Switchos™ II

Advanced Microcolumn Switching Unit

User's Manual

P/N 162013

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D850R3

Notice: The Switchos™ II Advanced Microcolumn Switching Unit is covered by a limited warranty. A copy of this warranty is included with this manual. The customer is required to perform routine maintenance as described in the User's Manual on a periodic basis to keep the warranty in effect.

All information in this manual is subject to change without notice and does not represent a commitment on the part of LC Packings, BV.

The material included in this manual is provided to assist users in the operation, maintenance and repair of the Switchos II Advanced Microcolumn Switching Unit. It is assumed that the individual using this manual has sufficient training in the use of analytical instrumentation and is aware of the potential hazards including (but not limited to) electrical hazards, chemical solvent hazards and the exposure to pressurized solvents.

Printed in the Netherlands.

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Warranty

LC Packings (Netherlands) BV, warrants that the products manufactured and sold by it to be free from defects in material and workmanship for normal use and service from the date of delivery to original purchaser for a period of one (1) year from the date of shipment. This limited warranty does not cover, and no warranty is provided, for parts that by their nature are required to be replaced periodically as a function of use of the normal operation of the system. These items include, without limitation: HPLC columns, fuses, tubing, detector sources, pump piston seals, injector rotors, check valves, filters, any software, etc. In addition, damage due to corrosion, misuse, negligence, accident, alteration of the system or repair by an unauthorized individual is not covered by the warranty. It is understood that the performance characteristics of the instrument require that the mobile phase be degassed with He as described in the User's Manual.

This warranty covers products sold under the LC Products trademark. If a different warranty than the above is indicated in the sales literature, the warranty indicated in the sales literature will prevail. If the system includes equipment supplied by LC Packings but manufactured by a third party, LC Packings makes no warranty of any kind, express or implied, including, without limitation, any warranty of merchantability or fitness for a particular purpose. LC Packings will make available to you, to the extent permitted, the warranties of the manufacturer of the relevant equipment following your timely written request.

If any product covered by this warranty becomes defective during the warranty period, it will be repaired or replaced by LC Packings at no charge to the customer (the repair/replace decision is solely at the option of LC Packings). All warranty requests must be received by LC Packings during the warranty period.

LC Packings will pay for surface transportation to the applicable LC Packings Office (North America – Sunnyvale CA, Europe and Asia - Amsterdam, the Netherlands), if the instrument proves defective within thirty (30) days from the date of shipment (this does not include air freight, drayage, labor, crating charges, customs clearance charges, etc.). The user should carefully follow the directions indicated on the Return Goods Instruction Sheet in the User's Manual. After thirty days, all transportation costs will be at the expense of the customer.

Software Warranty

If, at any time during the period ending ninety (90) days after delivery of any product to you, you report and document any error in any software provided with such product and developed by LC Packings or any failure of any such software substantially to conform to LC Packings software description that limits or prevents use of the software by you, we will use reasonable efforts to correct any such error or failure, will replace such software or will terminate your license to use the software and refund the price of the related product. In connection with any such termination and refund, you will return the related product to LC Packings upon request.

The warranty will apply only to those portions of the software that were developed by LC Packings and that incorporated all program corrections and modifications, if any, delivered to you. It will not apply to any error or failure due to machine error or to the misuse by or negligence of any person or entity other than LC Packings or to any software, which is modified by any person, or entity other than LC Packings.

Liability

Under no circumstances shall LC Packings be liable for damage to persons or property. This warranty is the only warranty given by LC Packings with respect to products and software provided with the products and is given in lieu of all other warranties, express or implied, including, without limitation, any warranty of merchantability or fitness for a particular purpose.

Your exclusive remedies and LC Packings's sole liability for any non-conformity or defect in the products and such software will be those expressed herein. Under no circumstances will LC Packings's liability arising from the performance or failure to perform of any product or software, in contract, in tort (including negligence), or otherwise, exceed the purchase price of the product and software. In no event will LC Packings be liable, in contract, in tort (including negligence), or otherwise for special, incidental, consequential or analogous damages, including, without limitation, damages resulting from loss of use, loss of profits, loss of business or loss of goodwill, even if LC Packings has been advised of the possibility of such damages.

This warranty comprises the entire warranty between LC Packings and the customer. It overrides any warranty related language that may appear in the customer purchase order or other documentation provided by the customer.

This warranty shall be governed by, and construed and enforced in accordance with, the laws of the Netherlands. It is non-transferable and shall run to the benefit of the original purchaser only. Any change, alteration or amendment to this warranty is not valid unless it has been approved in writing by an officer of LC Packings.

North America
LC Packings / Dionex

500 Mercury Drive
Sunnyvale, CA 94088-3603
USA

Technical Call Center
USA/CA: (800) 346-6390

Europe and Asia
LC Packings (Netherlands) BV
A Dionex Company
Abberdaan 114
1046 AA Amsterdam
The Netherlands

Phone: + 31 20 683 9768
Fax: + 31 20 685 3452

Instructions for Returning Instruments

Before you return any item for repair, please contact the nearest LC Packings office or its local distributor for instructions and obtain a return authorization number and the 'Health and Safety Form' (if applicable).

Pack the equipment carefully, preferably in its original shipping container and ship it to the LC Packings Service Department, using the appropriate address.

North America
LC Packings / Dionex

500 Mercury Drive
Sunnyvale, CA 94088-3603
USA

Technical Call Center
USA/CA: (800) 346-6390

Europe and Asia
LC Packings (Netherlands) BV
A Dionex Company
Abberdaan 114
1046 AA Amsterdam
The Netherlands

Phone: + 31 20 683 9768
Fax: + 31 20 685 3452

IMPORTANT:

1) Make certain that the return authorization number together with the HEALTH AND SAFETY form (if applicable) is attached outside of the package so that we can properly track and account for your system.

2) Please include the following

   a) Company letterhead with the following information.

      1. Your Name
      2. Complete Mailing Address
      3. Telephone Number, fax number and e-mail address
      4. Return Material Authorization (RMA) Number
      5. A detailed description of the problem.
      6. The name of the LC Packings personnel to whom you have spoken to regarding the problem
      7. Return Shipping Information (if appropriate)

   b) Relevant chromatograms

   c) A purchase order (if the system is not in warranty)

Note: The completed and signed HEALTH AND SAFETY form must be returned to LC Packings service department (fax or mail) prior to the return of any component, or attached outside the shipping package. In addition, the provided RMA number must be clearly marked on the outside of the shipping package. Failure to complete and return this form will result in the package returned without the parts being inspected or credit issued.

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Warnings

The Danger sign, Warning sign and the Caution sign shown below are included in various locations in this manual. These signs provide the following information:

Danger: The information in a danger statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to personal injury or loss of life.

Warning: The information in a warning statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to severe injury and/or damage or destruction to parts or all of the equipment.

Caution: The information in a caution statement relates to a condition that could lead to damage to equipment and/or lead to invalid analytical results.

Note: The information in a note statement relates to important information that should be read and understood before continuing.

Safety Precautions

Note: The following precautions should be followed to minimize the possibility of personal injury and/or damage to property.

Note: Make certain that you are familiar with the contents of this manual before working on the system.

1) The system should be installed in a well-ventilated laboratory. If the mobile phase includes volatile or flammable solvents, make certain that they are not allowed to enter the workspace.

2) If the mobile phase includes volatile or flammable solvents, avoid open flames and sparks.

3) If a leak occurs, turn off power to the instrument and remedy the situation immediately.

4) All components of the system should be plugged into a common power line that is directly connected to a true ground.

5) When the panels are removed, dangerous electrical connections will be exposed. Disconnect the instrument from all power sources before removing the panels.

6) Always replace blown fuses with fuses of the same size and rating indicated on the fuse holder and panel. Refer to Section 6.3.6 of this manual for more information on Fuses.

7) Repair or replace faulty power cords and all communication cables.

8) Many organic solvents and buffers are toxic. Make certain that you know the toxicological properties of all mobile phases that you are using.

9) The toxicological properties of many samples may not be well known. If you have any doubt about a sample, treat it as if it contained a potentially harmful substance.

10) Wear protective eye goggles when handling mobile phases or operating the instrument. An eye wash facility and a sink should be close to the unit. If any mobile phase splash on the eyes or skin, wash the affected area and seek medical attention.

11) Dispose of all waste mobile phase in an environmentally safe manner that is consistent with all local regulations. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose flammable and/or toxic solvents through the municipal sewage system

12) PEEK tubing is used in a variety of locations. While this polymer has superb chemical resistance to most organic solvents, it tends to swell when it is contact with $CHCl_3$, DMSO and THF. In addition, it is attacked by concentrated acids such as Sulfuric Acid and Nitric Acid (swelling or attack by acid is not a problem with short flushing procedures).

    Do not use PEEK tubing that is stressed, bent or has a kink.

13) Wear protective eye goggles when handling fused silica tubing (i.e. installation, cutting etc.)

14) If a buffer is used as a part of the mobile phase, flush the system with several volumes of a methanol/water (50/50) before it is shut down. This will prevent salt buildup inside the unit.

15) Do not use the instrument in ways other than those indicated in the instructions given in this manual.

CE

DECLARATION OF CONFORMITY

We

LC Packings Nederland BV
A Dionex Company
Abberdaan 114
1046 AA Amsterdam
The Netherlands

declare that our product

Switchos™ II Advanced Microcolumn Switching Unit

is in confirmation with the following documents:

EEC directives 89/392, incl. 91/368 and 93/44 (machine safety) and EEC directives 73/23 and 93/68 (low voltage safety), applied with the following standard:

EN61010-1	Safety requirements for laboratory equipment (Class I, Installation cat. II, Pollution degree II)

WARNING

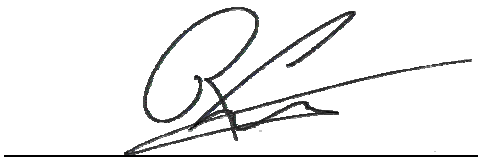
LC Packings will not accept any liability for damages direct or indirect caused by connecting this instrument to devices which do not meet relevant safety standards.

EEC directives 89/336 and 92/31 (EMC requirements), applied with the following standards:

EN 50081-1	Generic emission standard
EN 50082-1	Generic immunity standard
EN 61000-3-2	Harmonic current emissions

Use shielded cables and connectors for all remote connections.

Amsterdam, January 11, 2001



Robert van Ling, QA manager

D934R1

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CHAPTER 1

Introduction

1.1 Features of the Switchos™ II Advanced Microcolumn Switching Unit

The Switchos™ II Advanced Microcolumn Switching Unit is an advanced switching system for use with micro-HPLC systems. The system incorporates the following features:

- Two Valco 10-port low-dispersion switching valves which allow for the connection of capillary, micro and nano HPLC columns without any dead volume.
- A high precision loading pump, fully controllable by the CHROMELEON® software.
- Four channel solvent selection valve.
- Automatic control of all valves by the CHROMELEON software and manual control via push buttons.
- Manual valve switching is possible at any time, even when a program is running.
- LED's which indicate the present status of the switching valves (microfluidic pathways), the position of the solvent selection valve and the control mode.

