

ÄKTA^{pilot}

User Manual

Important user information

All users must read this entire manual to fully understand the safe use of ÄKTApilot.

WARNING!



The WARNING! sign highlights instructions that must be followed to avoid personal injury. Do not to proceed until all stated conditions are clearly understood and met.

Caution!

The Caution! sign highlights instructions that must be followed to avoid damage to the product or other equipment. Do not to proceed until all stated conditions are clearly understood and met.

Note

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

CE Certifying

This product meets all requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked Amersham Biosciences instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Biosciences except for alterations described in this manual.

WARNING!

This is a Class A product. In a domestic environment, it may cause radio interference, in which case the user may be required to take suitable measures.

Terms and Conditions of Sale

Unless otherwise agreed in writing, all goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group which supplies them. A copy of these terms and conditions is available on request.

Should you have any comments on this product, we will be pleased to receive them at:

Amersham Biosciences AB

SE-751 84 Uppsala
Sweden

Trademarks

BPG, Drop Design, FineLINE, GSTPrep, HiLoad, HiPrep, INdEX, STREAMLINE, UNICORN, ÄKTA and ÄKTApilot are trademarks of Amersham Biosciences Limited.

Amersham and Amersham Biosciences are trademarks of Amersham plc.

Microsoft and Windows are either registered trademarks or trademarks of Microsoft Corporation in the United States and/or other countries.

Office Addresses

Amersham Biosciences AB

SE-751 84 Uppsala
Sweden

Amersham Biosciences UK Limited

Amersham Place
Little Chalfont
Buckinghamshire
England HP7 9NA

Amersham Biosciences Corp.

800 Centennial Avenue
PO Box 1327
Piscataway NJ 08855
USA

Amersham Biosciences Europe GmbH

Munzinger Strasse 9
D-79111 Freiburg
Germany

Amersham Biosciences K.K.

Sanken Building
3-25-1 Hyakunincho, Shinjuku-ku
Tokyo 169-0073
Japan

Amersham Biosciences Limited

13/E., Tower I
Ever Gain Plaza
88 Container Port Road
Kwai Chung, New Territories
Hong Kong

www.amershambiosciences.com

© Copyright Amersham Biosciences AB 2003

- All rights reserved

Contents

1 Introduction

1.1	ÄKTApilot system	7
1.2	The User Manual	8
1.3	Principle of ÄKTApilot	9
1.4	System pump and sample pump	10
1.5	Membrane valves	10
1.6	Mixer	11
1.7	Detectors and monitors	11
1.8	Pressure sensors	11
1.9	Air trap and air sensors	12
1.10	Associated documentation	12

2 Starting ÄKTApilot

2.1	Required installations	13
2.2	Starting the system	13

3 System preparation

3.1	Tubing and connectors	15
3.2	Priming the pumps	19
3.3	Columns	20
3.4	Sample application	25
3.5	Using the air trap	26
3.6	Sanitization	27
3.7	Gradient mixing	31
3.8	Fraction collection	32

4 Operation

4.1	Creating a method	35
4.2	Final preparation	37
4.3	Starting a run	39
4.4	During the run	40
4.5	Viewing and printing the result	41
4.6	After the run	44

Appendix A Feedback tuning

A.1	Introduction	45
A.2	Tuning description	45
A.3	Setting up feedback tuning	46
A.4	Optimizing the PID parameters	47

Appendix B Gradient performance



1 Introduction

1.1 ÄKTApilot system

ÄKTApilot™ is a high performance, automated liquid chromatography system. The system is designed for process development, process scale-up and scale-down, and small scale production.

ÄKTA™ pilot is biocompatible, hygienic and sanitizable. It meets all GLP and cGMP demands for Phase I–III in drug development and small-scale production.

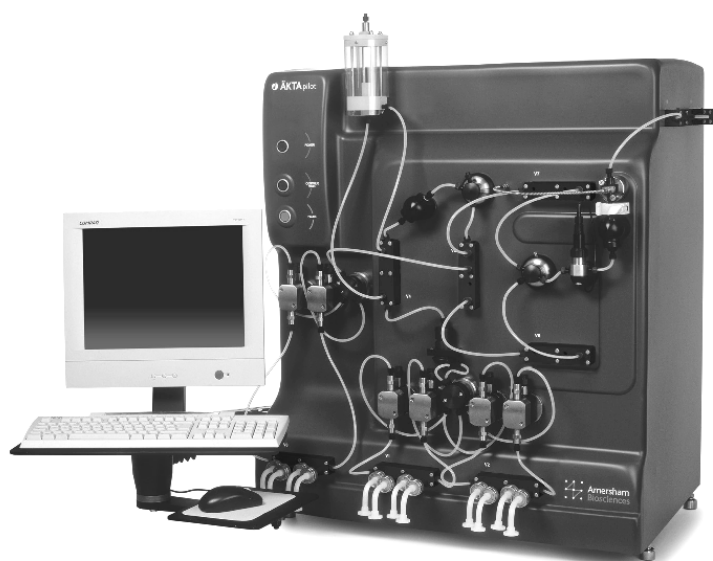


Fig 1-1. ÄKTApilot system

ÄKTApilot system consists of ÄKTApilot separation unit, the connected computer including a flat-screen monitor, and UNICORN™ control system.

ÄKTApilot features:

- Liquid flow rates from 4 to 800 ml/min (limited gradient performance at 400–800 ml/min).
- Operating pressure up to 20 bar.

1.1.1 Separation unit components

The location of the components of the separation unit is shown in the following figure.

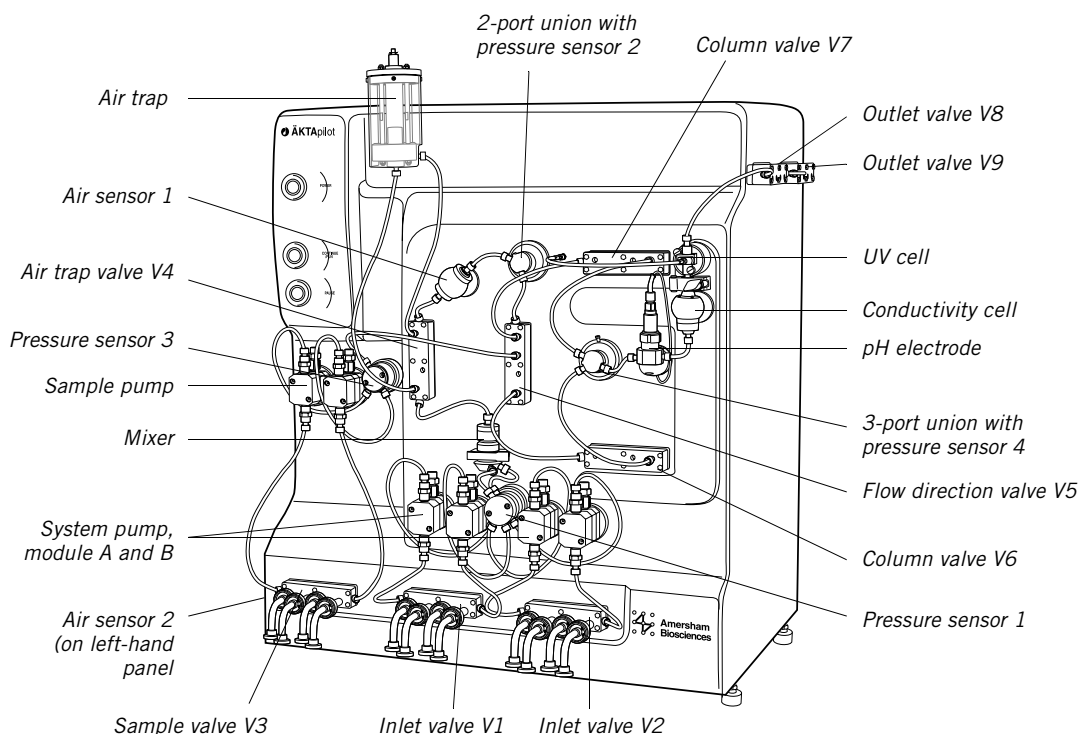


Fig 1-2. Location of components

1.2 The User Manual

This user manual describes the operation of ÄKTApilot. It provides instructions for preparing the system and performing a run using UNICORN control system. General sanitization procedures for the system are given in Section 3.6 Sanitization.

1.2.1 Typographical conventions

Menu commands, the names of dialog boxes, windows and option buttons are written in a **bold** typeface. Menu commands are written with the menu name and the command separated by a colon. For example, **File:Open** refers to the **Open** command in the **File** menu.

