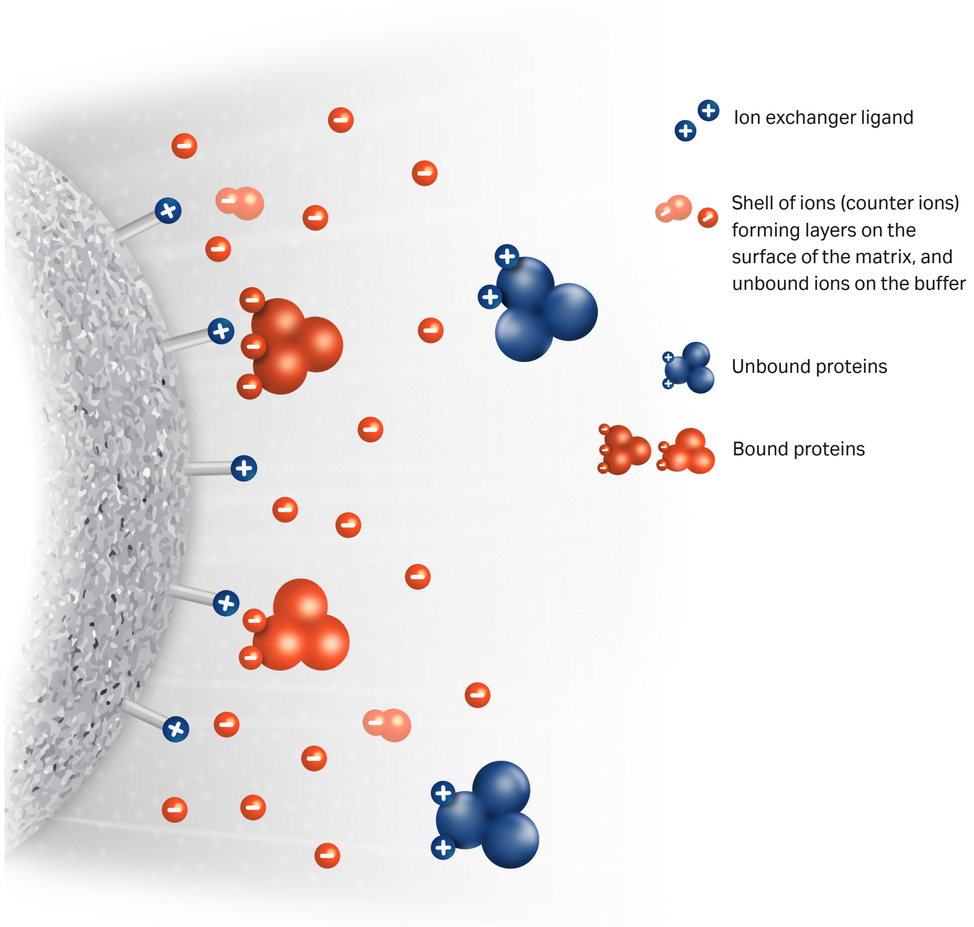


Fig A7.8. Schematic depicting anion exchange chromatography (AIEX) with a positively charged ligand. The same principle applies to cation exchange chromatography (CIEX), but the ligand is negatively charged.

Ion exchange chromatography (IEX)

IEX separates proteins with differences in surface charge to give high-resolution separation with high sample-loading capacity (Fig A7.8). The separation is based on the reversible interaction between a charged protein and an oppositely charged medium. Target proteins are concentrated during binding and collected in a purified, concentrated form. IEX media can be used at high flow rates because binding kinetics for IEX are fast, and rigid chromatography particles can be used.

The net surface charge of proteins varies according to the surrounding pH (Fig A7.9). Typically, when above its isoelectric point (pI) a protein will bind to a positively charged anion exchanger; when below its pI a protein will bind to a negatively charged cation exchanger.

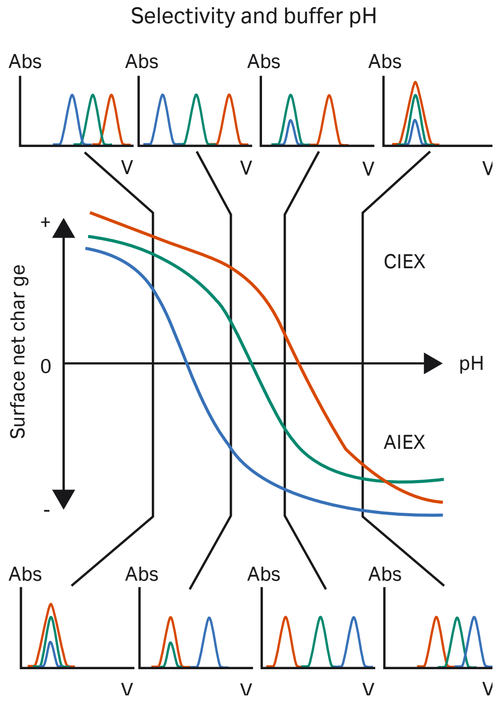


Fig A7.9. Schematic view of the effects of pH on protein elution patterns. The middle diagram shows the surface net charge of three proteins (blue, green, and red). The four chromatograms on top describe the behavior of these proteins in cation exchange chromatography (CIEX) with salt gradient elution run at varying pH values as indicated by the vertical lines. The bottom chromatograms show the behavior in anion exchange chromatography (AIEX).

Principles

Proteins bind as they are loaded onto a column at low ionic strength. The conditions are then altered so that bound substances are desorbed differentially. Elution is usually performed by increasing salt concentration or changing pH in a gradient (Fig A7.10), or stepwise (Fig A7.11). The most common salt is NaCl, but other salts can also be used to modulate separation, for example, salts containing K^+, Ca^{2+}, Mg^{2+}, CH_3COO^-, SO_4^{2-}, I^-, or Br^- ions. The buffer used can also impact separation. Ions that bind to the protein might change its behavior in IEX.

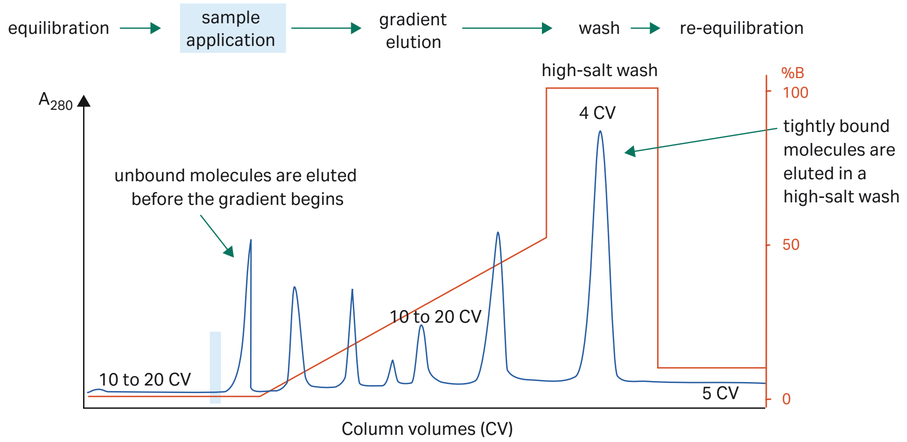


Fig A7.10. Typical IEX purification with gradient elution.