A broad range of applications can be performed using micro-column switching with the Switchos II including sample preconcentration, sample cleanup, multi-dimensional separations (2-D separations), desalting and selective extraction Typical examples of the use of the unit (which are described in Section 2.6.4 and Appendix C) include immuno-affinity extractions, isolation of phosphorylated peptides from complex protein digests, automated removal of detergents, extraction of drugs from biological fluids, high throughput analysis, etc.

Switchos II is configured with an LC Packings UltiMate™ Micropump and is normally used with the LC Packings UltiMate Capillary HPLC System. The system is fully compatible with other instrumentation (a minimum of three contact closures, TTL or Open Collector terminals are required).

1.2 General Design of the Switchos II Advanced Microcolumn Switching Unit

A schematic diagram of general design of the Switchos II Advanced Microcolumn Switching Unit is presented in FIGURE 1-1.

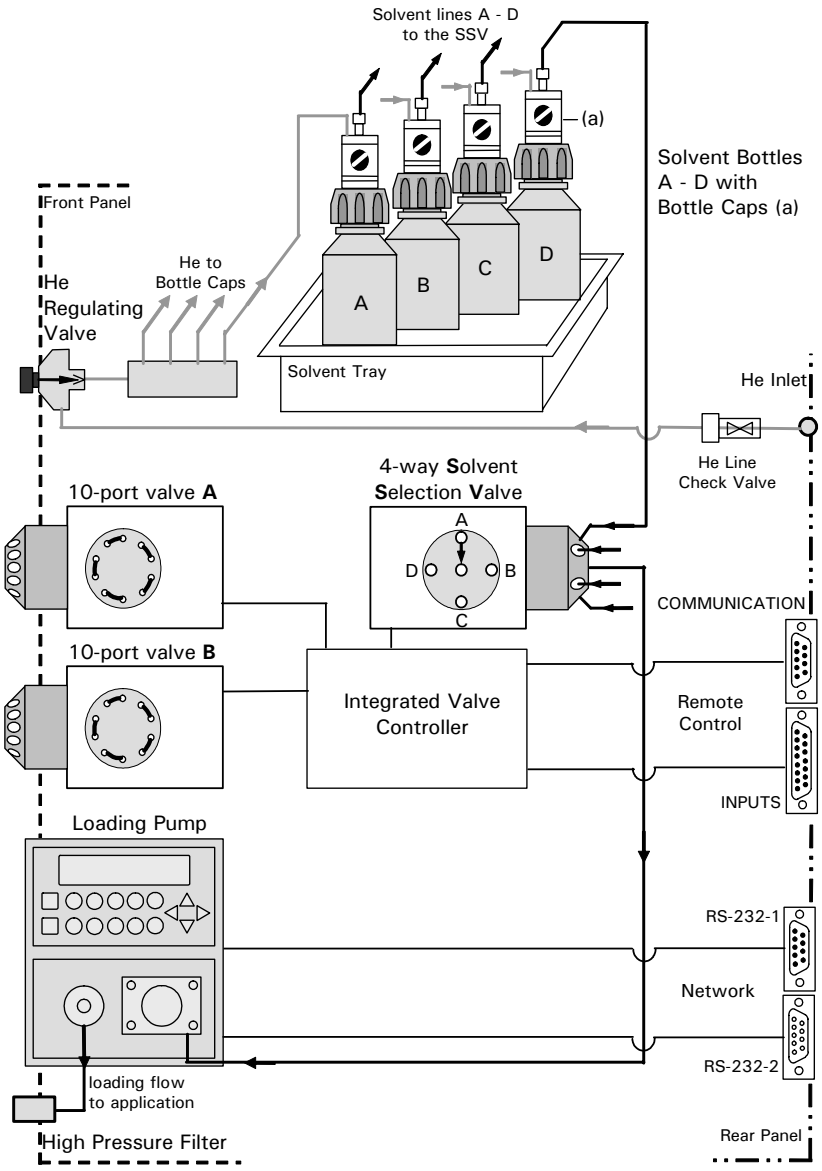
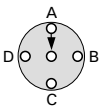


FIGURE 1-1. Schematic Diagram of the Switchos II Advanced Microcolumn Switching Unit

The Switchos II Advanced Microcolumn Switching Unit includes the following components:

- **10-Port Switching Valves** - low dispersion valves assure dead volume free connection of any Micro- or Nano LC column (e.g., Fusica, NanoSeries, Micro- and Nano-Precolumns, etc.). Two fast motor-driven actuators control the valve positions (6 port valves are incorporated into some systems).



- **4-Way Solvent Selection Valve** - allows for the automatic selection of up to 4 different solvents without the need of pump shut down or tedious purging. One fast motor-driven actuator switches the valve into the selected position.

- **Loading Pump** - designed for the use in the LC Packings UltiMate Capillary HPLC system, the micropump guarantees highest performance and perfect compatibility. It is fully controllable via the CHROMELEON software.

- **Solvent Bottles and He Degassing System** - provides mobile phase to the system. The Helium degassing system is provided to improve check valve reliability and diminish baseline noise.

- **Integrated Valve Controller** - micro-controller based electronics controls the valve actuators and monitors the position of the three valves (valve A, valve B, solvent selection valve). Additionally, it reads the COMMUNICATION port and reads out the status of the push buttons, switches and the remote control input connector.

- **High Pressure Filter** - serves to remove particulate matter from the mobile phase.

Note: For highest performance, complete automation and ease of operation, the Switchos II Advanced Microcolumn Switching Unit should be used in combination with the LC Packings UltiMate™ Capillary HPLC system and the FAMOS Microautosampler. These units can be readily connected via a local area network (Chapter 2.3) for secure communication between the various modules.

1.3 Front View of Switchos II

The front view of the Switchos II Advanced Microcolumn Switching Unit is presented in FIGURE 1-2.

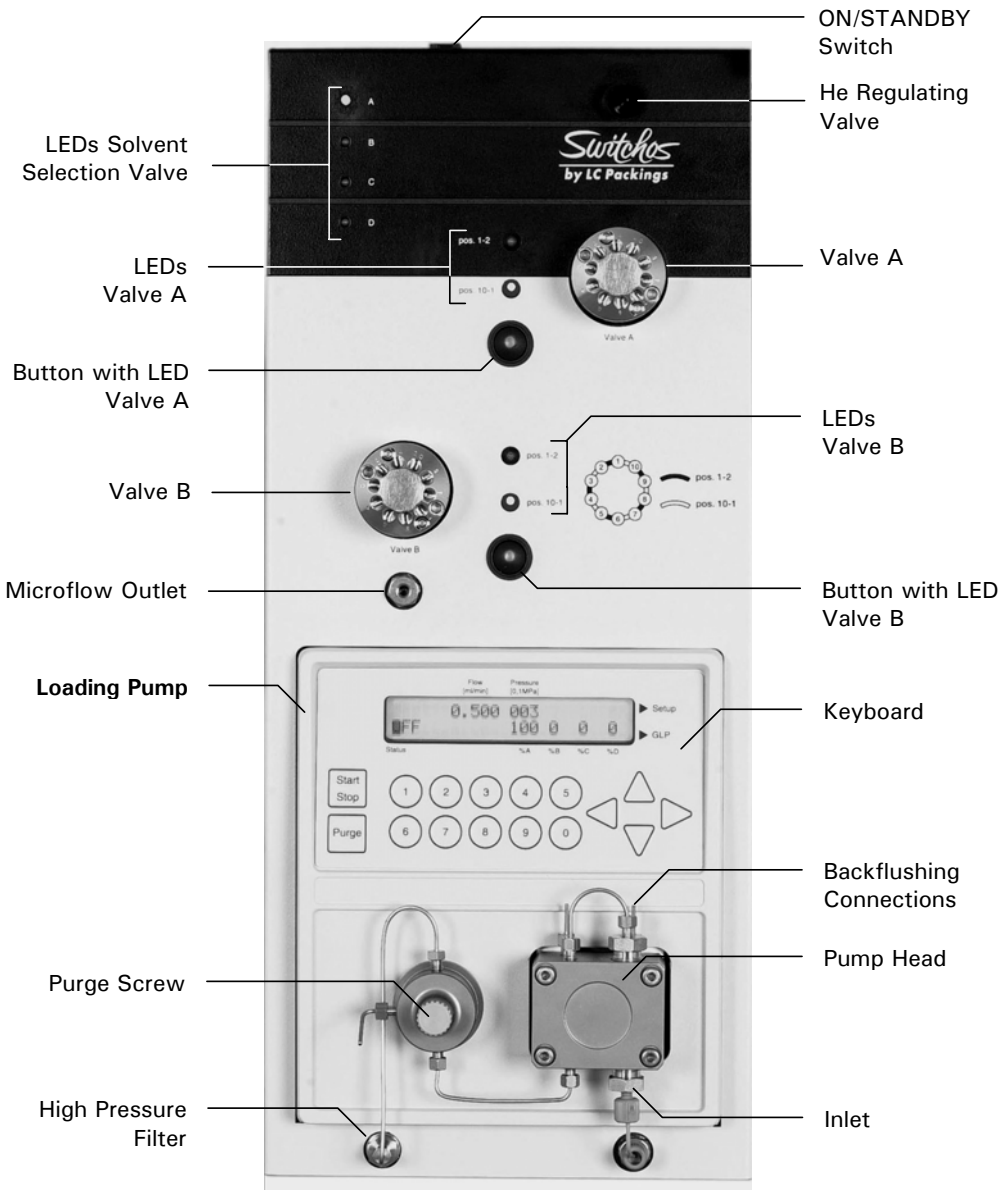


FIGURE 1-2 Front View of the Switchos II Advanced Microcolumn Switching Unit

1.4 Rear View of Switchos II

The rear view of the Switchos II Advanced Microcolumn Switching Unit is presented in FIGURE 1-3.