1.3 Principle of ÄKTApilot

ÄKTApilot contains all the fluid handling components required to perform buffer delivery and mixing, sample application, and in-line monitoring.

1.3.1 Liquid flow path

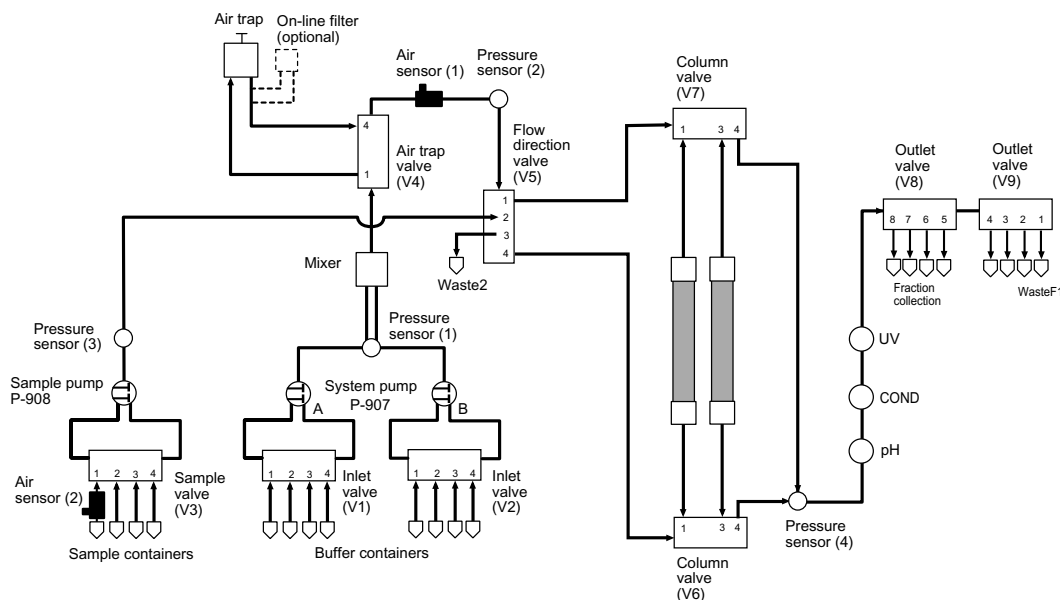


Fig 1-3. The liquid flow path of ÄKTApilot

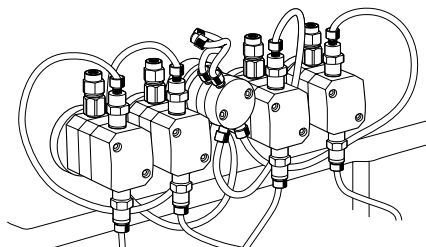
- 1 The sample valve selects an appropriate sample inlet tubing. The sample pump then delivers the sample solution to the column via a pressure sensor, the flow direction valve and the selected column valve. An air sensor can be used on the inlet tubing to, for example, prevent air from entering the column during sample loading.
- 2 The inlet valves select buffer inlet tubing. The system pump then delivers the buffer through a pressure sensor and a mixer to the air trap valve. If desired, the flow can be diverted by the air trap valve through an air trap (used to prevent air from entering the column).
- 3 After the air trap valve, the buffer flow is directed through an air sensor and a pressure sensor to the flow direction valve. The air sensor is used for column protection.
- 4 The flow path continues through one of the column valves to the column. The flow passes through the column (or the columns) where the separation takes place.

- 5 The flow then passes via a pressure sensor through the pH electrode located in a pH cell holder, the conductivity cell, and the UV cell.
- 6 The flow path continues to the outlet valves, which are used to divert the flow to waste or fraction collection.

Pressure sensors continuously monitor the pressure in the flow path.

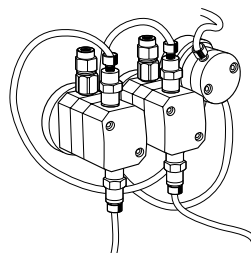
1.4 System pump and sample pump

The system pump, P-907, is a high performance pump producing an accurately controlled liquid flow. It is a low pulsation pump equipped with two pump modules; A and B. This allows for binary gradients with efficient mixing.



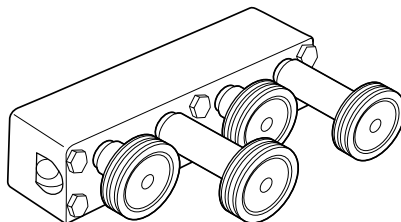
A pressure sensor is connected to pump module A (the left-hand pump heads). The pressure is almost the same in pump module B. The pressure sensor provides an over-pressure alarm function, which is preset in UNICORN.

The sample pump, P-908, is identical with the system pump but has only one pump module. P-908 is also equipped with a pressure sensor.



1.5 Membrane valves

Stepper motor-actuated membrane valves control the liquid flow in the separation unit. The valves are located in nine valve blocks. The valve blocks are individually designed regarding the inlet and outlet configuration. The illustration shows an inlet valve.



1.6 Mixer

Buffers delivered by the system pump are dynamically mixed by an electrically operated mixer. A 5 ml mixer chamber is available.

1.7 Detectors and monitors

ÄKTApilot is equipped with detectors for continuous in-line measurement of pH, conductivity and UV absorbance. The detectors provide accurate and reliable monitoring through self-test and self-calibration.

The flow cells are connected close together, which minimizes band broadening and time delay between the detectors. The flow cells are easily accessible from the front panel to facilitate user maintenance.

The pH electrode is mounted in the pH cell holder after pressure sensor 4 in the flow path. The pH monitor provides pH measurement in the range 0–14 (specifications valid between 2 and 12).

The conductivity cell is placed after the pH electrode in the flow path. It is used to verify gradients and to follow peak positions relative to ionic strength.

The relationship between the pH and the output signal from the pH electrode is temperature dependent. Therefore, the conductivity cell contains a temperature sensor which can be used to compensate the pH measurement during temperature changes.

The UV cell is placed after the conductivity cell in the flow path. It is used for measuring the UV absorbance of the liquid. Up to three wavelengths can be measured simultaneously in the range 190–700 nm.

1.8 Pressure sensors

There are four pressure sensors continuously measuring the pressure in the flow path. Two of them are located directly after the pumps, the third is placed before the column, and the fourth after the column.

1.9 Air trap and air sensors

The air trap is used for removing air bubbles flushed through the tubing by the buffer. It enhances system performance and prevents air from damaging the column performance. The air trap can also be used for removing air from the sample if the flow path, i.e. the tubing routing, between pressure sensor 3 and V5 is changed to include the air trap. Changes in the methods are also required.

Note: Directing the sample through the air trap will dilute the sample due to the large volume of the air trap.

The air sensor at the inlet is used to, for example, prevent air from entering the column during sample loading. The sensor can be connected to any of the three inlet valves.

The air sensor located before the flow direction valve V5 is used to prevent air in the buffer from entering the column.

1.10 Associated documentation

The documentation listed below is also included with ÄKTApilot.

ÄKTApilot Instrument Handbook contains technical information, maintenance schedules and instructions for troubleshooting and user maintenance.

ÄKTApilot Safety Handbook contains safety information.

UNICORN control system includes three manuals:

- *Getting Started*
- *User Reference Manual (2 pcs)*
- *Administration and Technical Manual*

2 Starting ÄKTApilot

2.1 Required installations

ÄKTApilot with the PC should be installed and prepared by personnel from Amersham Biosciences.

2.2 Starting the system

2.2.1 Start the separation unit

- 1 Switch on mains power to ÄKTApilot by turning the switch (located on the left side panel) to position **1**.

The **POWER** indicator on the separation unit flashes rapidly for a few seconds during the internal communication test.

After the test, the indicator flashes slowly.

2.2.2 Start and log on to UNICORN

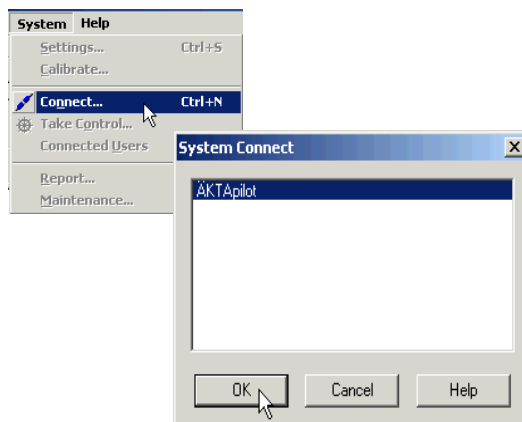
- 1 Switch on mains power to the PC and the monitor. Log on to Windows.
- 2 Start UNICORN by double-clicking on the icon on the Windows desktop.
- 3 When the **Logon** dialog appears, select a user from the **Users** list and enter the password. If you log on for the very first time, select user **default** and enter the password **default**. Click **OK**.