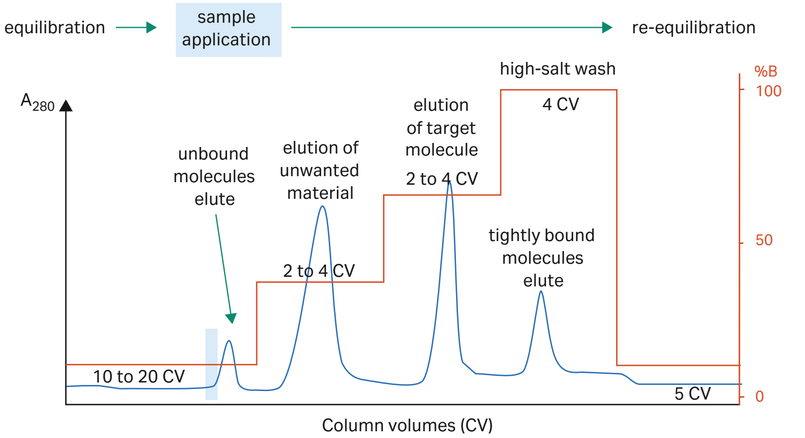


Fig A7.11. Typical IEX purification with step elution.

Strong vs weak ion exchangers

Ion exchange matrices are categorized as strong or weak for both anion and cation exchangers. A strong IEX medium has the same charge density on its surface over a broad pH range, whereas the charge density of a weak ion exchanger changes with pH. The selectivity and the capacity of a weak ion exchanger are different at different pH values. A recommendation is to first try strong ion exchangers. If other selectivity is desired, try a weak ion exchanger.

IEX applications

IEX can be used in any part of a multistep purification procedure: as a first step, in which high binding capacity and high flow rates allow capturing of both target protein and bulk impurities from a large-volume sample; as an intermediate purification step; or as a final step for high-resolution purification to remove remaining impurities. Typically IEX is used to bind the target molecule, but it can also be used to bind impurities, letting the target protein pass through the column. IEX can be repeated at different pH values to separate several proteins that have distinctly different charge properties. Alternatively, a purification step using CIEX can be followed by a second purification step using AIEX at the same pH.

Multimodal ion exchangers

Multimodal ion exchangers, for example, Capto adhere and Capto MMC media, have been developed to offer novel selectivities. The charged ligands are complemented with extra functional groups that introduce additional cooperative interactions (combinations of hydrogen bond, hydrophobic, and van der Waals' interactions). Capto adhere can be used for the removal of aggregated monoclonal antibodies (MAbs) to obtain pure monomers.

Hydroxyapatite chromatography (HAC) can also be considered a multimodal ion exchange method. Crystals of hydroxyapatite ($Ca_3[PO_4]_3OH$) can be used as chromatography medium. Proteins are believed to bind cooperatively to both calcium and phosphate ions on the hydroxyapatite. The hydroxyapatite has a negative charge at neutral pH, and proteins that bind AIEX media tend to also bind to hydroxyapatite. HAC is a less common purification method, partly because of its unpredictable separation mechanism and low binding capacity.

Further information — Handbooks

Ion exchange chromatography and chromatofocusing, principles and methods, 11000421
Multimodal chromatography handbook, 29054808
Strategies for protein purification, 28983331
Purifying challenging proteins, principles and methods, 28909531

Hydrophobic interaction chromatography (HIC)

HIC separates proteins with differences in hydrophobicity. The method is well-suited for the capture or intermediate step in a purification protocol. Separation is based on the reversible interaction between a protein and the hydrophobic surface of a chromatography medium (Fig A7.12). This interaction is enhanced by high ionic-strength buffer, which makes HIC an excellent purification step after precipitation with ammonium sulfate or elution in high salt during IEX. There is no universally accepted theory on the mechanisms involved in HIC. For a brief discussion of the mechanisms, see *Hydrophobic Interaction and Reversed Phase Chromatography: Principles and Methods*, 11001269.