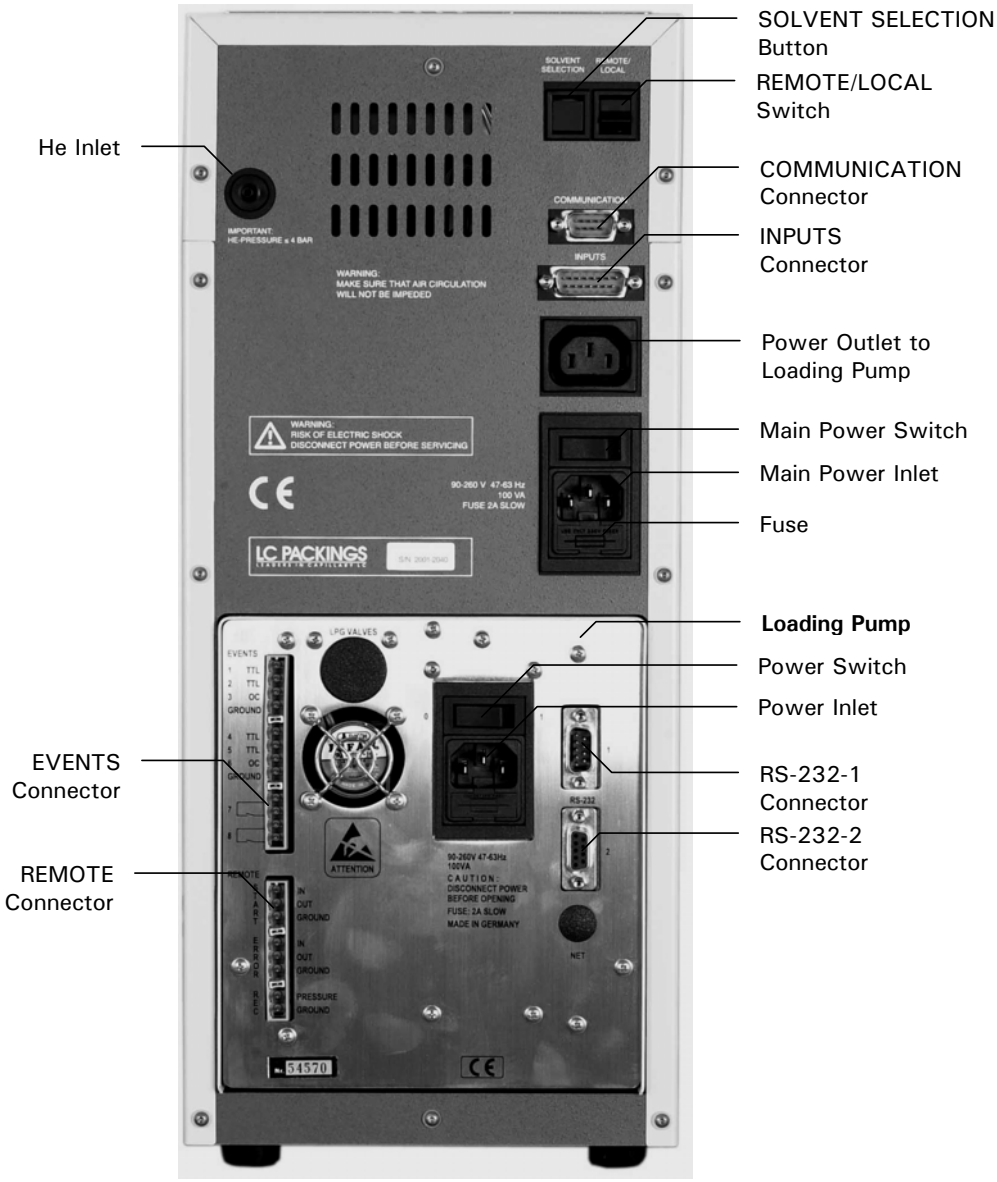


FIGURE 1-3 Rear View of the Switchos II Advanced Microcolumn Switching Unit

1.5 Contents of this Manual

Note: This manual covers the standard version of the Switchos II Advanced Microcolumn Switching Unit as well as the inert version. If you are using an inert version, please refer to Appendix D, which includes important information about how to connect fused silica tubing to the inert switching valves.

This manual includes the following information:

Chapter 2: *Installation and Getting Started* describes how to install the Switchos II Advanced Microcolumn Switching Unit.

Chapter 3: *The User Interface* describes the system controls, explains how the unit is used in manual mode and provides a short overview of the micropump controls. A detailed discussion of the micropump is presented in the UltiMate Micropump User's Manual, which is supplied with the system.

Chapter 4: *Software Control* discusses how to control the Switchos II Advanced Microcolumn Switching Unit by the CHROMELEON software and how to setup the software modules.

Chapter 5: *Testing the Switchos II* describes a series of operations to determine that the unit is functioning in an acceptable manner.

Chapter 6: *Maintenance and Troubleshooting* describes a variety of maintenance procedures to optimize the performance of the microcolumn switching unit. In addition, it discusses how the operator can determine the cause of a difficulty in the operation of the instrument and includes a list of spare/replacement parts.

Chapter 7: *Specifications* presents the specifications of the Switchos II Advanced Microcolumn Switching Unit.

In addition, a series of appendices that provide information about the valves that are incorporated in the unit, interfacing the unit to the local area network, and typical applications:

Appendix A: *Switching Valves* describes the valves that are included in the Switchos II Advanced Microcolumn Switching Unit. In addition, it presents information about the disassembly and reassembly of the valve.

Appendix B: *Network Identification* provides explains how the Switchos II is identified on the system network so that it can communicate with other components.

Appendix C: *Cleanup of Proteins* presents an explanation of how the Switchos II is used for sample clean-up, protein digestion and peptide separation.

Appendix D: *Connecting Fused Silica Capillary* explains how to connect a NANO column or other fused silica capillary to the inert PAEK switching valves of the Switchos II.

1.6 For Additional Information

The loading pump that is incorporated in the Switchos II Advanced Microcolumn Switching Unit is identical to the micropump supplied with the LC Packings UltiMate™ system. If you are using the Switchos II Advanced Microcolumn Switching Unit with the LC Packings UltiMate™ and/or CHROMELEON software, please refer to the documentation provided with these products for supplemental information and to the online help of CHROMELEON (F1 key).

In addition to the 'Switchos II Advanced Microcolumn Switching Unit - User's Manual' this binder contains a copy of the User's Manual for the LC Packings UltiMate™ Micropump. The 'UltiMate Micropump User's Manual' contains a detailed discussion about the operation and maintenance of the Micropump.

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CHAPTER 2

Installation and Getting Started

2.1 Installation

The instructions provided below are provided for installation of the Switchos™ II Advanced Microcolumn Switching Unit as part of the LC Packings UltiMate™ Capillary HPLC System as well as for installation of the instrument as a stand-alone component in an HPLC system.

When the Switchos Advanced Microcolumn Switching Unit is used in conjunction with the UltiMate system and the FAMOS™ Microautosampler, all instruments are controlled by the CHROMELEON® software. The loading pump will be included in the instrument network and the switching valves will be controlled by a COM port of the PC.

If no additional COM port is available or if the Switchos is to be installed with different or third-vendor software, please refer to Sections 2.4 and 2.5 for information about how to control the switching valves by relay outputs. (e.g. the Event outputs of the Switchos loading pump or the Auxiliaries of the FAMOS Microautosampler, if present).

Once you have set up the Switchos, refer to Section 2.7 for information about routine operation of the system.

Please refer to the user's manuals of the UltiMate system and the FAMOS Microautosampler and the online help of CHROMELEON for additional information.

2.1.1 Location of Switchos in the Laboratory

The Switchos Advanced Microcolumn Switching Unit should be installed in a facility with the following environmental conditions:

- The temperature range should be maintained between 10 and 40°C. The system should be installed in an area in which the temperature is fairly constant (do not place the system near a window, an air conditioning duct or a heating duct). The humidity should be maintained between 20 and 80 % relative humidity.

- If flammable or toxic solvents are to be used, a suitable ventilation system should be provided.
- The use of open flames in the laboratory should be prohibited.
- Corrosive vapors or dust should not be present as these materials can adversely affect the long-term performance of the system.

The Switchos Advanced Microcolumn Switching Unit requires approximately 190 mm (7.5") of linear bench space. The lab bench should be capable of supporting the entire system (for the LC Packings UltiMate, FAMOS and Switchos we recommend that the lab bench be capable of supporting at least 100kg (225 lb.). The power consumption of the Switchos is 100 VA (the power consumption of the UltiMate Capillary HPLC System is 250 VA and of the FAMOS Microautosampler is 250 VA).

Danger: The Switchos Advanced Microcolumn Switching Unit must be connected to a power source that is connected to a true ground. In addition, all other components of the system (e.g. the HPLC pump, the detector) should be connected to the same ground.

Caution: Do not install the Switchos Advanced Microcolumn Switching Unit in areas subject to shock, dust, or in direct sunlight.

2.2 Unpacking

When the Switchos Advanced Microcolumn Switching Unit is received, carefully unpack the unit and verify receipt of all components according to the packing list (some components include sub-packing lists). It is recommended that all packing materials be saved in the event that it is necessary to return any item to the factory.

Note: When lifting the instrument from the shipping container, make sure that the unit is kept upright. Lift the unit by placing your hands under the instrument.

If there is external damage to the shipping box, the damage should be reported to the shipping agent and LC Packings upon receipt of the goods. If internal damage is observed or if any items are missing, this should be reported to the shipping agent and to LC Packings as soon as it is observed.

Note: If there is any apparent damage to the instrument, the user should investigate the nature of the damage before plugging the unit into the mains to ensure that powering up of the instrument will not create a hazardous condition or damage internal components. If the damage appears significant, call LC Packings or its local representative before connecting the unit to the mains.

2.3 Installing the Switchos with the UltiMate Capillary HPLC System and the FAMOS Microautosampler

When the Switchos is used as a component in the LC Packings UltiMate Capillary HPLC system, all instruments are controlled by the CHROMELEON software. The loading pump will be included in the instrument network and the switching valves are controlled by a COM port of the PC.

2.3.1 Electrical Connections

All electrical connections are made on the rear panel of the instrument (FIGURE 2-1).