Refer to the UNICORN user manual *Getting Started* for a quick and easy guide to UNICORN software. It gives a brief introduction to the software structure and the work flow.

2.2.3 Connect UNICORN to the separation unit

- 1 In the **System Control** module, select **System:Connect...**



- 2 Select the appropriate system name and click **OK**.

When UNICORN is connected to the separation unit:

- There is a constant light on the **POWER** indicator on the separation unit.
- The green **RUN** indicator in the status bar in UNICORN is lit.



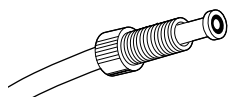
3 System preparation

This chapter describes how to prepare and optimize the system for different applications. The options available are discussed in the following sections:

- Tubing and connectors (section 3.1)
- Priming the pumps (section 3.2)
- Columns (section 3.3)
- Sample application (section 3.4)
- Using the air trap (section 3.5)
- Sanitization (section 3.6)
- Gradient mixing (section 3.7)
- Fraction collection (section 3.8)

3.1 Tubing and connectors

3.1.1 System tubing



It is important to use the correct system tubing. On delivery, the tubing from the inlet valves V1–V3 to the outlet valve V9 has i.d. 2.9 mm and o.d. 3/16". The tubing is made of ETFE and pre-flanged with 5/16" connectors.

3.1.2 Optional tubing

Tubing with i.d. 1.6 mm is available to enhance system performance at low flow rates. See also Ordering information in *ÄKTApilot Instrument Handbook*. This i.d. 1.6 mm tubing replaces the original tubing from the sample valve and the inlet valves to the outlet valves.

Note: *If tubing other than the original i.d. 2.9 mm system tubing is used, the sanitizability of the flow path cannot be guaranteed.*

Note: *When using a smaller tubing, the system pressure increases if the flow rate is not adjusted accordingly.*

3.1.3 Buffer and sample inlet tubing

Equilibration, washing and elution buffers, as well as cleaning solutions, are introduced into the system through the inlet valves V1–V2. The sample is applied through valve V3. There are eight buffer inlets, four in each valve, and four sample inlets available. Each inlet is equipped with a TC connector.

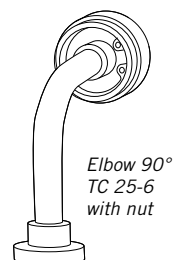
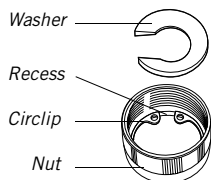
The inlet tubing included in the system is 120 cm long and has TC connectors at both ends. The tubing is made of PVC and has an inner diameter of 7 mm. An Elbow 90° TC 25-6, a TC clamp and an attachment nut is required to connect the tubing to the valve.

Note: Aggressive solvents, e.g. acetonitrile, propanol, ethanol, or tetrahydrofuran, should not be used with PVC tubing.

Assembling the attachment nut

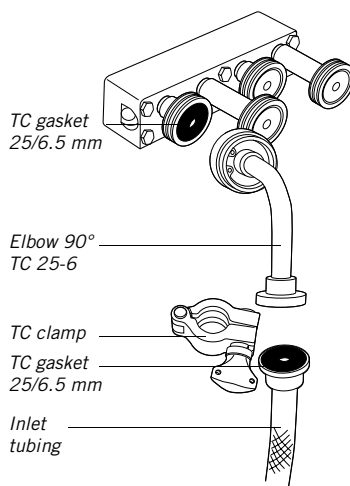
Required tool: Circlip pliers

- 1 Thread the attachment nut onto the Elbow 90° TC 25-6.
- 2 Using the circlip pliers, fit the circlip in the recess at the bottom of the nut.
- 3 Place the washer in the nut with the flat side facing the bottom of the nut.



Connecting the inlet tubing to a valve

- 1 Fit a rubber sealing in the TC connector of the chosen valve inlet.
- 2 Fit the Elbow 90° TC 25-6 to the TC gasket 25/6.5 mm and tighten the attachment nut firmly.
- 3 Fit a TC gasket 25/6.5 mm to a TC connector on the inlet tubing.
- 4 Fit the TC connector on the inlet tubing to the Elbow 90° TC 25-6.
- 5 Complete the connection using a TC clamp.



Connecting air sensor 2 to an inlet tubing

The distance between the air sensor and the valve depends on how the air sensor is monitored by UNICORN. It can be monitored in two ways:

- Alarm monitoring
- Watch monitoring

When using Alarm monitoring, the system is immediately set to PAUSE mode at air detection and the pump stops before air enters the valve. In this case, the air sensor can be connected directly to the Elbow 90° TC 25-6.

When using Watch monitoring (used in the Method Wizard), the system needs more time to activate the desired Watch instructions after air detection. In this case, an extra piece of PVC-tubing is required between the air sensor and the Elbow 90° TC 25-6 as a delay for the detected air.

As a rule of thumb, the extra piece of tubing should be at least 20 cm long at 200 ml/min, and 70 cm at 400 ml/min.

***Note:** The Elbow 90° TC 25-6 should always be connected when using air sensor 2.*

To connect air sensor 2 to an inlet tubing:

- 1 If using Watch monitoring, connect the extra piece of tubing to the Elbow 90° TC 25-6 using a TC clamp.
- 2 Fit two 5/16" male/TC unions to air sensor 2.
- 3 Connect air sensor 2 to the tubing (or to the Elbow 90° TC 25-6 if using Alarm monitoring) using a TC clamp.
- 4 Connect the ordinary inlet tubing to the other connector on air sensor 2 using a TC clamp.
- 5 To choose pump, select **System:Settings**. Air sensor 2 is found under **Instructions**.
- 6 If the air sensor is connected to a sample inlet (V3), select **SamplePump**.

If the air sensor is connected to a buffer inlet (V1, V2), select **SystemPump**.

3.1.4 Outlet tubing

Fractions can be collected through ports 5–8 in valve V8 and ports 2–4 in valve V9. Port 1 in valve V9 is used for the waste outlet.

A selection of pre-flanged outlet tubing pieces with connectors is included in the system. The outlet tubing has the same inner diameter as the system tubing; 2.9 mm.

- 1 Choose a tubing length that suits the distance to the fraction collection container, for example, to Fraction Collector Frac-950, or to the waste container.
- 2 Connect the tubing to the chosen fraction collection or waste outlet port. Note that Frac-950 should be connected to outlet valve V9, port 2.
- 3 Put the tubing in the fraction collection container or the waste container.

For connecting the tubing to Frac-950, see *Fraction Collector Frac-950 User Manual*.

3.2 Priming the pumps

3.2.1 Automated priming

To prime the pumps and the inlet tubing with buffer (or sample), it is convenient to use the pump wash instructions that are available in the System Control module:

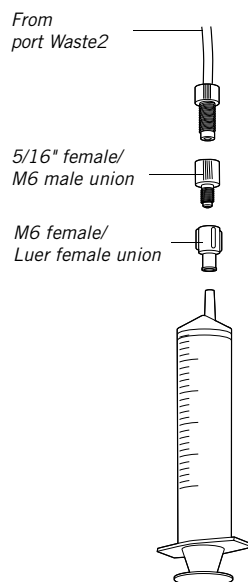
- SystemPumpWash
- SamplePumpWash

3.2.2 Manual priming

If a pump wash instruction fails to fill the pump or tubing, the flow path in the pump might be dry, which reduces the pumping capacity. If so, the pump has to be primed manually, or at least wetted, using a syringe and appropriate fittings.

To prime the pump and inlet tubing manually:

- 1 Fill a flask with distilled water and immerse the end of the appropriate inlet tubing in the water.
- 2 Connect a tubing equipped with 5/16" connectors at both ends to port 3 (Waste2) in the flow direction valve V5.
- 3 Connect a 5/16" female/M6 male union to the other end of the tubing.
- 4 Connect a M6 female/Luer female union to the first union.
- 5 Fit an empty male Luer syringe (> 25 ml) to the Luer union.
- 6 In the **System Control** module, select **Manual:Flowpath**.
- 7 Open Waste2 and an inlet valve that is connected to the pump module you want to prime.