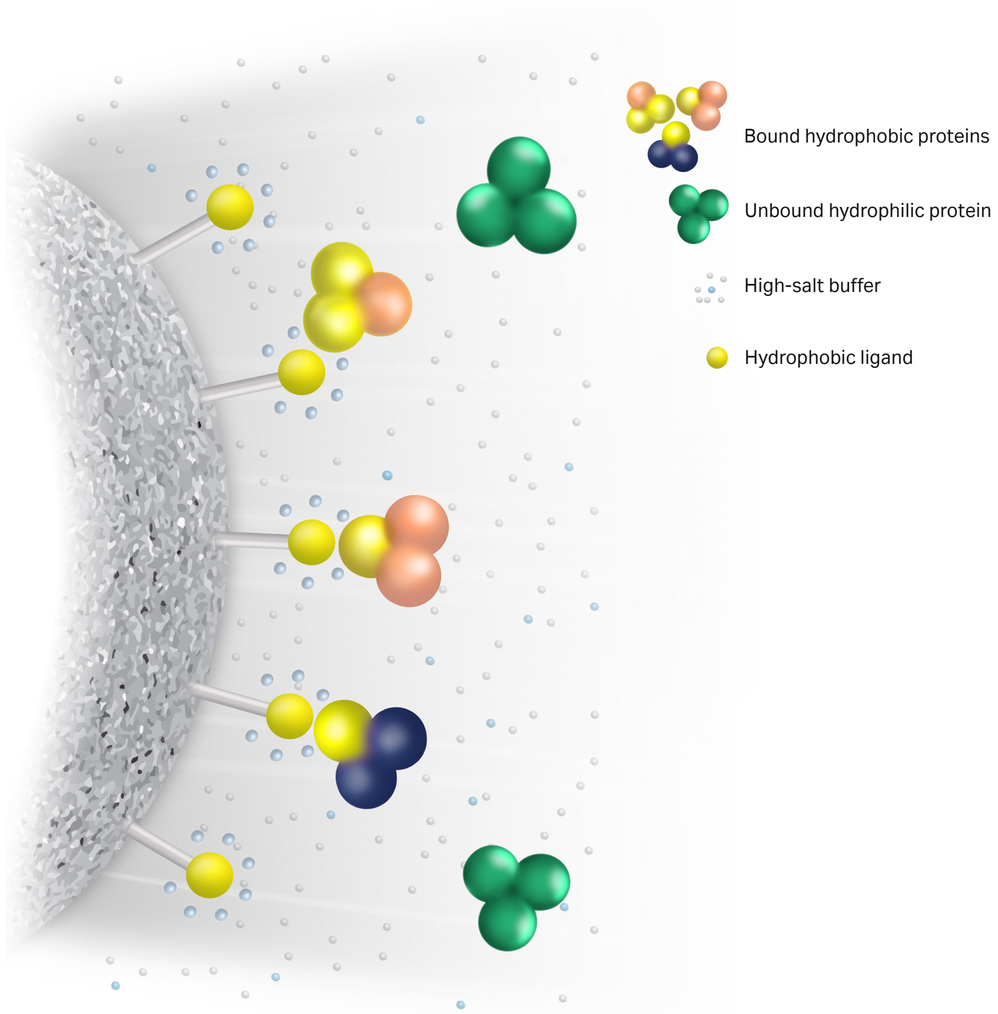


Fig A7.12. Schematic depicting HIC.

Principles

Many sample components bind a HIC column in high ionic-strength solution, typically 1 to 2 M ammonium sulfate or 3 M NaCl. Conditions are then altered so that the bound substances are eluted differentially. Elution is usually performed by decreasing the salt concentration. Changes are made with a continuous decreasing salt gradient (Fig A7.13) or stepwise (Fig A7.14). Most commonly, samples are eluted with a decreasing gradient of ammonium sulfate. Target proteins are concentrated during binding and are collected in a purified and concentrated form. Other elution procedures include reducing eluent polarity (ethylene glycol gradient up to 50%), adding chaotropes (e.g., urea, and guanidine hydrochloride) or detergents, or changing pH or temperature.

Optimization involves screening several HIC media with different ligands and ligand concentrations of the HIC medium, and scouting conditions for optimal binding selectivity and capacity. High concentrations of salt, especially ammonium sulfate, might precipitate proteins. Therefore, check the solubility of the target protein under the binding conditions to be used.

Further information — Handbooks

Hydrophobic interaction and reversed phase chromatography, principles and methods, 11001269
Strategies for protein purification, 28983331
Purifying challenging proteins, principles and methods, 28909531

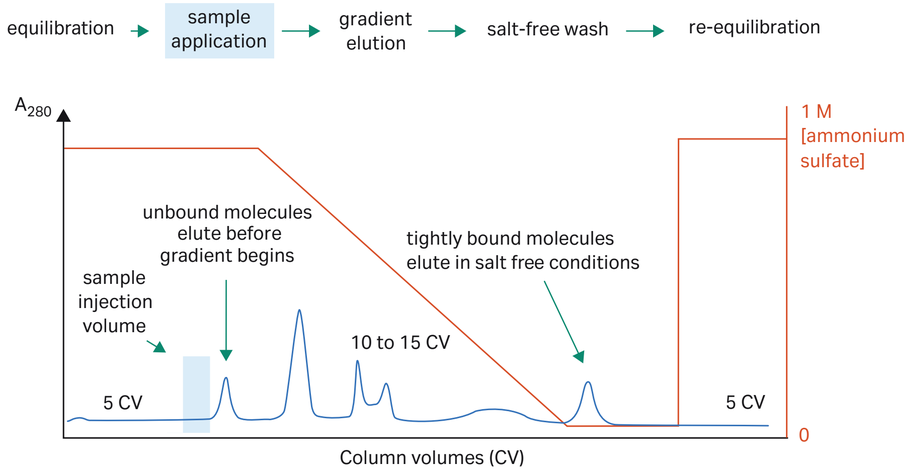


Fig A7.13. Typical HIC purification with gradient elution.

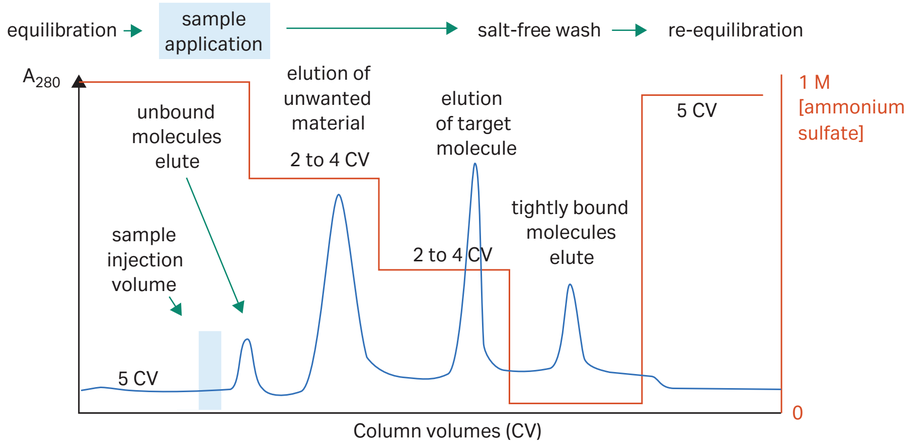


Fig A7.14. Typical HIC purification with step elution.

Reversed phase chromatography (RPC)

RPC separates proteins and peptides on the basis of hydrophobicity (Fig A7.15). RPC is a high-resolution method, requiring the use of organic solvents.