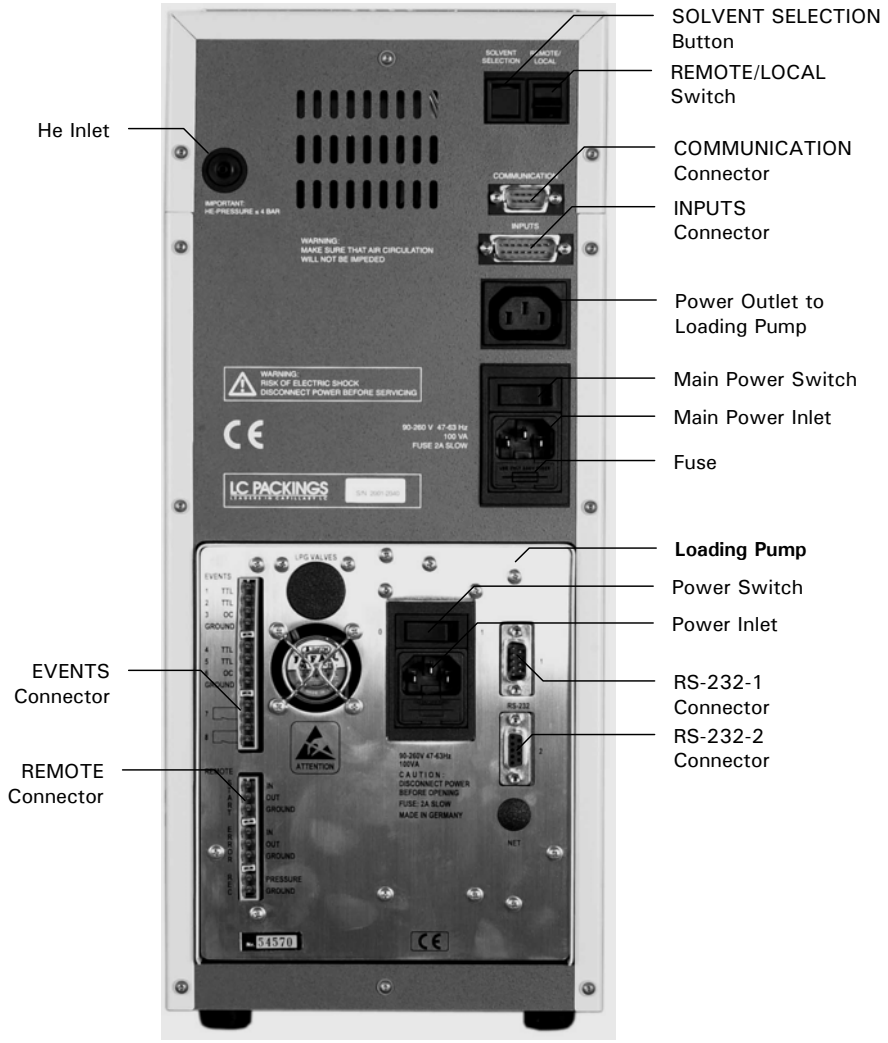


FIGURE 2-1 Rear Panel of the Switchos Advanced Microcolumn Switching Unit

CAUTION

Caution: Avoid touching the electrical contacts on the terminal strips. Electrostatic discharges could damage internal components.
The manufacturer will not accept any liability for damages directly or indirectly caused by connecting the Switchos Advanced Microcolumn Switching Unit to instruments which do not meet relevant safety standards.

2.3.2 RS-232 Connectors

The two RS-232 serial interfaces enable digital data transfer between the loading pump, the UltiMate Micropump, the UltiMate UV Detector (if present) and the PC. These devices communicate with each other to form an integrated network.

To set up the RS-232 network connections:

- Connect the RS-232 **1** connector of the Switchos loading pump, the RS-232 **2** connector of the UltiMate UV Detector and the COM port of the PC using the Y-cable (item a, FIGURE 2-2).
- Connect the RS-232 **2** connector of the Switchos loading pump and the RS-232 **1** connector of the UltiMate Micropump using the Serial Communication Cable (item b, FIGURE 2-2).
- Connect the RS-232 **2** connector of the Micropump and the RS-232 **1** connector of the UV Detector with the same type of cable.

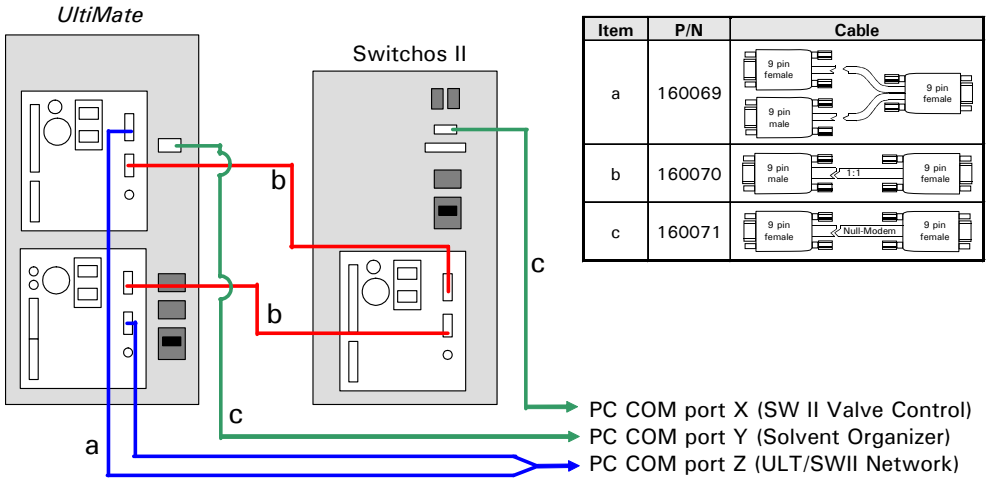


FIGURE 2-2 Setting up the RS-232 Network Connections

If your configuration does not include an UltiMate UV Detector, connect the y-cable to the RS-232 **1** connector of the UltiMate Micropump, the RS-232 **2** connector of the Switchos loading pump and the COM port of the PC. Use a Serial Communication Cable to connect the other RS-232 connectors of both pumps.

CAUTION

Caution: The RS-232 sockets are to be used only with LC Packings software products (e.g. CHROMELEON) or third vendor software that support the LC Packings UltiMate System.

2.3.3 COMMUNICATION Connector

Connect the COMMUNICATION port to a free COM port on the computer using the Solvent Organizer Com cable (item c, FIGURE 2-2).

2.3.4 INPUTS Connector

CHROMELEON controls the valve positions via the COMMUNICATION port (Section 2.3.3). Refer to Sections 2.4 and 2.5 if the valves need to be controlled by digital input signals (e.g. the instrument is to be installed in conjunction with a software package which does not support the serial communication).

2.3.5 Triggering a Mass Spectrometer (or an Other Device)

If you want to provide a mass spectrometer (or other external device) with a trigger signal from the CHROMELEON software at some event (e.g. on the beginning of the gradient program), connect the appropriate input of the mass spectrometer (or other device) to the EVENT 8 output (relay contact) of the UltiMate Micropump as shown in FIGURE 2-3.

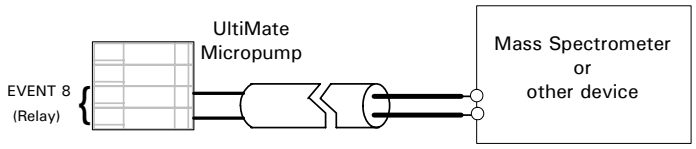


FIGURE 2-3 Cable to trigger the MS (or another Device) by EVENT 8

2.3.6 Power Connector

Since the Switchos is fitted with a universal power supply for input voltages from 90 to 260 V, manual setting of the supply voltage is not required. The power cord should be inserted in the socket directly below the Main Power switch on the right side of the rear panel (FIGURE 2-1). In addition, the loading pump should be plugged into the socket above the Main Power switch.

Danger: The Switchos must be connected to a power source that is connected to a true ground.

2.4 Using the Event Outputs or the FAMOS Auxiliaries

If no additional COM port is available or if the Switchos Advanced Microcolumn Switching Unit is to be installed with a different or third vendor software package, the switching valves on the Switchos can be controlled by:

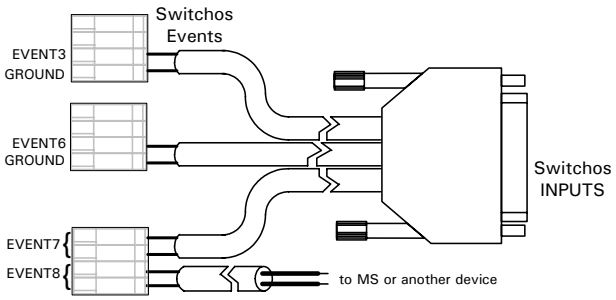
a) the event outputs of the Switchos Loading Pump.

b) the Auxiliaries (P5) outputs of the FAMOS Microautosampler.

To connect the INPUTS connector:

a) Valves are controlled by the Loading Pump

Connect the INPUTS connector to the EVENTS outputs of the loading pump using the connection cable P/N 160172 presented in FIGURE 2-4.

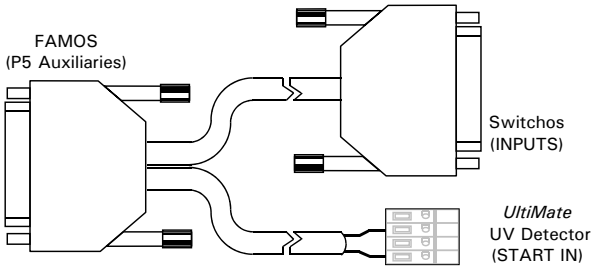


Micropump	Switchos II
EVENT3	Valve A
EVENT6	Valve B
EVENT7	SSV
EVENT8	Trigger

FIGURE 2-4 Cable to control Switchos by the UltiMate System

b) Valves are controlled by the FAMOS Microautosampler

Connect the INPUTS connector of the Switchos, the P5 (AUXILIARIES) connector of the FAMOS Microautosampler and the START IN input of the UltiMate UV Detector using the connection cable P/N 160171 presented in FIGURE 2-5.



FAMOS	Switchos II
AUX1	Valve A
AUX2	Valve B
AUX3	SSV
AUX4	START IN

FIGURE 2-5 Cable to control Switchos by the FAMOS P5 Connector

2.5 Installing the Switchos with Other HPLC Systems

The electrical connections that are required depend on the nature of the instrumentation and the desired application. In this section, we provide general information about how the instrument can be interfaced to equipment from other manufacturers. The user should also refer to the documentation provided with this equipment. The Switchos is controlled via the INPUTS connector on the rear panel and the keypad of the loading pump.

CAUTION

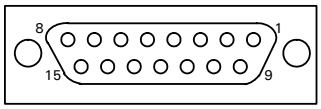
Caution: If you are interfacing the Switchos to instrumentation from other manufacturers, ensure that input voltages and output voltages are within the ranges indicated in the specifications (Chapter 7). If you have any questions, please call LC Packings. The LC Packings warranty will not be valid if the Switchos is damaged due to interfacing of the system to instrumentation from third parties.

2.5.1 INPUTS Connector

The positions of the switching valves A and B and the Solvent Selection Valve (SSV) are controlled by the INPUTS connector (FIGURE 2-6). Contact closure (relay), TTL and open collector outputs can drive the Switchos inputs.

CAUTION

Caution: Check to make certain that the maximum ratings of the outputs matches with the requirements of the Switchos Advanced Microcolumn Switching Unit (Chapter 7).