Note: Only *one* inlet valve should be opened.

- 8 Use the syringe to draw water through the inlet tubing and pump until it starts to enter the syringe.

3.3 Columns



WARNING! OVER-PRESSURE. Use columns that withstand expected pressures. If not, the columns might rupture, resulting in injury.



WARNING! OVER-PRESSURE. The flow rate may under no circumstances exceed the specified column maximum flow rate. The flow might affect the packed column, causing the pressure to exceed the specified column maximum pressure.

The system can be used with small, pre-packed columns as well as with standard laboratory and pilot-scale columns. This section provides a list of recommended columns. It also includes a description of how to connect a column.

More information about columns and separation media can be found in the Product catalog on the company web page.

3.3.1 Recommended columns

Table 3-1 lists empty columns that are recommended for used in ÄKTApilot.

<i>Code no.</i>	<i>Column name</i>	<i>Max. pressure [MPa]</i>
18-1000-72	XK 26/20	0.5
18-8768-01	XK 26/40	0.5
18-8769-01	XK 26/70	0.5
18-8770-01	XK 26/100	0.5
18-1000-71	XK 50/20	0.3
18-8751-01	XK 50/30	0.3
18-8752-01	XK 50/60	0.3
18-8753-01	XK 50/100	0.3
18-1115-06	INdEX™ 70/500	0.3
18-1115-07	INdEX 70/950	0.3
18-1104-15	INdEX 100/500	0.3
18-1104-16	INdEX 100/950	0.3
18-1115-08	INdEX 140/500	0.3
18-1115-09	INdEX 140/950	0.3
18-1103-01	BPG™ 100	0.8
18-1113-08	BPG 140	0.6
18-1103-11	BPG 200	0.6
18-1102-02	FineLINE™ Pilot 35	2
18-1152-98	FineLINE 70/375	2
18-1152-99	FineLINE 70/750	2
18-1105-35	FineLINE 100	2
18-1119-56	FineLINE 100/750	2
18-1110-50	STREAMLINE 25 (hydraulic)	0.1
18-1110-51	STREAMLINE 25 (manual)	0.1
18-1038-01	STREAMLINE 50	0.1
18-1126-34	STREAMLINE 100	0.1

Table 3-1. Empty columns recommended for ÄKTApilot

3.3.2 Optional columns

In the following families with small, prepacked columns, most of the columns can be used with ÄKTApilot:

- GSTPrep™
- HiLoad™
- HiPrep™

However, to fully utilize the performance of these columns, the system tubing should be changed to a tubing with a smaller inner diameter, especially the tubing after the column. Tubing with i.d. 1.6 mm is available to enhance system performance at low flow rates. See Ordering information in *ÄKTApilot Instrument Handbook*.

Note: When using a smaller tubing, the system pressure increases if the flow rate is not adjusted accordingly. Study the column instructions and change the method as required.

Note: If tubing other than the original i.d. 2.9 mm system tubing is used, the sanitizability of the flow path cannot be guaranteed.

3.3.3 Mounting a column on the frame

Small laboratory columns can be mounted on the frame between the column valves to reduce the tubing lengths and thus the dead volume.

Fasten the column on the frame using the appropriate attachment devices.

3.3.4 Connecting a column

Install the column according to the instructions below. Read through the complete installation procedure before attempting to install a column. Refer also to the column installation information provided in the instructions for the chosen column.

- 1 Fill the system with an appropriate buffer for column installation (see the column instructions).
- 2 Remove any stop plugs or tubing connectors from the ports on column valves V6 and V7 to which the column is to be connected. Ports 1 and 3 are used for connecting columns, while port 4 is always used for column bypass.
- 3 Connect a tubing to the chosen port in valve V6.
- 4 Fill the tubing manually with buffer using the pump
- 5 Connect the other end of the tubing to the bottom of the column.

Note: *Combine the TC-unions, connectors and tubing supplied with the system with those included in the column to create a proper connection between the valve and the column.*

Note: *Avoid using thicker tubing than required between the valves and the column. TC connectors should be connected as close as possible to the column.*

- 6 Connect a tubing between the chosen port in valve V7 and the top of the column.

The column is now ready for use and should be equilibrated before sample application.

3.3.5 Automated column packing

The Method Wizard in ÄKTApilot includes a method called Column packing, which is especially made for column packing. The system controls the flow and the pressure during the packing procedure, and hence provides automated and unattended column packing.

Preparing column packing

Refer also to the packing instructions provided with the column instructions.

- 1 Connect the tubing from column valve V7, port 1 (or 3), to the upper column packing port.
- 2 Connect the tubing from column valve V6, port 1 (or 3), to the lower column packing port.
- 3 Prepare a container with a suitable packing solvent.
- 4 Immerse inlet tubing A1 and B1 in the packing solvent.
- 5 Route the appropriate waste tubing to waste.
- 6 Fill the column according to the column instructions.

Packing the column

- 1 In the **System Control** module, select **File:Instant Run**.
- 2 In the **Instant Run** window, select system. Click **Run**.
- 3 In the **Method Wizard**, choose **Special Method**.
- 4 Select **Column packing**.
- 5 Select the appropriate parameter values in the dialog boxes that follow.
- 6 Click **START** in the last dialog box.

The **Variables** page shows the parameters that determine the control of the flow and pressure during column packing.

- 7 The default parameter values suit the most frequent situations, but can be further optimized if required. More information about changing the tuning parameters can be found in Appendix A Feedback tuning.

3.3.6 Removing a column

When removing a column, disconnect and plug the bottom connection first, and then disconnect and plug the top connection.

3.4 Sample application

The sample can be applied either by using the sample pump or by using the system pump. The sample pump is most practical since it does not have to be washed directly after sample application. Furthermore, the hold-up volume is smaller when using the sample pump.

In the Method Wizard, the **Sample Loading** dialog contains the parameters that specify sample application.

***Note:** When using the Method Wizard for creating a method, only the sample pump is enabled for sample application.*

3.4.1 Priming the sample tubing

Before a gel filtration run, prime the sample tubing all the way up to the valve V5 in order to reduce dilution of the sample. This is especially important when the sample volumes are small.

When using adsorption techniques, e.g. hydrophobic interaction, affinity or ion exchange, the general recommendation is to prime the sample inlet tubing up to the sample inlet valve.

3.4.2 Using air detection

An air sensor can be connected on the inlet tubing to prevent air from entering the column. The system can then be set to stop the sample flow and to, for example, allow the tubing to valve V5 to be flushed with buffer through another port, or let elution buffer enter the system through the inlet valves. The air sensor thus enables unattended filling of samples with unknown volumes.

Instructions for connecting the air sensor are found in section 3.1.3 Buffer and sample inlet tubing.

To enable air detection in the Method Wizard, choose **Watch Air Sensor 2** in the dialog.

To define which pump is controlled by the air sensor, select **System:Settings**. Air sensor 2 is found under **Instructions**. If the air sensor is connected to a sample inlet (V3), select **SamplePump**. If the air sensor is connected to a buffer inlet (V1, V2), select **SystemPump**.

3.4.3 Using feedback tuning

Feedback tuning provides unattended, continuous control of the flow rate and pressure during sample application. Feedback tuning is particularly useful in applications where high back-pressure can be expected, or when the back-pressure fluctuates, for example, when using viscous samples. With feedback tuning the flow rate is controlled so that the pressure never exceeds the maximum pressure of the column, which otherwise interrupts the sample application.

The default values of the parameters that determine the feedback tuning properties are suitable for most purposes.

***Note:** If the flow rate falls below the MinFlow setting, an Alarm is triggered and the system set to Pause. We recommend using a Watch instruction for the flow that is activated above the MinFlow setting. A suitable action is to continue to the next block. Use a lower flow rate in this block than used before the Alarm.*

Appendix A Feedback tuning contains more information about the parameters, how to use them, and how to optimize them.

Feedback tuning in the Method Wizard

When creating a method in the Method Wizard, feedback tuning is included by choosing **Automatic Pressure Flow Regulation**.

The tuning parameters set in the Method Wizard apply to both the sample pump and the system pump. However, the parameter values for the pumps can be changed separately on the Variables page.

The default feedback tuning set in the Method Wizard is activated only during sample application and wash-out of unbound sample.

3.5 Using the air trap

The air trap is used for removing air bubbles flushed through the tubing by the buffer.