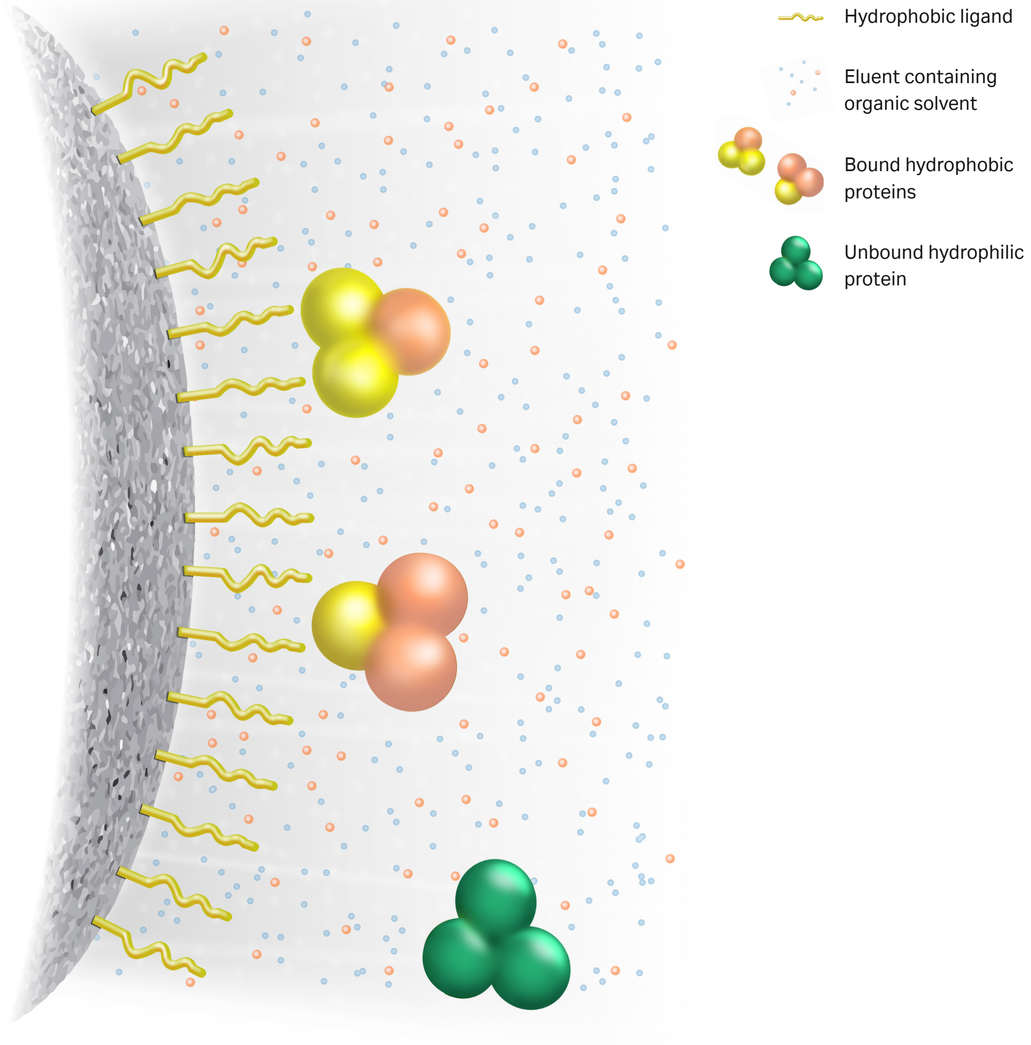


Fig A7.15. Schematic depicting RPC.

Principles

RPC is widely used for purity check analyses when activity and tertiary structure are not a focus. Because many proteins are denatured by organic solvents, the method is not generally recommended for preparative protein purification — the recovery of activity and native tertiary structure is often compromised. Proteins tend to denature and bind strongly to the RPC medium, and can be very difficult to elute. However, in the polishing phase, when the majority of protein impurities have been removed, RPC is excellent, particularly for small target proteins that are less commonly denatured by organic solvents. Sample components bind as they are loaded onto the column. Conditions are then altered so that the bound substances are eluted differentially. Due to the nature of the reversed-phase matrices, binding is usually very strong.

Binding may be modulated by the use of organic solvents and other additives (ion pairing agents). Elution is usually performed by an increase in organic solvent concentration, where acetonitrile, methanol, ethanol, and propanol are most commonly used. The target protein is purified and concentrated in the process. The key stages in a separation are shown in Figure A7.16.

Further information — Handbooks

Hydrophobic interaction and reversed phase chromatography, principles and methods, 11001269
Strategies for protein purification, 28983331
Purifying challenging proteins, principles and methods, 28909531

Chromatofocusing (CF)

CF separates proteins according to differences in their isoelectric point (pI). It is a powerful method and can resolve very small differences in pI (down to 0.02 pH units) and thus separate very similar proteins. However, the capacity of the method is low; CF should preferably be used for partially pure samples.

A pH gradient is generated on the column as buffer and medium interact. The medium is a weak anion exchanger, and the buffer is a polyampholyte elution buffer containing a mixture of polymeric buffering species that buffers a broad pH range. Proteins with different pI values migrate at different rates down the column as the pH gradient develops, continually binding and dissociating while being focused into narrow bands and finally eluted.

CF applications

CF is useful for high-resolution analytical separations and in preparative purification if IEX or other methods do not give a satisfactory purification.

Further information — Handbooks

Ion exchange chromatography and chromatofocusing, principles and methods, 11000421

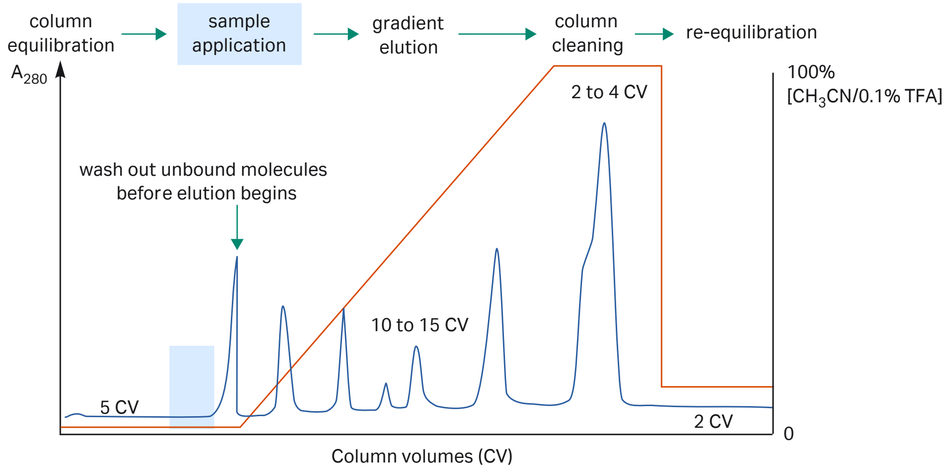


Fig A7.16. Typical RPC purification with gradient elution.

Appendix 8
Columns for ÄKTA systems

High-quality column packing is essential for a good separation. A poorly packed column gives rise to uneven flow dispersion, peak broadening, and loss of resolution. A wide variety of available columns are described below, covering different principles, matrices, and sizes.

For packing a column, a range of empty columns is available. See Table A8.1 for guidelines on how to combine media and columns.

Prepacked columns

Prepacked columns from Cytiva ensure reproducible results and excellent performance.

For more information refer to the guide *Prepacked chromatography columns for ÄKTA systems,* 28931778.

RESOURCE™ columns are prepacked with SOURCE™ 15 media for IEX, HIC, and RPC. RESOURCE columns are made of PEEK (polyetheretherketone), which has high pressure tolerance and high chemical resistance (Fig A8.1). The RPC media are packed into steel columns. SOURCE media are based on a hydrophilic matrix made from monodispersed, rigid, polystyrene/divinyl benzene. The media demonstrate very high chemical and physical stability. The small particle size allows fast binding and dissociation to facilitate high resolution, and the uniformity and stability of the particles ensure high flow rates at low back pressure. RESOURCE columns cannot be opened and repacked.