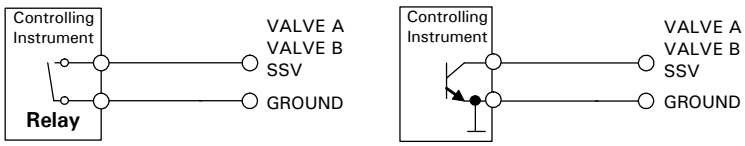


PIN #	SIGNAL
1	VALVE A
2	GROUND
4	VALVE B
5	GROUND
7	SSV
8	GROUND

FIGURE 2-6 Pin-Out INPUTS Connector

To control the switching valves A and B and the SSV:

- Connect two outputs of the controlling instrument to the Switchos input pins VALVE A and VALVE B of the INPUTS connector (FIGURE 2-7).
- Connect the common terminals or the ground terminals of the controlling instrument to the corresponding GROUND pins of the INPUTS connector (FIGURE 2-7).
- Connect the SSV pin and the corresponding GROUND in the same way to another output of the controlling instrument (FIGURE 2-7).



A) Contact Closure (Relay) Output

B) TTL / Open Collector Output

FIGURE 2-7 Different Output Configurations of the Controlling Instrument

VALVE A(B) - these two pins control the two switching valves. An open contact closure or an inactive TTL (or open collector) output will drive the corresponding valve into position "1-2", while an active input will drive the corresponding valve into position "10-1". The current positions are indicated by the LEDs on the front panel (FIGURE 1-2).

SSV – this pin is used to select the position of the SSV. An active input signal shorter than 1.5 seconds will switch the valve to the next solvent channel (from A to B, B to C, ... and finally from D back to A). Additionally, the SSV can be reset to solvent channel A by applying an active signal for longer than 1.5 seconds. FIGURE 2-8 shows the timing diagram. The selected solvent channel is indicated by one of the four LEDs on the upper front panel (FIGURE 1-2).

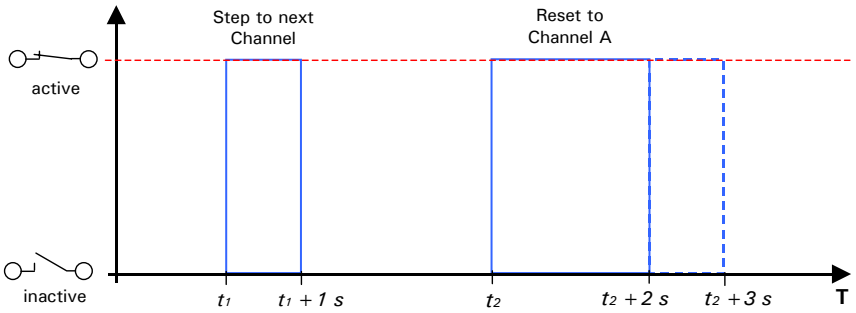


FIGURE 2-8 Timing Diagram of the SSV Input

2.5.2 Power Connector

Connect the Switchos to the power line as described in Section 2.3.6.

DANGER

Danger: The Switchos must be connected to a power source that is connected to a true ground.

2.6 Fluidic Connections - Switchos to the HPLC System

2.6.1 Preliminary Operations

To connect the Switchos Advanced Microcolumn Switching Unit to an HPLC system and to prepare the instrument for operation:

- Place the Switchos Advanced Microcolumn Switching Unit in its operating location, preferably on the left side of the HPLC system. Make sure the ventilation holes are not obstructed. Allow the instrument to acclimatize for 1 hour.
- Connect the Helium line (Section 2.6.2).
- Power-up the system. The display of the loading pump and the various LED's will indicate that the self-test and initialization have been executed. A detailed discussion about the control and the various display messages of the loading pump is presented in the 'UltiMate Micropump User's Manual'.
- Sparge the mobile phase and flush the solvent lines (Section 2.6.3).
- Prepare the fluidics connection according to your needs. A typical example is shown in Section 2.6.4 (additional examples are presented in Appendix C).

Caution: Do not use a stainless steel nut and/or ferrule with the inert (PAEK) injection/switching valves. The use of stainless steel nuts or ferrules may damage the valve. Use only the supplied fittings (PEEK) and follow the instructions below. If you are using an inert version, please refer to Appendix D, for important information about how to connect fused silica tubing to the inert switching valves.

Caution: Use the fittings supplied with the instrument (Valco) or equivalent fittings to ensure that the dead volume of the system is minimized. Use of Rheodyne fittings should be avoided. Rheodyne fittings are designed differently; they will lead to unswept volume and possible system damage.

2.6.2 He Connection

Connect the Helium line (1/4 " O.D.) that is supplied to the Helium inlet on the rear panel of the Switchos (FIGURE 2-1). To connect the Helium line of the Switchos to the Helium line of the UltiMate Capillary HPLC System, use the T-piece (P/N 161470) that is supplied with the Switchos instrument. The Helium pressure should be set to approximately 1 bar.

Caution: Do not operate the He lines at a higher pressure than 4 bar (60 psi). Close the He-shut off valves when the system is in idle state or not used.

2.6.3 Sparging the Mobile Phase and Flushing the Solvent Lines

Although the Switchos loading pump is controlled via the CHROMELEON software, initial system preparation is done with the pump on an off-line basis (i.e. the pump is operated locally).

To prepare the system for a separation:

a) Power up the Switchos and make certain that power is provided to the pump.

b) Check that the pump head backflushing system is operating. A solution of isopropanol/water (1:1) is commonly used but other solvents can be used (if any of the buffer components are not soluble in this mixture, reduce the fraction of propanol). If desired you can reduce evaporation of the backflushing solution by connecting the two backflush ports. A 5 mL syringe is provided to backflush the head and fill the reservoir.

Note: The pump head of the pump should be backflushed with propanol/water (1:1). If crystalline materials are deposited in the pump head, irreversible damage to seals and or the piston may result; this will dramatically shorten the life of these components.

c) Inspect all fittings. If there is a salt deposit by a joint, it is probable that a leak has occurred and the fitting should be cleaned and tightened. When you tighten a fitting, do not overtighten. Check that the solvent filters are clean, if not they are not clean, they should replaced.

d) Fill the solvent reservoirs with the mobile phases to be used for the application.

CAUTION **Caution: Only use the shielded solvent reservoirs supplied with the Switchos.**

CAUTION **Caution: Do not operate the He lines a higher pressure than 4 bar (60 PSI). Close the He-shut off valves when the system is in idle state or not used.**

Note: All four solvent bottles must be filled and purged (even if the application requires less than four mobile phases) to assure proper function of the system. Fill solvent bottles that will not be used with methanol/water (1/1).

Note: The solvents must be degassed via the He degassing technique described below. If other techniques are used (e.g. vacuum degassing) the performance of the system will be seriously degraded and the performance specifications will not be obtained.

e) Open the He Shut-off valves by rotating the knob so that the line on the valve is vertical (FIGURE 2-9) and open the He Regulating Valve for maximum sparging (FIGURE 2-10A). Allow sparging to continue for approximately 10 minutes at a rapid rate, then lower the He flow rate to maintenance mode (FIGURE 2-10B).

FIGURE 2-9 He Shut-Off Valve

(A) (B)

FIGURE 2-10 (A) Rapid Sparging (B) Sparging – Maintenance Mode (Shield removed to provide clarity)

f) Connect the 5 mL syringe to the purge outlet on the purge valve on the loading pump using 1/16″ ID silicon tubing (FIGURE 2-11).

FIGURE 2-11 The Purge Valve

g) Open the purge valve on the loading pump by turning the purge valve knob (FIGURE 2-11) approximately 1 turn counterclockwise.

h) Press the PURGE key on the loading pump, set the flow rate of the to 0.0 mL and select solvent channel A using the SSV button on the rear side (Section 3.3). Withdraw solvent from bottle A using the syringe until no air is observed. Repeat this process for all four channels.

i) Set the purge flow to 0.5 mL/min. Allow the system to purge for at least 3 min. After line A has been purged, repeat the process for all other lines.

j) Close the purge valve on the loading pump by turning the purge valve knob clockwise.

k) Close the He shut-off valve(s) on top of the solvent bottles that will not be used (the white line should be horizontal).

l) Place the loading pump under computer control and deliver mobile phase through the entire HPLC system at the flow rate and from the solvent reservoir that are used for the initial conditions for the analysis that you intend to perform. As the system is delivering mobile phase, check for leaks, monitor the baseline and check that the pressure is similar to what was observed when the system was last used.

2.6.4 Setting-up the Fluidic Connections

The Switchos is used in a broad range of applications and the user can configure the unit to meet the specific needs of the laboratory. In this section we show the fluidic setup of a pre-concentration application using the Switchos Advanced Microcolumn Switching Unit in conjunction with the FAMOS Microautosampler and the UltiMate Capillary HPLC System (FIGURE 2-12). Additional examples are presented in Appendix C. While these examples may not meet the specific needs of the analyst, it is likely that they can be used with minor modification.

Pre-Concentration using the Switchos and the FAMOS Microautosampler

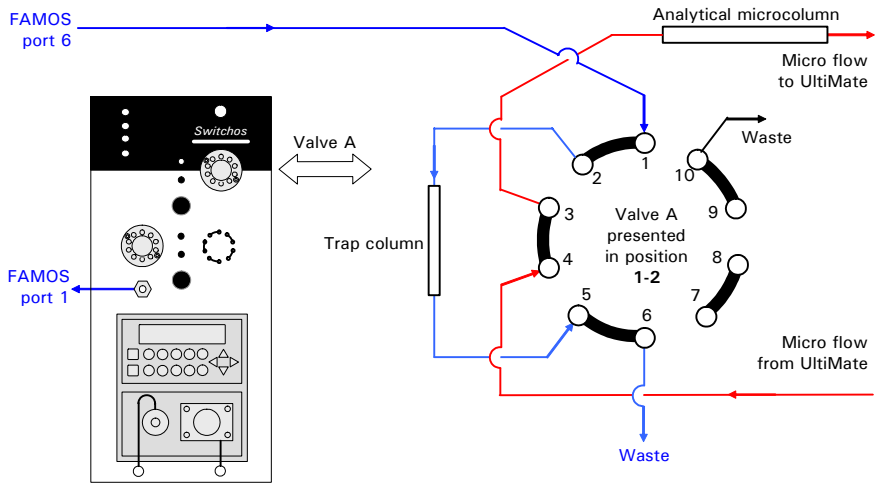


FIGURE 2-12 Typical Pre-Concentration Setup (Loading the Trap Column)

To setup the Switchos for the pre-concentration application presented in FIGURE 2-12, connect the instruments as follows:

a) Connect port 1 of the injection valve of the FAMOS Microautosampler to the Nano/Micro flow outlet of the Switchos loading pump using the 130 µm I.D. PEEK tubing provided with the instrument:

Dimension	Standard Version	Inert Version
50 cm x 130 µm I.D.	P/N 160180	P/N 160180
100 cm x 130 µm I.D.	P/N 160181	P/N 160181

b) Connect port 6 of the injection valve of the FAMOS Microautosampler to the port 1 of valve A of the Switchos II as described in item a).

c) Connect the outlet flow of the UltiMate System (e.g. on the left side panel or from the upper T-Piece of the flow splitter) to port 4 of valve A of the Switchos. Use the appropriate tubing for your application.