The air trap can also be used for removing air from the sample. This function requires changes in the methods and in the tubing routing between pressure sensor 3 and valve V5 in order to include the air trap.

The air trap is equipped with a vent connector at the top. This used for adjusting the buffer level in the air trap before the run.

To include the air trap in the flow path, choose **Include Air Trap** in the Method Wizard. When using instructions, set **Airtrap_Filter** to **Inline**.

3.6 Sanitization

ÄKTApilot is designed to be hygienic and sanitizable. To facilitate sanitization, the Method Wizard in UNICORN contains two ready-made sanitization methods for Cleaning-in-place (CIP): CIP System and CIP Column.

CIP System is used for sanitizing the entire flow path, including selected inlet and outlet tubing. The columns must be replaced by bypass tubing before the sanitization. The bypass tubing is provided with the system. The pH electrode should also be removed and the dummy electrode inserted in the cell holder instead.

CIP Column is for sanitizing columns. The method is adapted to the column specified when setting up the method in the Method Wizard.

3.6.1 Preparing for a CIP System sanitization



WARNING! CORROSIVE CHEMICALS. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

CAUTION! Always make sure that the chromatography media, columns and system components are compatible with sodium hydroxide at the concentration, time, and temperatures used.

General preparation

The sanitizing agent that is used, NaOH (1 M), is widely accepted for sanitizing chromatography systems. However, as with any sanitizing agent, certain precautions should be taken and compatibility with both chromatography media and system determined.

- 1 Prepare four suitable containers/flasks with the following contents:
 - 10 l of 1 M NaOH.
 - 16 l of water of appropriate quality.
 - 1 l of 1 M NaOH (for rinsing system).
 - 1 l of 20% ethanol (for rinsing system)
- 2 Immerse the ends of all inlet tubing that should be sanitized in the water.
- 3 Route all outlet tubing that should be sanitized to waste.

- 4 Replace the column(s) with by-pass tubing.
- 5 Replace the pH electrode with a dummy electrode.
- 6 Clean the tip of the pH electrode with the 70% ethanol.
Immerse the tip in a 1:1 mixture of pH 4 buffer and 2 M KNO₃.

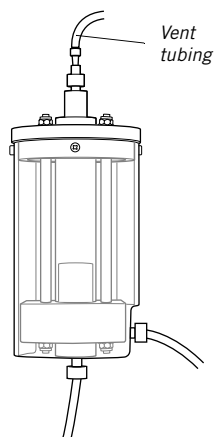
Preparing the inlet tubing and the air trap

Note: Make sure that the inlet tubings and the pumps are filled with water.

Before the sanitization, the air trap has to be filled with water.

Preparing the air trap for filling:

- 1 Connect the air trap vent tubing to the vent connector.
- 2 Route the tubing to a suitable waste container.
- 3 Open the vent connector by unscrewing it a few turns.
- 4 Immerse the ends of all inlet tubing that should be sanitized in the water container.



Filling the air trap:

- 1 In the **System Control** module, select **Manual:Flowpath**.
- 2 Set **Airtrap_filter** to **Inline** and click **Insert**.
- 3 Set **InletA** to **InletA1** and click **Insert**.
- 4 Set **InletB** to **InletB1** and click **Insert**.
- 5 Set **Waste2** to **Open** and click **Insert**.
- 6 Click **Execute**.
- 7 Start the pump at more than 200 ml/min to fill the air trap.
- 8 When the air trap is full and water enters the tubing at the top, set **Airtrap_filter** to **Bypass** and click **Execute**.
- 9 Click **End** on the toolbar to stop the pump and to close the inlet and outlet valves.
- 10 Close the vent connector by tightening it.

3.6.2 Running the CIP System sanitization

Starting the method

- 1 In the **System Control** module, select **File:Instant Run...**
- 2 In the **Instant Run** window, select system. Click **Run**.
- 3 In the **Method Wizard**, choose **Special Method**.
- 4 Select **CIP System**.
- 5 Select the appropriate parameter values in the subsequent dialogs.

By default, all inlets and outlets are included in the sanitization method. Inlet A1, Inlet B1, Sample 1, Waste2 and WasteF1 are **always** included in the sanitization.

If a valve is deselected in the Method Wizard, for example, Inlet A3, the time in the method for sanitizing Inlet A3 will be transferred to Inlet A1. In the same way, time for Inlet B2–B4 is transferred to Inlet B1, time for Sample 2–4 to Sample 1, and time for Waste F2–F8 to Waste F1. Consequently, the total time for running CIP System always remains the same.

- 6 Click **Run** in the last dialog box. The start protocol opens.
- 7 Go through the dialogs in the start protocol by clicking **Next**.

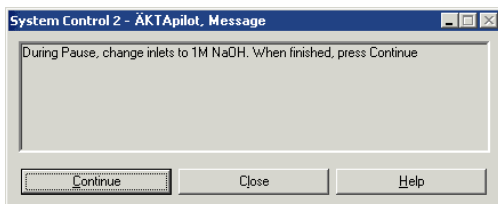
Note: *Do not close any inlet or outlet valves on the Variables page. The system will be set to Pause when trying to deliver liquid through a valve that has been closed on the Variables page.*

- 8 Click **START** in the last dialog to start the sanitization.

The CIP System method takes about 2.5 hours.

Operator actions during the run

The liquid that is used has to be changed manually a few times during the sanitization. Therefore, the system is set to Pause and a dialog (see example below) showing the required action appears in UNICORN when it is time to move tubing to another container or flask.



The sanitization is continued by clicking **Continue**.

Note: *When moving the tubings, air might accidentally enter the tubing. When the sanitization continues, the air will eventually get trapped in the air trap. Remove the air from the air trap using the vent connector to make sure that the air trap remains completely filled.*

The system is paused three times during the sanitization:

- 1 After the water rinsing (13 min from method start), the system will be rinsed with NaOH.
 - All inlets tubing ends should be moved to the large NaOH container.
 - All inlet and outlet tubing ends of the rinsing system should be moved to the small NaOH flask.
- 2 After the NaOH rinsing (25 min from method start), NaOH will be recirculated during the main part of the sanitization.
 - The tubing ends of the outlet tubing from valve V8, V9 and Waste2 should be moved from the waste container to the large NaOH container, where the inlet tubing ends are immersed.
- 3 After the recirculation of NaOH (approx. 125 min from method start), the system will be neutralized by using water.
 - The inlet tubing ends should be moved to the water container and the outlet tubing ends to waste.
 - The inlet and outlet tubing ends of the rinsing system should be moved to the 20% ethanol flask.

3.7 Gradient mixing

Gradients are mixed by using two separate buffers, each pumped by a separate pump module of the system pump.

3.7.1 Mixer chamber

The actual mixing takes place in the mixer chamber, which has a stirrer to make the mixing more efficient. The mixer chamber has a volume of 5 ml.

3.7.2 Gradient performance

The *specified* gradient performance is limited due to restrictions in the system pump as shown in the figure below. Note the limitations at flow rates above 400 ml/min, i.e. when running the system pump in dual mode. See also Appendix B Gradient performance for details.

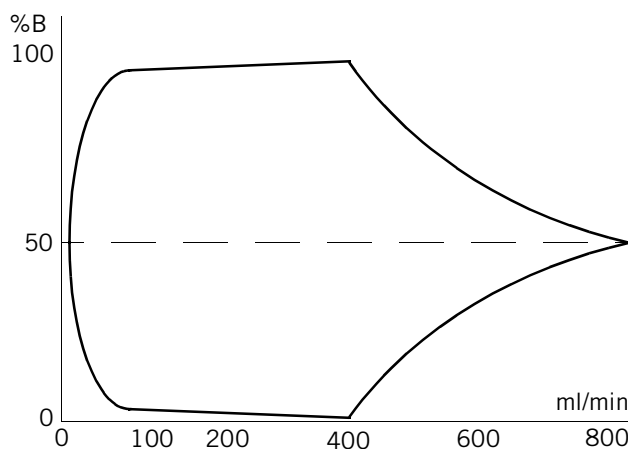


Fig 3-1. Gradient performance

Note: Gradients outside the specified range can be made at flow rates below 400 ml/min. However, the specified performance is then not guaranteed.

3.8 Fraction collection

Fractions can be collected through ports 5–8 in valve V8 and ports 2–4 in valve V9, or by connecting Fraction Collector Frac-950 to port 2 in valve V9. Port 1 in valve V9 is used for the waste outlet.

The fractionation parameters, e.g. fractionation volumes and collecting windows, are set in UNICORN when creating the method.