Fig A8.1. RESOURCE columns.

Fig A8.2. Precision columns. Examples: Mini Q PC 3.2/3 (at left); and Superdex Peptide 3.2/300 (at right).

Precision columns are designed for micropurification and analysis of proteins and peptides (Fig A8.2). The columns are used extensively in peptide sequencing and protein structure/function studies. They are excellent for the polishing step of small-scale protein purification procedures and for purity check analysis. The small volume of the columns decreases the total area of the prepacked medium, which minimizes nonspecific binding and dilution effects. The column volumes have been scaled down 10-fold compared with Tricorn™ columns. Precision columns are available for SEC and IEX. SEC media are: Superdex Peptide, Superdex 75 and 200, Superose™ 6 and 12, Superose 6 Increase, and Superdex 200 Increase. IEX media are: Mono Q™, Mono S™, Mini Q™, and Mini S™. Precision columns require a special Precision column holder for use on ÄKTA systems, and the columns cannot be opened and repacked.

Fig A8.3. HiTrap prepacked columns.

HiTrap columns are convenient and reliable columns (1 or 5 ml) with a bed height of 2.5 cm for fast and easy preparative purifications, either alone or connected in series (Fig A8.3). They are designed for use with a syringe, peristaltic pump, or chromatography system. There are HiTrap columns for a broad range of chromatography media: for AC, immobilized metal affinity chromatography (IMAC), IEX, desalting, and HIC. A range of Sephadex™, Sepharose High Performance, Sepharose XL, Sepharose 4B, and Sepharose Fast Flow columns, as well as Capto ImpRes, Capto, MabSelect™, MabSelect Xtra™, MabSelect SuRe, and MabSelect SuRe LX media. The HiTrap column inlet is molded with 1/16" female threads, and the outlet has 1/16" male threads for direct coupling to ÄKTA systems without the need for extra connectors. HiTrap columns cannot be opened and repacked.

Fig A8.4. HiScreen columns.

HiScreen columns are part of the process development platform available from Cytiva (Fig A8.4). The columns are prepacked with a range of BioProcess™ chromatography media (for AC, IMAC, IEX, and HIC) and designed for parameter screening and method optimization. HiScreen columns have small bed volumes (4.7 ml) thus requiring low sample and buffer volumes. Process fluid velocities can be applied, because the 10 cm bed height gives enough residence time, and the results can then serve as the basis for linear process scale-up. If necessary, two columns can easily be connected in series to give a bed height of 20 cm. The small volume makes HiScreen columns suitable also for laboratory-scale purification. HiScreen columns cannot be opened and repacked.

FigA8.5. HiPrep prepacked columns.

HiPrep prepacked columns are designed for convenient scale-up purification (Fig A8.5). HiPrep columns are available for SEC, desalting, AC, IEX, and HIC in four different sizes, 20 ml, 53 ml, 120 ml, and 320 ml. HiPrep columns for SEC are prepacked with Sephacryl™ High Resolution media, in 120 ml and 320 ml sizes. The HiPrep Desalting column has a column volume of 53 ml for convenient desalting/buffer exchange of sample volumes up to 15 ml. IEX and HIC chromatography media are available in 20 ml HiPrep columns. The column inlet and outlet are molded with 1/16" female threads for direct connection to ÄKTA systems. HiPrep columns cannot be opened and repacked.

Fig A8.6. HiLoad columns.

HiLoad™ columns are prepacked with high-performance Superdex media for convenient and reliable SEC (Fig A8.6). HiLoad columns are available in 120 ml and 320 ml formats prepacked with Superdex 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade to cover a wide range of high-resolution separation of proteins of different molecular weights. The columns have an outer plastic tube that protects the column and provides personal safety in the event of breakage.

Fig A8.7. Tricorn columns.

Tricorn high-performance columns are designed for high-resolution protein purification at laboratory scale, making them an excellent choice for the polishing step in multi-step purification protocols (Fig A8.7). Tricorn columns are available with a range of chromatography media for SEC (Superose, Superose Increase, Superdex, Superdex Increase), IEX (Mono Q, Mono S, SOURCE 15Q, and SOURCE 15S), chromatofocusing (Mono P), and HIC (SOURCE 15PHE).

The columns are simple to use, with specially designed fittings for easy connection to ÄKTA systems and other high-performance LC systems. The columns are coated with a protective plastic film that protects the column and provides personal safety in the event of breakage. Tricorn columns are also available empty for packing with a chromatography medium of choice (see next page).

Tricorn columns are designed for high-performance chromatography media such as MonoBeads, Sepharose High Performance, Superdex, and SOURCE (Fig A8.8). When working with capture media such as Capto, MabSelect, or Sepharose Fast Flow, a Tricorn Coarse Filter Kit is recommended to use for reducing the risk of clogging. Tricorn columns are available with an i.d. of 5 mm with lengths of 20, 50, 100, 150, and 200 mm, and with an i.d. of 10 mm and in lengths of 20, 50, 100, 150, 200, 300, and 600 mm. The maximum pressure is 100 bar for the 5 mm (i.d.) column and 50 bar for the 10 mm (i.d.) column.

Fig A8.8. Tricorn columns.

XK columns are specified to run most chromatography media including Superdex prep grade and Sepharose High Performance (Fig A8.9). They are jacketed and available as 16, 26, and 50 mm (i.d.) columns (XK16, XK26, and XK50) with lengths from 20 to 100 cm. The maximum pressure is 5 bar for XK 16 and XK 26 columns and 3 bar for XK 50 columns. Prepacked XK columns go under the name HiLoad.

Fig A8.9. XK columns.

HiScale™ columns are designed for preparative laboratory-scale purification and for process development using standard chromatography media (Fig A8.10). HiScale columns are available with i.d. of 16, 26, and 50 mm and lengths of up to 20 or 40 cm. The maximum pressure is 20 bar. The QuickLock mechanism of the adapter shaft facilitates rapid and easy movement of the adapter, simplifying adjustments as well as disassembly and cleaning. Turning the column end caps enables controlled axial compression of the medium bed, which is suitable during packing of rigid media.

Fig A8.10. HiScale columns.

Empty columns

To obtain a column with high-quality packing and that can resist the pressure caused by the pressure drop across the selected chromatography bed, select the appropriate empty column based on the guidelines given in Table A8.1. During packing, follow the instructions supplied with the chromatography medium and empty column.