Application (I.D.)	Standard Version	Inert Version
Capillary LC (50 µm)	P/N 161479	P/N 161261 (1)
Nano LC (20 µm)	P/N 160178	P/N 161259 (1)
		(1) use PEEK fingertight fittings only

d) Connect port 3 of valve A of the Switchos to the UltiMate column bulkhead or directly to the micro column located in the UltiMate column compartment. Use the appropriate tubing for your application.

Application (I.D.)	Standard Version	Inert Version
Capillary LC (50 µm)	P/N 161480	P/N 161262 (1)
Nano LC (20 µm)	P/N 160179	P/N 161260 (1)
		(1) use PEEK fingertight fittings only

e) Connect ports 6 and 10 of valve A of Switchos to waste (e.g. using 200 - 500 µm I.D. PTFE tubing).

f) Connect the trap column between ports 2 and 5 of valve A of the Switchos II, using the appropriate tubing supplied with the trap column.

A typically loading solvent is 100% water with 0.05% TFA. Fill solvent bottle A with this solvent. Fill bottle B with either the same solvent or a mixture of methanol/water (1:1), bottles C and D may be left empty).

After pre-concentrating the sample on the trap column, the valve A of Switchos will be switched into position 10-1 to elute the sample (FIGURE 2-13).

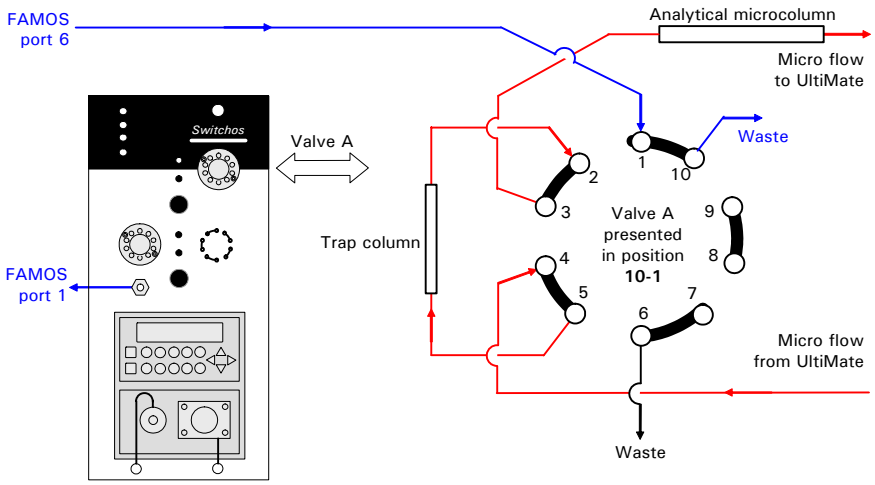


FIGURE 2-13 Eluting Sample adsorbed on the Trap Column (in Backflush Mode)

2.7 Routine Operation of the System

2.7.1 Sample and Mobile Phase Considerations

The Switchos Advanced Microcolumn Switching Unit is used in an HPLC system and the "standard" operating precautions for HPLC should be employed:

- Ensure that samples and mobile phases do not contain particulate matter. All samples and mobile phases should be filtered through a 0.22 μm membrane filter. If organic solvents are used, make sure that extractable materials are not present in the filter.

- The sample should be soluble in the mobile phase. If a gradient is used, make certain that the sample is soluble in the mobile phase at all mobile phase compositions to be used in the separation.

- After you have finished using the system, flush it with a water/methanol or water/acetonitrile mobile phase before shutting it down.

- Solvent should be degassed by sparging with Helium.

- Make certain that the sample and the buffer are soluble in all compositions of the mobile phase that will be used in the separation. This test should be run in a beaker or test tube so that particulate matter does not enter the system. If any cloudiness is observed in the test, the gradient should be adjusted and repeated.

Caution: It is strongly recommend that only bottled HPLC water and solvent be used. If water from water purification systems is used, polymeric contamination may seriously damage the column and the flow cell. This is especially true if sample pre-concentration or 2D separations are performed. This polymeric contamination may also seriously damage the flow cell (e.g. coating of the capillary walls).

CHAPTER 3

The User Interface

3.1 Overview

This chapter describes the general mode of operation of the Switchos™ Advanced Microcolumn Switching Unit. It includes the following information:

- Powering Up the Switchos (Section 3.2)
- A description of the controls on the front and rear panel (Section 3.3)
- LOCAL vs. REMOTE Control (Section 3.4)
- Basic Operations for the Loading Pump (Section 3.5)

3.2 Powering up the Switchos

When the Switchos Advanced Microcolumn Switching Unit is powered up via the main power switch on the rear panel (FIGURE 2-1), it will go through an initialization/self-test protocol. During this period, all LEDs on the front panel will be illuminated for a short period of time and a number of messages are displayed on loading pump indicating that various components are functioning properly.

After completion of this procedure, the Main Screen appears on the display of the loading pump (FIGURE 3-1) and the Solvent Selection Valve will be switched to channel A. The LEDs indicating the current status of the Switchos (e.g. LED 1-2 in the upper front panel indicates that valve A is in position '1-2').

Note: The initialization procedure takes a few seconds. The instrument is ready to use when LED A of the SSV LEDs is illuminated.

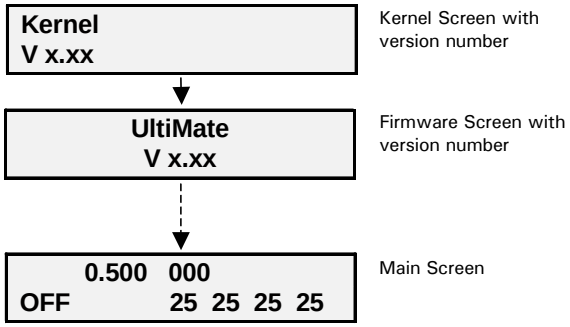


FIGURE 3-1. Loading Pump Start-Up Screens

The system can be switched into standby mode (e.g. when not used over night) by pressing the ON/STANDBY push button (FIGURE 3-2) for greater than **2 seconds.** This turns off the power to the loading pump, illuminates the Standby LED and places the Switchos into standby mode. When the system is in standby mode, pressing the button for a short time will power on the system again (and switch the Standby LED off).

FIGURE 3-2 The ON/Standby Switch

3.3 Controls on the Front and Rear Panel

When the Switchos Advanced Microcolumn Switching Unit is used on a local basis, the user sets the desired operating conditions via the controls on the front panel and the push button and switch located on the rear panel. The present status of the system is indicated by a series of LEDs on the front panel. The loading pump is controlled via the keypad on the lower front panel of the Switchos (see Section 3.5).

This section describes the use of the various controls and LEDs on the Switchos. Basic information about how to use the keypad of the loading pump is presented in Section 3.5, and detailed information is presented in the 'UltiMate Micropump User's Manual'.

The **upper front panel** of the Switchos (FIGURE 3-3) includes the following:

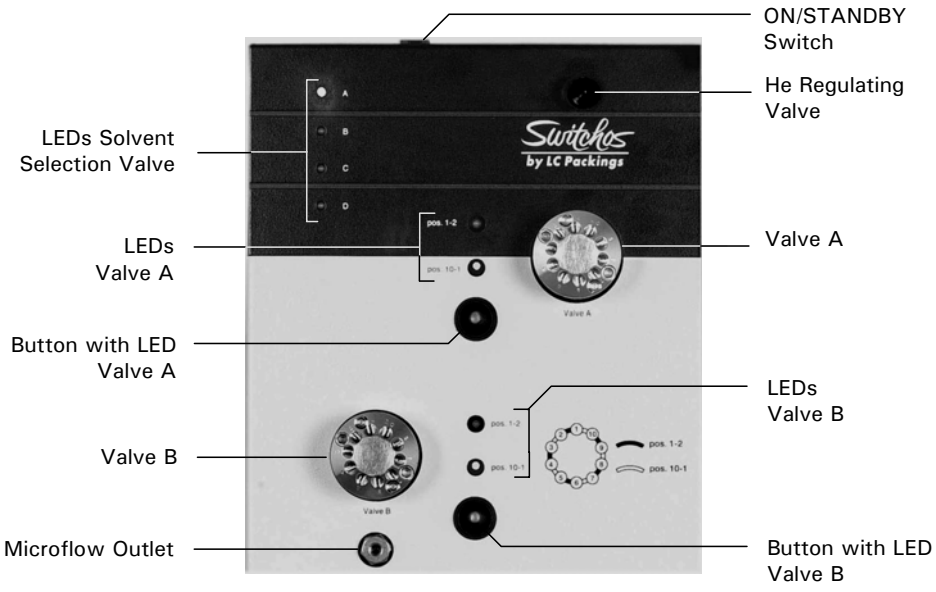


FIGURE 3-3 Control Elements on the Upper Front Panel

- **Valve A and B Controls**– the 10-port switching valves can be controlled by two push buttons when the Switchos is set into local mode. The local mode is indicated by the built-in LEDs. Pressing the appropriate button will toggle the position of the corresponding valve.

- **LEDs - Valve A** – indicate the position of valve A. When Valve A is switched into position "1-2" (e.g. the fluidic connection is between inlet 1 and inlet 2, the upper LED (1-2) is illuminated).

- **LEDs - Valve B** – indicate the position of valve B. When valve B is switched into position "1-2" (e.g. the fluidic connection is between inlet 1 and inlet 2, the upper LED (1-2) is illuminated).

- **LEDs - Solvent Selection Valve** – indicate the solvent bottle from which solvent is being withdrawn at the present time.

- **He Regulating Valve** – used to control the flow of He for sparging (see Section 2.5).

The **upper rear panel** of the Switchos (FIGURE 3-4) includes the following:

FIGURE 3-4 Control Elements on the Upper Rear Panel

- **SSV (Solvent Selection Valves) Button** – pressing the button will step to the next solvent channel, from A to B, B to C, ... and finally from D back to A.
- **LOCAL/REMOTE Switch** – used to select the control mode.

3.4 LOCAL Mode vs. Remote MODE

The control mode of the Switchos Advanced Microcolumn Switching Unit can be set via the REMOTE/LOCAL switch on the rear panel (FIGURE 3-4):

LOCAL mode - when the REMOTE/LOCAL switch is set to LOCAL mode (the switch is set to the upper or lower position), the unit can be controlled on a local basis by the push buttons and switches on front and rear panel. Any change that is made will override the present condition (regardless of whether it was set via Local or Remote Control). When the unit is in LOCAL mode, the INPUTS connector is disabled and the built-in LEDs in the push buttons are illuminated.