The most frequent fractionation options are:

- Flow through fractionation
- Eluate and peak fractionation

Flow through fractionation means that fixed volumes are collected before the eluate fractionation starts.

Eluate fractionation allows you to collect fixed volumes during the elution within a set interval of %B. Eluate fractionation can be combined with peak fractionation, which means collecting peaks during the elution. A peak can be collected in one fraction only or in several fractions.

3.8.1 Fraction Collector Frac-950

Fraction Collector Frac-950 is supported by UNICORN, i.e. it is easy to initiate as a pre-defined component and the software already contains parameters and commands for controlling Frac-950.

***Note:** When using Fraction Collector Frac-950, the maximum allowed flow rate is 100 ml/min.*

Fraction Collector Frac-950 should be connected to valve 9, port 2. More installation instructions are found in the *Fraction Collector Frac-950 User Manual*.

Preparing Frac-950 for operation

- 1 In the **UNICORN Main Menu** module, select **Administration: System Setup**.
- 2 Select the appropriate system and click **Edit**.
- 3 Click **Component**.
- 4 Select **Frac-950** and click **OK**.
- 5 Click **OK** and then **Close**.

The instructions associated to Frac-950 appear when creating a method or editing an existing method.

To find the Frac-950 instructions:

- 1 In the **System Control** module, select **Manual:Frac**.
- 2 Study the instructions available for Frac-950 to familiarize yourself with the new functions available.



4 Operation

This section describes the following topics:

- Creating a method.
- Final preparation before a run.
- Performing a run.
- After-run procedures.
- Viewing and printing the result.

4.1 Creating a method

To create a new method, there are two alternatives:

- In the Method Wizard, customized methods for most purposes are made by setting appropriate values for the method variables.
- In the Text Instructions editor in the **Method Editor** module, more advanced editing facilities are available. For more information, see the UNICORN user manuals.

To create a new method using the Method Wizard:

- 1 In the **Method Editor** module, click the Method Wizard icon .

If necessary, select the system to use.

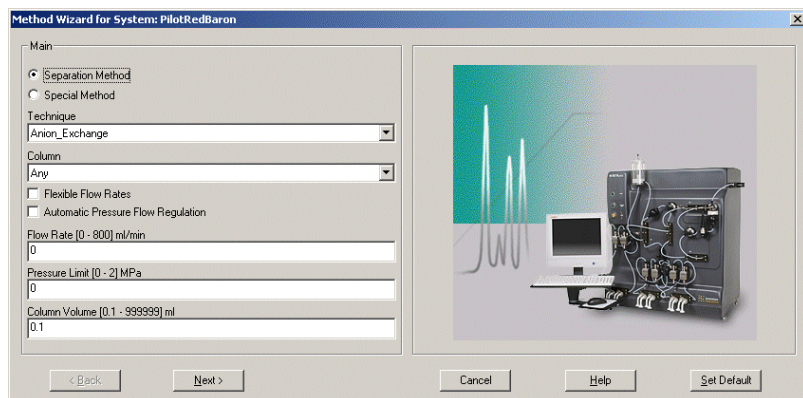


Fig 4-1. ÄKTApilot Method Wizard

- 2 In the first dialog box, select **Separation method** or **Special method**.

In **Separation method**, you find frequently used purification techniques.

In **Special method**, you find, for example, a method for sanitizing the system.

- 3 In each dialog box, select the appropriate parameter values and click **Next** to continue.
- 4 Click **Finish** in the last dialog box.
- 5 The Run Setup window that appears consists of a number of tab pages. On the **Variables** page, chosen columns and running conditions are displayed. These values can be changed if desired.

Block	Variable	Value	Range
Main	Column	FineLine70_Source30Q [Global]	
Start_Settings	Inlet_A	InletA1	
	Inlet_B	InletB1	
	ColumnValve	Col1Up_Col2Bypass	
	Wavelength_1 (nm)	280	190 - 700
	Wavelength_2 (nm)	254	190 - 700
	Wavelength_3 (nm)	215	190 - 700
Start_Concentration	StartConcentration_B (%B)	0.00	0.00 - 100.00
Flow_Rate	FlowRate (ml/min)	100.0	0.0 - 800.0
Sample_Loading	Sample_Inlet	Sample1	
Sample_Volume	Sample_Volume (ml)	50.00	0.00 - 99999.00
Outlet_Fractionation	Fraction_Volume (ml)	40	40 - 100000

☐ Show details
☐ Show unused variables
☒ Display tooltip for extended variable cells

[Edit Variable...](#)
[Help](#)

Fig 4-2. The Variables page

- 6 Default values for the selected columns are set for:

- column volume
- recommended flow rate
- maximum pressure limit

Additional customized columns can be added to the column list by selecting **Edit:Column List** in the **Method Editor** module.

More information about the tab pages in the Run Setup window is provided in the UNICORN user manuals.

- 7 Select **File:Save** to save the method.

4.2 Final preparation

This section describes the final preparations that should be done before starting a run.

4.2.1 Buffers

- 1 Immerse the ends of the buffer inlet tubing in the appropriate buffer containers.
- 2 Check that there are sufficient buffer volumes available.

4.2.2 Samples

- 1 Put the ends of the sample inlet tubing in the appropriate sample containers.
- 2 Check that there is sufficient sample available.

4.2.3 Fractionation

- 1 If fractionation is included in the method, put the outlet tubing from the outlet valves V8 and V9 into appropriate fractionation bottles. When using Fraction Collector Frac-950, connect it to valve V9, port 2.
- 2 Check that the fractionation bottles will accept the volumes diverted to them during the run.

4.2.4 Waste

- 1 Put the waste tubing from flow direction valve V5, port 3, and outlet valve V9, port 1, into a waste container.
- 2 Check that the waste container is not full and will accept the volume diverted to it during the run.

4.2.5 Columns

- 1 Check that the correct columns are fitted to the correct positions in the column valves V6 and V7 (see Section 3.3.4 Connecting a column).

Note: Port 4 in the valves is used for bypass and no column should be connected to this position.

- 2 Check that the columns have been packed properly and equilibrated (if not included in the method).

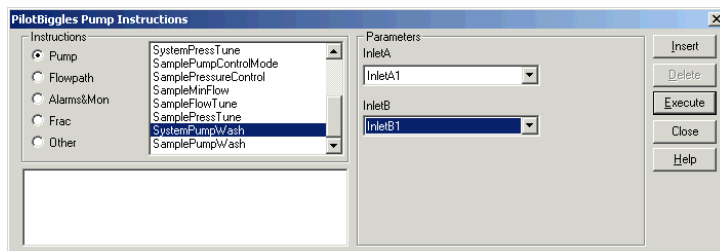
4.2.6 Calibration

Calibrate the pH electrode if required. Refer to *ÄKTApilot Instrument Handbook*.

4.2.7 Filling the inlet tubing

To fill the buffer and sample inlet tubing with the correct liquids:

- 1 In the **System Control** module, select **Manual:Pump**.



- 2 Select the instruction **SystemPumpWash**.
- 3 Select the appropriate inlet on V1 and V2.
- 4 Click **Execute** to fill the buffer inlet tubing. The flow direction valve V5 will automatically switch to the Waste2 port during the pump wash.
- 5 Select the instruction **SamplePumpWash**.
- 6 Select the appropriate inlet on V3.
- 7 Click **Execute** to fill the sample inlet tubing. The flow will be diverted to the Waste2 port through V5.

4.3 Starting a run

- 1 In the **System Control** module, select **File:Run**.
- 2 Select the method to start. Click **OK**.

A Start Protocol appears consisting of a number of dialog boxes.

- 3 On the **Variables** page, it is possible to fine-tune the method before proceeding.

Block	Variable	Value	Range
Main	Column (ml)	50.000	0.100 - 999999.000
Start_Settings	Inlet_A	InletA1	
	Inlet_B	InletB1	
	ColumnValve	Col1Up_Col2Bypass	
	Wavelength_1 (nm)	280	190 - 700
	Wavelength_2 (nm)	OFF	190 - 700
	Wavelength_3 (nm)	OFF	190 - 700
Start_Concentration	StartConcentration_B (%B)	0.00	0.00 - 100.00
Flow_Rate	FlowRate (ml/min)	100.0	0.0 - 800.0
Sample_Loading	Sample_Inlet	Sample1	
Sample_Volume	Sample_Volume (ml)	10.00	0.00 - 999999.00
Outlet_Fractionation	Fraction_Volume (ml)	40	40 - 100000

☐ Show details
☐ Show unused variables
☒ Display tooltip for extended variable cells

< Back Next > Cancel Help

Fig 4-3. Start Protocol – Variables dialog box

- 4 Make sure that the sample volume is correct.
- 5 Click **Next** or **Back** to go through the dialog boxes and add the information that is required as well as your own comments.
- 6 Click the **START** button in the **Result Name** dialog box. This will initiate the method run.