Table A8.1 Empty column and chromatography media guide

	Recommended column		
Bulk media	**Tricorn**	**XK**	**HiScale**
Size exclusion			
Sephadex	o	•	o
Sepharose	•	•	o
Sephacryl	•	•	o
Superdex prep grade	•	•[2]	o
Superose	•	•	o
Ion exchange			
Capto	•	–	•
Capto ImpRes	•	–	•
Capto S ImpAct	•	–	•
Sepharose Fast Flow	•	•	o
Sepharose High Performance	•	•[1, 2]	o
Sepharose XL	•	•	o
SOURCE	•	–	•
Affinity			
Capto	•	–	•
Capto Blue	•	–	•
Sepharose 6B/4B/CL-4B	•	•	o
Sepharose Fast Flow	•	•	o
Sepharose High Performance	•	•[1]	o
MabSelect/MabSelect Xtra/MabSelect SuRe/MabSelect SuRe LX	•	o[1]	•
Reversed phase			
SOURCE	•	–	–

	Recommended column		
Bulk media	**Tricorn**	**XK**	**HiScale**
Hydrophobic interaction			
Capto	•	–	•
Capto Phenyl	•	–	•
Capto Butyl	•	–	•
Sepharose Fast Flow	•	•	o
Sepharose High Performance	•	•[1, 2]	o
SOURCE	•	–	•
Systems			
ÄKTAmicro	•	–	–
ÄKTApurifier 10	•	•	–
ÄKTApurifier 100	•	•	•
ÄKTA pure 25	•	•	•
ÄKTA avant 25	•	•	•
ÄKTA pure 150	–	•	•
ÄKTA avant 150	–	•	•
ÄKTA start	o	–	–

• Recommended combination
o Can technically be used, but not an optimal combination
- Not recommended or not applicable
[1] Not recommended for XK 50
[2] For optimal performance use prepacked columns where purification parameters are predefined

For more information visit www.cytiva.com/protein-purification, www.cytiva.com/bioprocess, or www.cytiva.com/purification_techsupport

Appendix 9
Chemical resistance guide

This section specifies the chemical resistance of ÄKTA laboratory-scale systems to some of the most commonly used chemicals in liquid chromatography.

ÄKTA start has certain chemical resistance limitations due to it's special pump tubing. Refer to *ÄKTA start operating instructions*, 29027057.

ÄKTA systems are designed for maximum biocompatibility, with biochemically inert flow paths constructed mainly from titanium, PEEK, and highly resistant fluoropolymers and fluoroelastomers. Titanium is used as far as possible to minimize contribution of potentially deactivating metal ions such as iron, nickel and chromium. There is no standard stainless steel in the flow path. Plastics and rubber materials are selected to avoid leakage of monomers, plasticizers or other additives.

Strong cleaning works well with 2 M sodium hydroxide, 70% acetic acid or the alcohols methanol, ethanol and isopropyl alcohol. Complete system cleaning with 1 M hydrochloric acid should be avoided due to sensitivity in the pressure sensors. For cleaning separation media with 1 M hydrochloric acid, use loop injections of the acid.

Make sure that the column is not mounted on column valve **V9-C** or **V9H-C** (which contains a pressure sensor).

If sodium hypochlorite is used as sanitizing agent instead of 2 M sodium hydroxide, use a concentration up to 10%.

Reversed phase chromatography of proteins works well with 100% acetonitrile and additives trifluoroacetic acid (TFA) up to 0.2% or formic acid up to 5%.

Strong organic solvents like ethyl acetate, 100% acetone or chlorinated organic solvents should be avoided. These might cause swelling of plastic material and reduce the pressure tolerance of PEEK tubing. For this reason, flash chromatography and straight ("normal") phase is generally not recommended on the system.

List of chemicals

A list of chemicals compatible with ÄKTA systems is described in the following tables. In composing the list, the following assumptions have been made:

- Synergy effects of chemical mixtures have not been taken into account
- Room temperature and limited overpressure is assumed

Chemical influences are time and pressure dependent. Unless otherwise stated, all concentrations are 100%.

A user can be exposed to large volumes of chemical substances over a long period of time. Material Safety Data Sheets (MSDS) provide the user with information regarding characteristics, human and environmental risks, and preventive measures. Make sure that you have the MSDS available from your chemical distributor and/or databases on the internet.

ÄKTA systems tolerate most aqueous buffers between pH 2 and pH 12 in continuous use.

Table A9.1. Strong chemicals and salts for CIP

Chemical[1]	Concentration	CAS number/EC number
Acetic acid	70%	75-05-8/200-835-2
Decon 90	10%	N/A
Ethanol	100%	75-08-1/200-837-3
Methanol	100%	67-56-1/200-659-6
Hydrochloric acid[2]	100 mM	7647-01-0/231-595-7
Isopropanol	100%	67-63-0/200-661-7
Sodium hydroxide	2 M	1310-73-2/215-185-5
Sodium hydroxide/ethanol	1 M/40%	N/A
Sodium chloride	4 M	7647-14-5/231-598-3
Sodium hypochlorite	10%	7681-52-9231-668-3

[1] Up to 2 h contact time at room temperature.

[2] Hydrochloric acid concentration should not exceed 100 mM in the system pressure monitor or in the pressure monitor in column valve V9-C and V9H-C. For other parts of the system up to 1 M HCl is acceptable for short periods of use. For cleaning of columns with HCl concentrations exceeding 100 mM, manually fill a loop with HCl and inject the cleaning agent.

Table A9.2. Solubilization and denaturing agents

Chemical[1]	Concentration	CAS number/EC number
Guanidinium hydrochloride	6 M	50-01-1/200-002-3
Sodium dodecyl sulfate (SDS)	1%	151-21-3/205-788-1
Triton™ X-100	1%	9002-93-1
Tween™ 20	1%	9005-64-5/500-018-3
Urea	8 M	57-13-6/200-315-5

[1] Continuous use, as additives in separation and purification methods.

Table A9.3. Chemicals used in reversed phase chromatography (RPC)

Chemical[1]	Concentration	CAS number/EC number
Acetonitrile[2]	100%	75-05-8/200-835-2
Acetonitrile/tetrahydrofuran[2]	85%/15%	109-99-9/203-726-8
Acetonitrile/water/TFA[3]	Max. 0.2% TFA	N/A
Ethanol	100%	75-08-1/200-837-3
Isopropanol	100%	67-63-0/200-661-7
Methanol	100%	74-93-1/200-659-6
Water/organic mobile phase/formic acid	Max. 5% formic acid	N/A

[1] Continuous use.
[2] Organic solvents can penetrate weaknesses in PEEK tubing walls more easily than water based buffers. Special care should therefore be taken with prolonged use of organic solvents close to pressure limits.
Depending on pressure, tubing between pump head and pressure monitor needs to be changed.
[3] Mobile phase system.