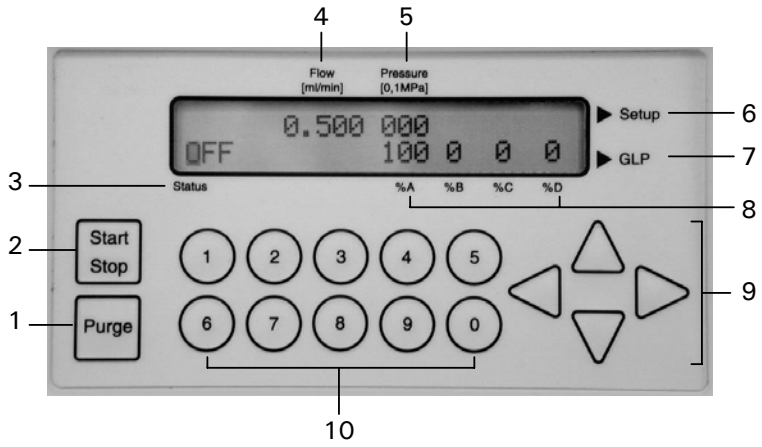
REMOTE mode - when the REMOTE/LOCAL switch is set to REMOTE mode (the switch is in the middle position) the instrument is controlled by an external device. In this mode, the INPUTS connector is enabled and the built-in LEDs and the push buttons are deactivated The switching valves A and B will be positioned according to the current signals of the INPUTS connector.

Note: If the instrument is in REMOTE control mode and the INPUTS connector on the rear panel is not connected, switching valves A and B are "locked" in position '1-2'.

3.5 Basic Operations for the Loading Pump

The main screen of the loading pump includes a series of parameters that are used to set the principal operating conditions for delivering the mobile phase, such as the flow rate and maximum pressure. In automated mode (networking) these parameters are set via CHROMELEON. The following sections provide basic information about the loading pump, a detailed discussion on pump control is presented in the 'UltiMate Micropump User's Manual'.

All communication between the user and the system is provided by the front panel of the pump (FIGURE 3-5).



1 START/STOP Purge
2 START/STOP Pump
3 Status of Pump
4 Flow Rate
5 Pressure
6 Setup Menu Access
7 GLP Menu Access
8 % value for solvents *(not used)*
9 Arrow Keys for Cursor
10 Data Input Keys

FIGURE 3-5 The Front Panel of the Loading Pump

3.5.1. Setting the Flow Rate

To set the flow rate, use the arrow keys to move the cursor to the *Flow* field (item 4, FIGURE 3-5) and enter the desired flow rate.

3.5.2 Setting the Maximum Pressure

The maximum pressure value for the pump can be set via the Pressure field (item 5, FIGURE 3-5). As an alternative, it can be set via the PRESSURE LIMITS screen of the SETUP Menu (global setting, see 'UltiMate Micropump User's Manual').

To set the maximum pressure, move the cursor to the Pressure field (item 5, FIGURE 3-5) and enter the desired value. When this field is being edited, it is bracketed by a pair of vertical lines to indicate that this is a programmed value rather than the actual pressure. During operation of the pump, the actual pressure is indicated. However, if the cursor is moved to the Pressure field during operation, the maximum pressure value will be indicated and the brackets will be presented.

3.5.3 Starting and Stopping Flow Delivery

When the desired parameters have been entered, the flow delivery can be started with the START/STOP key (item 2, FIGURE 3-5). This key is also used to stop the flow delivery.

3.5.4 Purging the Loading Pump

To purge the loading pump, opening the purge valve and then press the PURGE key on the pump (item 1, FIGURE 3-5). To assure proper purging of the solvent lines, set the flow rate to 0.5 mL/min. Select each of the four solvent bottles A, B, C and D by the SSV button on the rear panel. Each of the four solvent channels should be flushed for approximately 2 minutes and the operator should ensure that no air bubbles are observed in the mobile phase.

Caution: Purging the system without opening the purge valve knob may cause damage to your column and/or the system.

CHAPTER 4

CHROMELEON® Control

4.1 Overview

This chapter provides information about how to setup the CHROMELEON® Software to control the Switchos II Advanced Microcolumn Switching Unit either by the RS-232 communication port or by the event outputs of the Switchos II loading pump. A detailed description of the software features is provided in CHROMELEON online help (F1 key) and a quick "Getting Started" reference can be found in the UltiMate User's Manual. It is assumed that the user has a basic understanding of the CHROMELEON software and its modules.

When the Switchos™ II Advanced Microcolumn Switching Unit is used in conjunction with the LC Packings UltiMate™ Capillary HPLC System and the CHROMELEON software, the flow rate of the loading pump as well as the position of the valves of the Switchos II can be programmed from the CHROMELEON software. The Switchos II has to be installed as described in Chapter 2.

The role of the valves of the Switchos II is dependent on the application for which the unit is configured (e.g. if the FAMOS Microautosampler is included in the system). The user should ensure that the electrical interface and the fluidic interface to other devices in the system meet the requirements of the analysis.

Note: All dialog boxes and information refer to CHROMELEON 6.5 SP3.

In this discussion, we will describe how to establish a typical column switching application with the following parameters:

- Sample load/clean-up time: 5 min (at a loading flow rate of 30µL/min)
- Gradient analysis time: 50 min

4.2 Server Configuration Setup

4.2.1 Adding and Configuring the Switchos II

To communicate with the Loading Pump of the Switchos II Advanced Microcolumn Switching Unit:

a) Start the CHROMELEON server, and then start the CHROMELEON Server Configuration. Select the name for the server (if more than one is configured) of which you want to modify the configuration. Click the '+' character in front of the server name to view its current configuration.

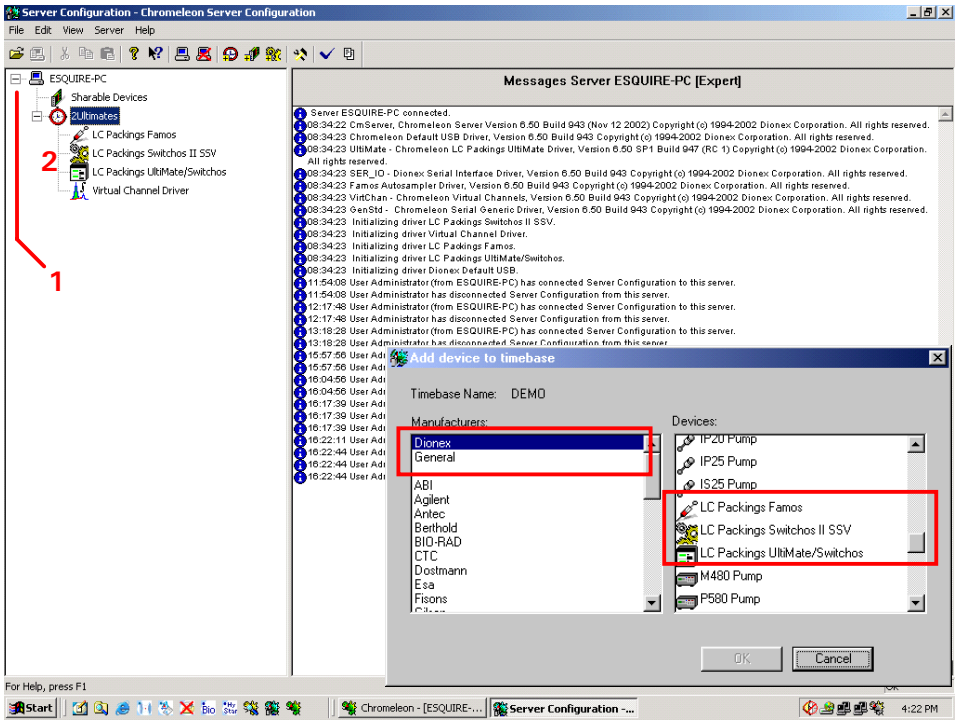


FIGURE 4-1 The Server Configuration Box

b) Double-click on the 'LC Packings UltiMate/Switchos' device (item 2, FIGURE 4-1).The *LC Packings UltiMate/Switchos box* appears (FIGURE 4-2).

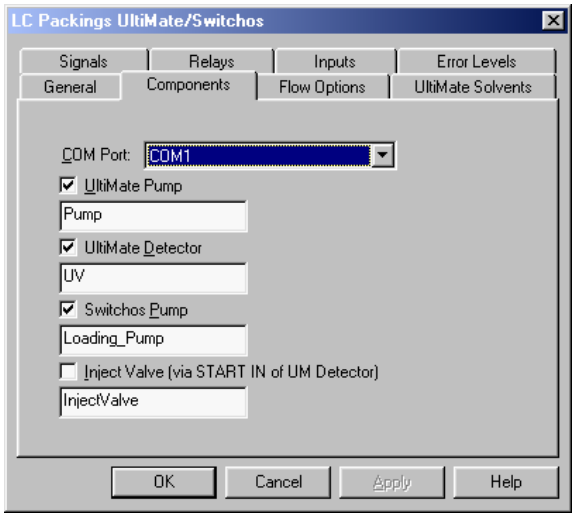


FIGURE 4-2 The UltiMate Configuration Box – Components Tab

c) Make sure that the **SwitchosPump** box is checked on the **Components** tab.

The valves of the Switchos II can either be controlled directly via the COMMUNICATION connector and via a free COM port of the PC (Section 2.3) or by the event outputs of the Switchos II Loading Pump via the INPUTS connector (Section 2.4).

4.2.2 Switchos II controlled by a RS-232 COM Port

To control the unit by serial communication:

a) Add the 'LC Packings Switchos II SSV' device to the Timebase (FIGURE 4-1). This will present the *LC Packings Switchos II SSV* configuration box (FIGURE 4-3).

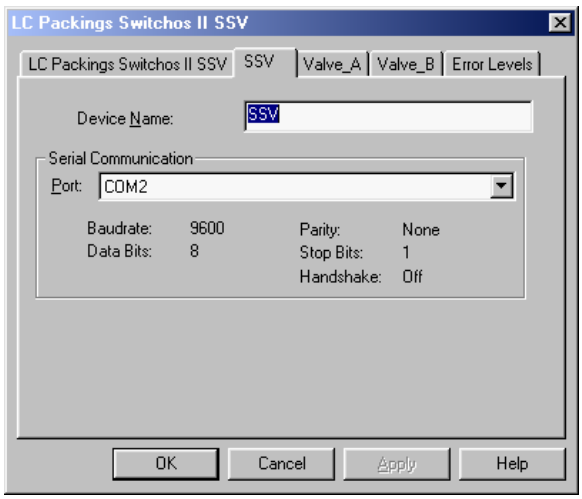


FIGURE 4-3 The Switchos II Configuration Box

Select the COM port which controls the unit and click **OK** to confirm the setting.

To verify or change the configuration, double-click on the 'LC Packings Switchos II SSV' device (item 2, FIGURE 4-1).

Note: If there are not enough COM ports available, the valves of the Switchos II can be controlled by the event outputs of the Loading Pump (Section 4.2.3).