4.4 During the run

The progress of the method being used can be viewed in detail in UNICORN. The **System Control** module displays the current status of ÄKTApilot and displays up to four view panes for monitoring different aspects of the run.

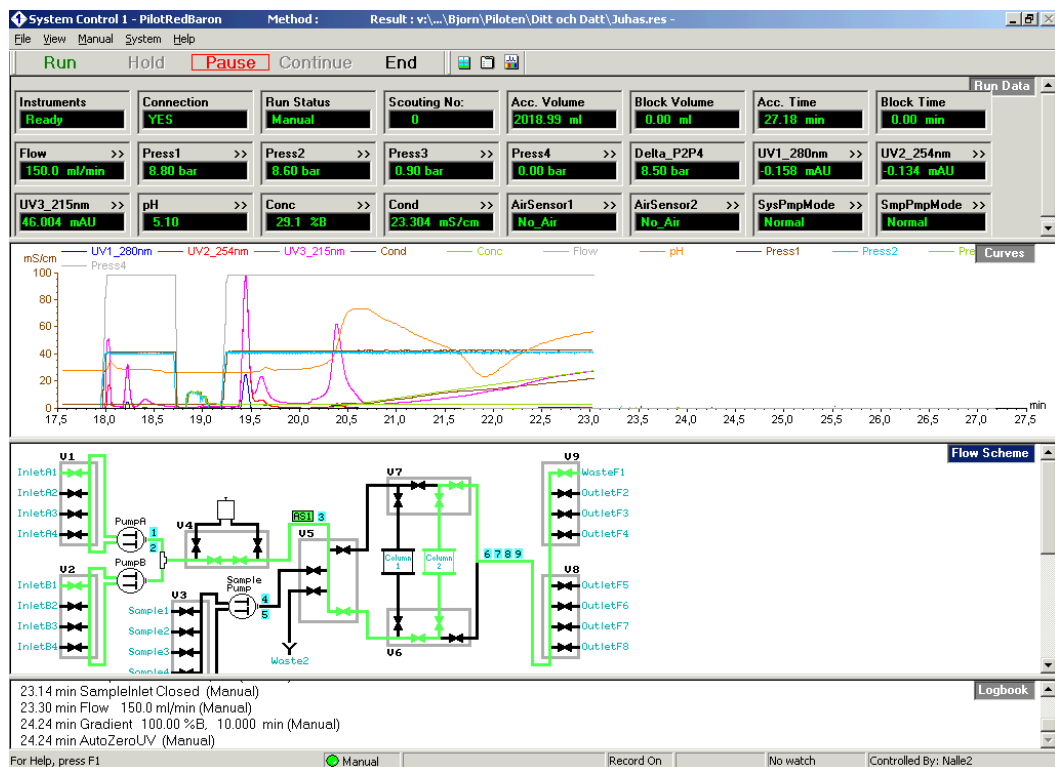


Fig 4-4. The System Control window

To customize the view panes **Run data**, **Curves**, **Flow scheme** and **Logbook**, right-click in the respective view pane and select **Properties...**

For more information about customizing the view panes, see the UNICORN user manuals.

To stop the run before it is finished, click the **End** button above the **Run data** view pane.

4.5 Viewing and printing the result

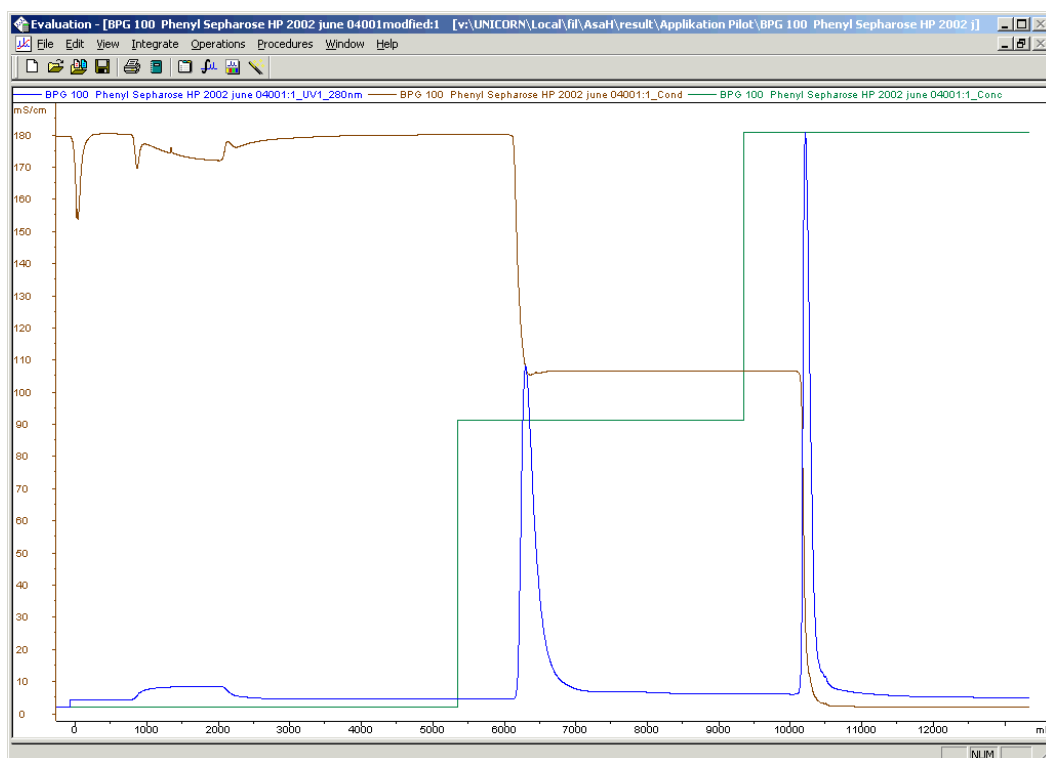
This section describes the basics of how to view and print the result in the **Evaluation** module.

4.5.1 Viewing the result

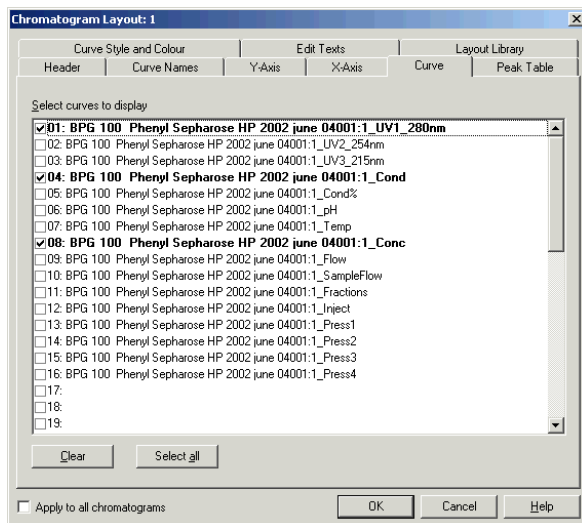
To view the result:

- 1 Locate the result file in the **Results** pane in the **Main Menu** module.
- 2 Double-click the file.

The result file opens in a **Chromatogram** window in the **Evaluation** module. See below.



- 3 To change the layout of the **Chromatogram** window, right-click in the window and select **Properties**.



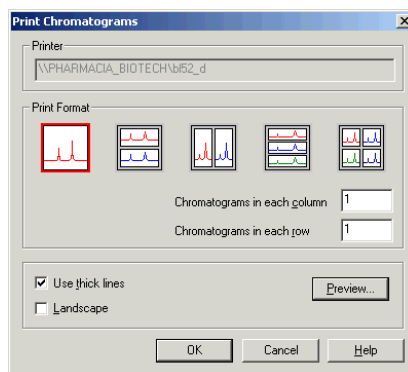
- 4 In the **Curve** tab page, click the check boxes to clear the curves you do not want to display.
- 5 Click **OK**.

Refer to the UNICORN user manuals for more information about viewing the result and customizing the layout.

4.5.2 Printing the result

To print the chromatograms:

- 1 Open all chromatograms you want to print in the **Evaluation** module.
- 2 Select the **File:Print** command.
- 3 In the **Print Chromatograms** dialog, select print formats and layout options.