Replace the mixer sealing ring in ÄKTA avant and ÄKTA pure with the highly resistant O-ring (product code 29011326) if the system is to be exposed to organic solvents or high concentrations of organic acids, such as acetic acid and formic acid, for a longer period of time.

Table A9.4. Salts and additives for hydrophobic interaction chromatography (HIC)

Chemical	Concentration	CAS number/EC number
Ammonium chloride	2 M	12125-02-9/235-186-4
Ammonium sulfate	3 M	7783-20-2/231-984-1
Ethylene glycol	50%	107-21-1/203-473-3
Glycerol	50%	56-81-5/200-289-5

[1] Continuous use.

Table A9.5. Reducing agents and other additives

Chemical[1]	Concentration	CAS number/EC number
Arginine	2 M	74-79-3/200-811-1
Benzyl alcohol	2%	100-51-6/202-859-9
Dithioerythritol (DTE)	100 mM	3483-12-3/222-468-7
Dithiothreitol (DTT)	100 mM	3483-12-3 /222-468-7
Ethylenediaminetetraacetic acid (EDTA)	100 mM	60-00-4/200-449-4
Mercaptoethanol	20 mM	37482-11-4/253-523-3
Potassium chloride	4 M	7447-40-7/231-211-8
Acetone	10%	67-64-1/200-662-2
Ammonia	30%	7664-41-7/231-635-3
Dimethyl sulfoxide (DMSO)	5%	67-68-5/200-664-3
Ethanol for long-term storage	20%	75-08-1/200-837-3
Phosphoric acid	100 mM	7664-38-2/231-633-2

[1] Continuous use.

Wetted materials

The table to the right list the typical materials that come into contact with process fluids in ÄKTA laboratory-scale systems.

Table A9.6. Materials used in primary flow path and pump rinse system

Material	Abbreviation
Primary flow path	
Ethylene ChloroTriFluoroEthylene	ECTFE
Ethylene TetraFluoroEthylene	ETFE
Fluorinated Ethylene Propylene	FEP
Fluorinated Propylene Monomer	FPM/FKM
Fully Fluorinated Propylene Monomer	FFPM/FFKM
PolyChloroTriFluoroEthylene	PCTFE
PolyEtherEtherKetone	PEEK
PolyPropylene	PP
PolyTetraFluoroEthylene	PTFE
PolyVinylidene DiFluoride	PVDF
UltraHighMolecularWeightPolyEthylene	UHMWPE
Aluminum oxide	
Elgiloy	
Hastelloy™ C-276	
Quartz glass	
Ruby	
Sapphire	
Titanium grade 2	
Titanium grade 5	
Pump rinse system	
EthylenePropyleneDiene M-class rubber	EPDM
PolyEtherEtherKetone	PEEK
PolyPropylene	PP
PolyPhenylene Sulfide	PPS
PolyVinylidene DiFluoride	PVDF
Silicone	

Related literature

	Product code
Handbooks	
GST Gene Fusion System	18115758
Affinity Chromatography, Principles and Methods	18102229
Antibody Purification, Principles and Methods	18103746
Purifying Challenging Proteins	28909531
Protein Sample Preparation	28988741
Strategies for Protein Purification	28983331
Recombinant Protein Purification, Principles and Methods	18114275
Size Exclusion Chromatography, Principles and Methods	18102218
Hydrophobic Interaction and Reversed Phase Chromatography, Principles and Methods	11001269
Ion Exchange Chromatography and Chromatofocusing, Principles and Methods	11000421
2-D Electrophoresis	80642960
Design of Experiments in Protein Production and Purification	29103850
Selection guide	
Prepacked chromatography columns for ÄKTA systems	28931778
User manuals for ÄKTA system	
Refer to www.cytiva.com and search for specific system's user manual within the Literature Documents and Downloads section.	
CDs	
Column Packing CD — The Movie	18116533
Data files, interactive selection guides, apps, and application notes	
Refer to www.cytiva.com/protein-purification	

Cue cards	
Good ÄKTA systems practice	29109616
ÄKTA routine maintenance	29130436

Ordering information

Description	Quantity/pack size	Product code
Tubing		
PEEK tubing i.d.: 0.25 mm, o.d.: 1/16"	2 m	18112095
ETFE tubing i.d.: 0.25 mm, o.d.: 1/16"	2 m	18112136
PEEK tubing i.d.: 0.50 mm, o.d.: 1/16"	2 m	18111368
ETFE tubing i.d.: 0.50 mm, o.d.: 1/16"	2 m	18112096
PEEK tubing i.d.: 0.75 mm, o.d.: 1/16"	2 m	18111253
ETFE tubing i.d.: 0.75 mm, o.d.: 1/16"	2 m	18111974
PEEK tubing i.d.: 1.0 mm, o.d.: 1/16"	2 m	18111583
ETFE tubing i.d.: 1.0 mm, o.d.: 1/16"	3 m	18114238
FEP tubing i.d.: 1/16", o.d.: 1/8"	3 m	18112116
FEP tubing i.d.: 1/8", o.d.: 3/16"	3 m	18111247
Fingertights and tubing connectors		
Fingertight connector 1/16" M - Narrow - Black	10	18117263
Fingertight connector 1/16" M - Narrow - Red	8	28401081
Fingertight connector 1/16" M	10	18111255
Tubing connector for o.d. 1/16"	10	18112707
Tubing connector for o.d. 1/8"	10	18112117
Tubing connector for o.d. 3/16"	10	18111249
M6 connector	10	18117264
Unions		
Union Luer F - M6 F	2	18102712

Description	Quantity/pack size	Product code
Union 1/16" F - 1/16" F	5	11000339
Union Fingertight i.d. 0.3 mm	4	11000852
Union 1/16" M - M6 F	8	18111258
Union 1/16" F - 1/16" F Ti	1	18385501
Union 1/16" F - M6 M	6	18111257
Union Luer F - 1/16" M	2	18111251
Union 1/16" M - 1/16" M - i.d. 0.13 mm	1	18112090
Union 1/16" M - 1/16" M - i.d. 0.25 mm	2	18112092
Union 1/16" M - 1/16" M - i.d. 0.5 mm	2	18112093
Union 5/16" F - M6 M	3	18112776
Union 5/16" F - 1/16" M	8	18114208
Union M6 F - 1/16" M	5	18385801
Union 1/16" M - 1/16" M - i.d. 0.5 mm	5	28954326
Union M6 F-M6 F SRTC-2	5	19214301
Ferrules		
Ferrules for 1/16" tubing connector - Blue	10	18112706
Ferrules for 1/8" tubing connector - Yellow	10	18112118
Ferrules for 3/16" tubing connector - Blue	10	18111248
Stop plugs		
Stop plug 1/16" Narrow	5	11000355
Stop plug 5/16" M	5	18111250
Stop plug 1/16" M	5	18111252

Description	Quantity/pack size	Product code
Sample loops		
Assorted sample loops, PTFE (25, 50, 100, 200, 500 µl)	1 each	18040401
Sample loops, PTFE (1 and 2 ml)	1 each	18589701
Sample loop, PTFE, 10 ml	1	18116124
Sample loop, PEEK, 1 ml	1	18111401
Sample loop, PEEK, 10 µl	1	18112039
Sample loop, PEEK, 100 µl	1	18111398
Sample loop, PEEK, 500 µl	1	18111399
Sample loop, PEEK, 2.0 ml	1	18111402
Sample loop, PEEK, 5.0 ml	1	18114053
Sample loop, ETFE, 10.0 ml	1	11000302
Loop extension kit for ÄKTAxpress, ETFE, 10.0 ml	5	28904438
Superloop 10 ml, 1/16" fittings	1	18111381
Superloop 50 ml, 1/16" fittings	1	18111382
Superloop 150 ml, M6 fittings	1	18102385
Solvent resistant O-rings to Superloop 10 and 50 ml		
O-ring to movable seal (11.3 × 2.4 mm) KAL	3	18630001
O-ring to movable seal (11.3 × 2.4 mm) FFPM/FFKM	2	18110497

Description	Quantity/pack size	Product code
Mixers		
Mixer M-925 Mixing chamber 90 µl	1	18114724
Mixer M-925 Mixing chamber 200 µl	1	18114721
Mixer M-925 Mixing chamber 0.6 ml	1	18111890
Mixer M-925 Mixing chamber 2 ml	1	18111891
Mixer M-925 Mixing chamber 5 ml	1	18111892
Mixer M-925 Mixing chamber 12 ml	1	18111893
ÄKTA avant and ÄKTA pure Mixer chamber 0.6 ml	1	28956186
ÄKTA avant and ÄKTA pure Mixer chamber 1.4 ml	1	28956225
ÄKTA avant and ÄKTA pure Mixer chamber 5 ml	1	28956246
ÄKTA avant and ÄKTA pure Mixer chamber 15 ml	1	28980309
Mixer ÄKTA start	1	29023960
UV/Vis flow cells		
Flow cell, 2 mm for UPC-900	1	18112825
Flow cell, 5 mm for UPC-900	1	18112824
Flow cell, 2 mm for UV-900	1	18111110
Flow cell, 3 mm for UV-900	1	18114725
Flow cell, 10 mm for UV-900	1	18111111
UV flow cell, 0.5 mm for U9-M	1	28979386
UV flow cell, 2 mm for U9-M	1	28979380
UV flow cell, 10 mm for U9-M	1	28956378

Description	Quantity/pack size	Product code
UV flow cell, 2 mm for U9-L	1	29011325
UV flow cell, 5 mm for U9-L	1	18112824
UV-900 cell, 1 mm calibration kit	1	18632401
UV-900 and UPC-900 cell, 2 mm calibration kit	1	18632402
UV-900 cell and UPC-900, 5 mm calibration kit	1	18632404
UV-900 cell, 10 mm calibration kit	1	18632405
UV-900 cell calibration file	1	18632406
pH detectors for ÄKTA systems		
pH electrode with cell and holder, round tip	1 each	18113484
pH electrode, round tip	1	18111126
pH electrode for ÄKTA avant and ÄKTA pure	1	28954215
Dummy electrode, round tip	1	18111103
Air sensors		
Air-900 N control unit	1	18112122
Air-912 N flow cell (1.2 mm i.d.)	1	18117415
Air-925 N flow cell (2.5 mm i.d.)	1	18117416
AirSensor L9-1.2	1	28956502
AirSensor L9-1.5	1	28956500
Racks and cassettes for ÄKTA avant and ÄKTA pure fraction collectors		
Cassette, holds 6 × 50 ml tubes	2	28956402
Cassette, holds 15 × 15 ml tubes	2	28956404
Cassette, holds 40 × 3 ml tubes	2	28956427

Description	Quantity/pack size	Product code
Cassette, holds 40 × 5 ml tubes	2	29133422
Cassette, holds 1 × 96-, 48-, or 24-deep-well plate	2	28954212
Rack, holds 55 × 50 ml tubes	1	28980319
Rack, holds 18 × 250 ml bottles	1	28981873
Cassette, holds 24 × 8 ml tubes	2	28956425
Racks and options for Frac-950		
Rack A, 18 mm and 30 mm tubes	1	18608311
Rack B, 12 mm tubes	1	18608312
Rack C, 4 × 96-well and 30 mm tubes	1	18608313
Rack D, 30 mm tubes	1	18608314
Prep mode		
Prep Mode Conversion Kit (for use with Rack E and Rack F)	1	18608318
Rack E for Prep mode using 30 mm tubes	1	18608315
Rack F for Prep mode using 250 ml bottles	1	18608316
Funnel to Flask Kit with funnels, tubing, and tubing organizer (for use with Rack E)	1	18608317
Microfractionation		
Microfraction Collection Kit	1	28948780
Racks for Frac-920 and F9-R		
Tube rack 95 × 10–18 mm, complete	1	18305003
Tube rack 175 × 12 mm, complete	1	19868403
Tube rack 40 × 30 mm, complete	1	18112467
Frac30 Bowl Assembly	1	29024045

[1] Inline filter is sometimes also referred to as online filter.

Description	Quantity/pack size	Product code
Filter assemblies		
Inline[1] filter (10 and 20 ml/min systems)	1	18111801
Inline[1] filter kit (10 ml/min systems)	2	18112094
Inline[1] filter holder (20, 50, and 100 ml/min systems)	1	18111244
Inline[1] filter kit (20, 50, and 100 ml/min systems)	10	18102711
Inlet filter set	10	18111442
Column holders		
ÄKTA avant and ÄKTA pure		
Column block for 5 columns	1	28956270
Column holder	1	28956282
Flexible column holder	1	28956295
Column clip	5	28956319
Column holder HiScale 50	1	28964499
Other ÄKTA		
Large column holder	1	28400737
Column holder, short, plastic	1	18111317
Column holder XK 50	1	18309460
Column holder, extra long, metal	1	18112632
Column clamp, small column	1	18114998
Clamp for lab rods	1	18111319
ÄKTA extension equipment holder	1	18115831

Description	Quantity/pack size	Product code
For external equipment		
AD-900 Analog/digital converter	1	18114862
I/O box E9	1	29011361
For extra modules		
Extension Box for ÄKTA avant and ÄKTA pure	1	29110806

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CY13989-02Dec20-HB

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