- 4 Click **Preview**.
- 5 In the **Customise Report** window, verify that the layout is correct.
- 6 Click **Edit Mode** to make changes. Click **Preview** to return to the preview mode.
- 7 Click **Exit** to return to the **Print Chromatograms** dialog.
- 8 Click **OK**.

Refer to the UNICORN user manuals for more information about printing results.

4.6 After the run

4.6.1 Cleaning between runs

If the system needs to be rinsed, flush with distilled water as follows:

- 1 Submerge the ends of the appropriate inlet tubing in distilled water.
- 2 Use appropriate instructions/methods to rinse the flow path.

The SystemPumpWash and SamplePumpWash instructions rinse the flow path to the flow direction valve V5. The flow is then diverted to Waste2.

The SystemWash method in the Method Wizard rinses the entire flow path to the outlet valves V8 and V9.

4.6.2 Leaving for a few days

- 1 Rinse the entire flow path with distilled water using the System Wash method found in the Method Wizard.
- 2 Repeat with a bacteriostatic solution, for example, 20% ethanol (not the pH electrode, see instruction below).

The pH electrode should always be stored in a 1:1 mixture of pH 4 buffer and 2 M KNO₃ when not in use. When the pH electrode is removed from the cell holder, the dummy electrode (supplied) must be inserted in the flow path.

CAUTION! Never leave the pH electrode in the electrode holder when the system is not used since this might cause the glass membrane of the electrode to dry out. Remove the pH electrode from the cell holder and fit the end cover filled with a 1:1 mixture of pH 4 buffer and 2 M KNO₃. Do NOT store in water only.

4.6.3 Cleaning-in-place

Two Cleaning-in-place methods are available in the Method Wizard:

- CIP System (for system sanitization)
- CIP Column

See section 3.6 Sanitization for instructions about using the Cleaning-in-place methods.

Appendix A Feedback tuning

A.1 Introduction

Feedback tuning can be used when high back-pressures are expected, for example, when packing a column using the system pump, or when applying samples with high viscosity. Feedback tuning provides continuous control of the pressure and the flow rate during the procedure, which thereby can be left unattended.

The purpose of feedback tuning is to eliminate discrepancies between the actual value and the requested value. The method instructions for controlling feedback tuning are found in UNICORN.

In ÄKTApilot, feedback tuning is used for:

- Maintaining the requested pump flow rate.
- Making sure that the maximum pressure limit is not exceeded.

Feedback tuning can be applied to the system pump as well as to the sample pump.

A.2 Tuning description

As long as the pressure is below the set point, the actual pump flow rate will be tuned to the set flow rate. If the pressure exceeds the set point, the system decreases the flow rate in order to reduce the pressure. When the pressure falls below the set point, the actual flow rate will again be tuned to the set flow rate, and so on.

Consequently, there are two regulators involved in tuning each pump:

- FlowTune, which is active when the pressure is below the set point.
- PressTune, which is active when the pressure is above the set point.

Note: *If the flow rate falls below the minimum flow rate limit, an Alarm is triggered and the system set to Pause. Therefore, we recommend using a Watch instruction (Watch_Flow_ and/or Watch_Sample_Flw) for the flow that is activated above this limit. A suitable action is to continue to the next block. To prevent pressure peaks when continuing, use a lower flow rate in the block after the Watch instruction than used when the Watch instruction was activated.*

A.3 Setting up feedback tuning

UNICORN uses so-called PID feedback tuning, where P, I and D are parameters that determine the tuning characteristics.

There are two ways to apply the feedback tuning instructions:

- In the Method Wizard or in the **Method Editor** module.
- In the **System Control** module by selecting **Manual:Pump**.

The feedback tuning instructions for the sample pump are listed in Table 4-1. The system pump has corresponding instructions.

<i>Instruction</i>	<i>Parameter description</i>
SamplePumpControlMode	To activate feedback tuning, select PressFlowControl . To deactivate feedback tuning, select Normal (default).
SamplePressureControl	Pressure set point for pressure sensor 3 (pressure sensor 1 when tuning the system pump)
SampleMinFlow	Sets the minimum flow rate limit of the sample pump. When the flow rate falls below this limit, an Alarm is triggered and the system set to Pause. When continuing from Pause to Run, the Alarm will be disabled for 60 s.
SampleFlowTune	Sets the values of the P, I and D parameters for tuning the actual sample pump flow rate to the set flow rate. SampleFlowTune is active when the pressure is below the set point in SamplePressureControl.
SamplePressTune	Sets the values of the P, I and D parameters for reducing the sample pump flow rate and thereby decreasing the pressure to below the pressure set point. SamplePressTune is active when the pressure is above the set point in SamplePressureControl.

Table 4-1. Feedback tuning instructions

A.4 Optimizing the PID parameters

The two regulators for each pump have separate PID parameters. The default PID parameters in UNICORN provide a robust feedback tuning that is suitable for most purposes. However, the parameters can be further optimized to suit a specific application.

The table below describes the three PID parameters.

Parameter	Description
P	The P parameter reduces the effect of an error but does not completely eliminate it. A simple P-regulator results in a stable stationary error between actual and requested flow.
I	The I parameter eliminates the stationary error, but results in a slight instability leading to oscillations in the actual flow. The I parameter can have values between 0 and infinity. Smaller values have a greater effect and a value of infinity has no effect. <i>Note:</i> The value infinity is set as 9999 in UNICORN.
D	In certain cases, the D parameter can reduce the oscillations introduced by a PI-regulator. D can have values between 0 and infinity, where larger values have a greater effect and a value of 0 has no effect. <i>Note:</i> Most often, a simple PI-regulator is preferable for control of flow rate, and ÅKTApilot is therefore configured by default with the D parameter set to zero.

Table 4-2. PID parameters

Rules of thumb for optimizing the PID parameters:

- Use the default parameter values as a start.
To set the default values, select **System:Settings** in the **System Control** module. The parameters are found in **Specials**.
- Keep the D parameter set to zero, i.e. use a simple PI-regulator.
- Start the pump before activating the regulator.
- Increasing P makes the regulator faster.
- Increasing I reduces oscillations.

See also the *UNICORN Administration and Technical Manual* for more information about feedback tuning.



Appendix B Gradient performance

The *specified* gradient performance in ÄKTApilot is limited due to the specified flow rate range of each pump module; 4–400 ml/min.

Note: Gradients outside the specified range can be made at flow rates below 400 ml/min. The specified performance is then invalid.

Gradient performance at 8–400 ml/min

Use the following formula to calculate the minimum %B (GradMin) and maximum %B (GradMax) at a given flow rate:

$$\text{GradMin} = 100 \cdot \frac{8}{\text{FlowSetp}}$$

$$\text{GradMax} = 100 - \text{GradMin}$$

where FlowSetp is the flow rate setting.

Gradient performance at 400–800 ml/min

Use the following formula to calculate the minimum %B (GradMin) and maximum %B (GradMax) at a given flow rate:

$$\text{GradMin} = 100 \cdot \frac{\text{FlowSetp} - 400}{\text{FlowSetp}}$$

$$\text{GradMax} = 100 - \text{GradMin}$$

where FlowSetp is the flow rate setting.

The table and diagram below illustrate the formulas. For gradient specification, see *ÄKTApilot Instrument Handbook*.

Flow rate [ml/min]	GradMin [%B]	GradMax [%B]
20	20	80
40	10	90
80	5	95
200	2	98
400	2	98
500	20	80
600	33	67
700	43	57
800	50	50

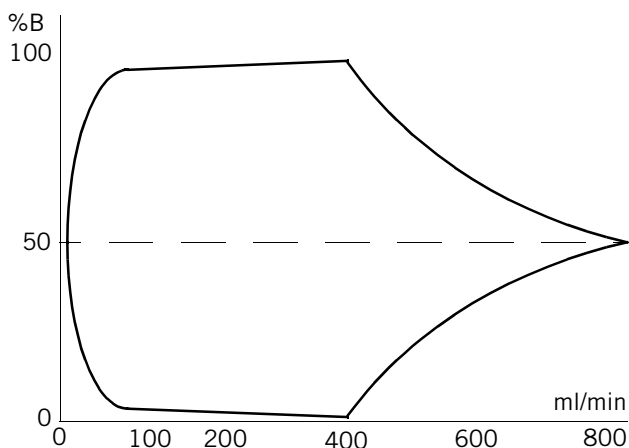


Table 4-3. Gradient performance in ÄKTApilot