4.2.3 Switchos II controlled by Event Outputs

If serial control is not possible (e.g. no free COM port available), the Switchos loading pump can be used to control the valve positions. The switching valves are then controlled by the event outputs of the Switchos II loading pump (TABLE 4-1) and EVENT8 can be used to trigger another device (e.g. a mass spectrometer).

TABLE 4-1 Control Outputs

Switchos II Input	Loading Pump	Default CHROMELEON Signal Name
Valve A	EVENT3	Valve_A
Valve B	EVENT6	Valve_B
SSV	EVENT7	SSV
Trigger	EVENT8	Switchos_Relay2

Note: The EVENT number as well as the type are indicated on the rear panel of the Micropump (e.g. EVENT 1 'TTL', EVENT 3 'OC', etc.). Make certain that the proper EVENT output is linked to the corresponding signal name of CHROMELEON (e.g. 'Valve_A' should control EVENT3 of the Micropump, 'SSV' should control EVENT7, etc.).

To control the unit by the event outputs of the Loading Pump:

a) Select the **Relays** tab of the *LC Packings UltiMate/Switchos* box (FIGURE 4-4).

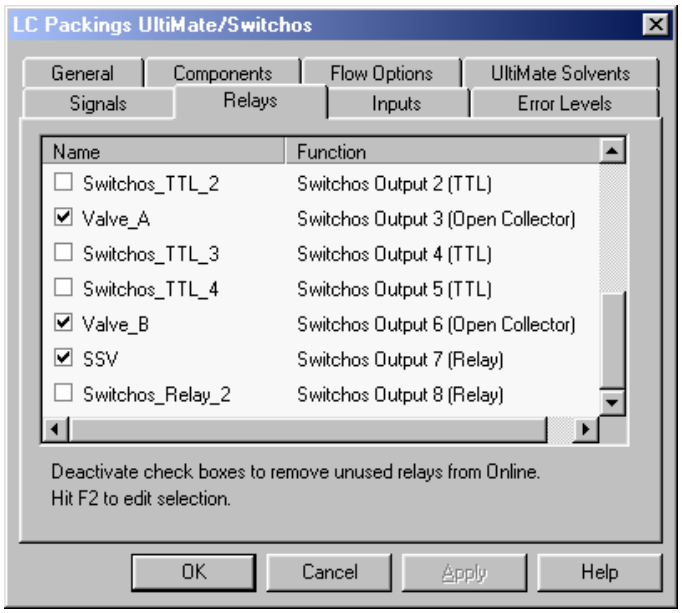


FIGURE 4-4 The UltiMate Configuration Box – Relays Tab

b) Check the outputs 'SSV', 'Valve_A', and 'Valve_B', check 'Switchos_Relay_2' if you want to trigger any instrument by the EVENT 8 output.

Note: The default signal names corresponds to the names used in the (predefined) panels and should not be changed. If you modify the signal names, they may not be recognized and some functions may not work properly.

4.2.4 Adding and Configuring the Virtual Channel Driver

To monitor and record various parameters of the UltiMate (e.g. the column pressure or the oven temperature) a Virtual Channel Driver is needed.

To add a Virtual Channel Driver:

a) Click on **Add Device** in the *Server Configuration* box and select **Virtual Channel Driver** from the **General** tab to present the *Virtual Channel Driver* box (FIGURE 4-5).

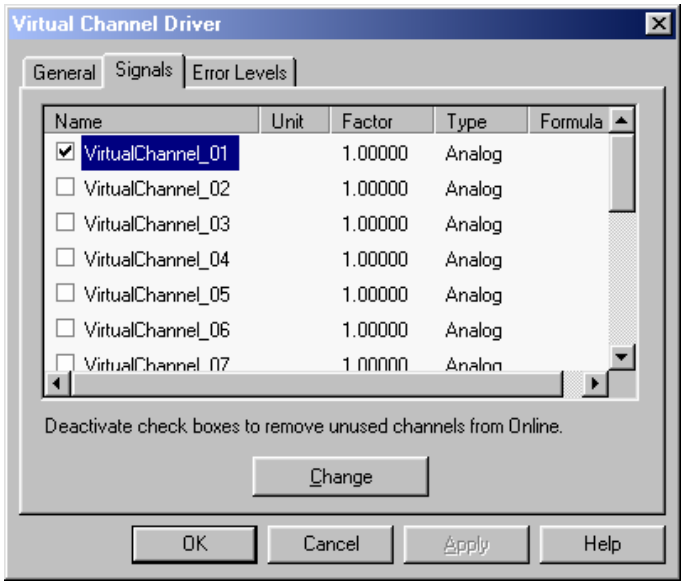


FIGURE 4-5 The Virtual Channel Driver Box

b) Check the first channel and click on **Change** to configure it. To readout the column pressure, configure the *Signal Configuration* box as indicated in FIGURE 4-6.

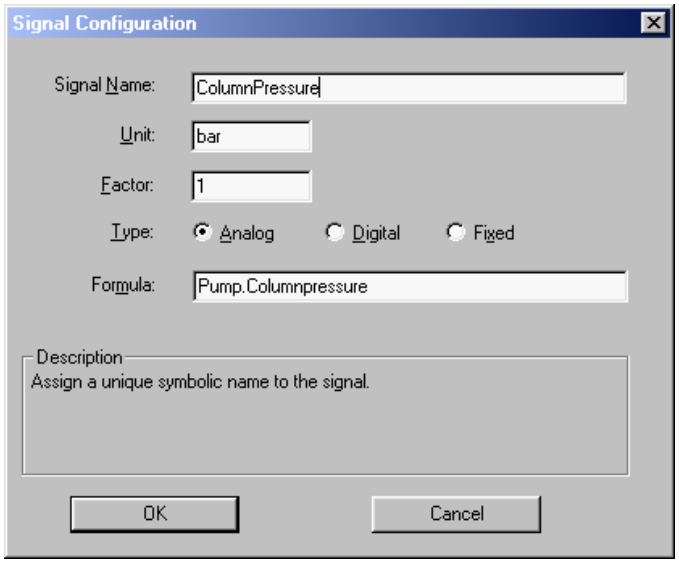


FIGURE 4-6 Virtual Channel Setup for Column Pressure Readout

To monitor other parameters (e.g. the UltiMate Micropump pressure), select a new channel (FIGURE 4-5) and configure the signal as presented in TABLE 4-2.

TABLE 4-2 Signal Name and Formula Definitions

Device [a]	Signal Name	Unit	Factor	Formula
ULT	PumpPressure	bar	1.0	pump.masterpressure
ULT	ColumnPressure	MPa	0.1	pump.columnpressure
SW II	TrapColumnPressure	psi	14.5	loading_pump.trapcolumnpressure
ULT	OvenTemperature	°C	1.00	Oven.Temperature
FMS	TrayTemperature			Sampler.Temperature

Note: a) ULT = UltiMate , FMS = FAMOS , SW II – Switchos II

A typical setup is presented FIGURE 4-1.

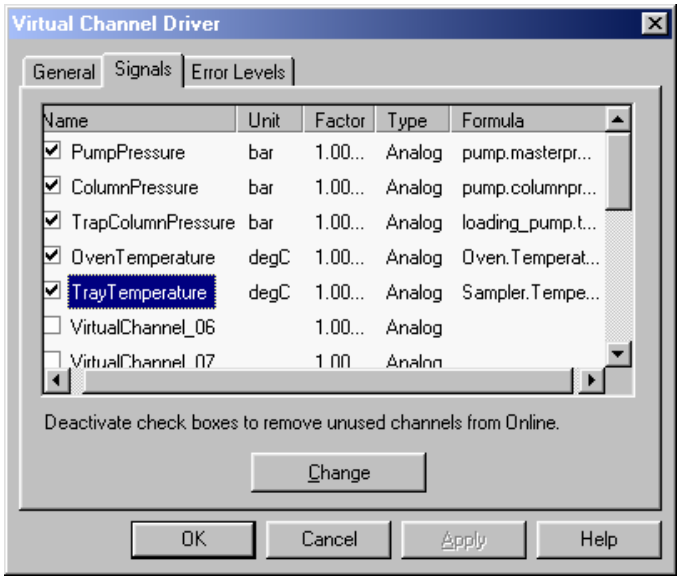


FIGURE 4-7 Example for a typical Virtual Channel Setup

Note: Make sure to use the same signal names and the same formulas as indicated in TABLE 4-2. If you modify the signal names or the formulas, they may not be recognized by the (predefined) panels and some functions may not work properly.

4.3 Using CHROMELEON

The following section provides information how to use the Switchos II Advanced Microcolumn Switching Unit in conjunction with the CHROMELEON software, the LC Packings UltiMate system and the FAMOS Microautosampler. In addition to the steps used to program the Switchos, basic information is provided how to control the other modules.

4.3.1 Starting CHROMELEON

Click on the CHROMELEON Server icon to start the CHROMELEON server (which is the interface between the CHROMELEON user interface and the instruments). Click on the CHROMELEON icon to start the software.

4.3.2 The Control Panel

The Control Panel (abbreviated: the 'panel') controls and monitors the instruments of one Timebase. With regard to appearance and function, it is a special type of window. You can determine the number of available controls and their functionality via the design tools, depending on your individual requirements (refer to the CHROMELEON user's manual for more detailed information).

A number of standard panels for the most commonly used UltiMate configurations are available. Refer to TABLE 4-3 and choose the panel which corresponds to your instrument configuration, and then load this panel from the 'Dionex Templates/ Panels/Dionex LC' directory.

TABLE 4-3 Panel Name vs. Instrument Configuration

Control Panel Name	Instrument Configuration
Ultimate.pan	UltiMate
Ultimate_FAMOS.pan	UltiMate and FAMOS
Ultimate_FAMOS_Switchos.pan	UltiMate, FAMOS, Switchos II (Switchos II valves are controlled by the Loading Pump – Section 4.2.3)
Ultimate_FAMOS_SwitchosII.pan	UltiMate, FAMOS, Switchos II (Switchos Valves are controlled by serial communication – Section 4.2.2)

Depending on your authorization, you can create a completely new control panel. In order to create or change properties of a control panel, change to the **Layout Mode** on the **Edit** menu. A new control panel is saved as a PAN file (*.pan) and is then available to the user (refer to the CHROMELEON user's manual for more detailed information).

4.3.3 Connecting the Control Panel to a Timebase

A panel needs to be connected to a certain Timebase to allow the control of the instruments of this Timebase.

To connect the control panel to a Timebase:

a) Click on the **Connect to Timebase**... command on the Control menu to select the Timebase you want to connect the panel to.

b) Use on the **Save as...** command on the File menu to save this new assignment.

4.3.4 Starting the Flow Delivery and Baseline Monitoring

To gain control of the different Ultimate modules, each module of the configuration need to be connected to the control panel. Check the **Connect** boxes to connect the individual modules (item 1; FIGURE 4-8).

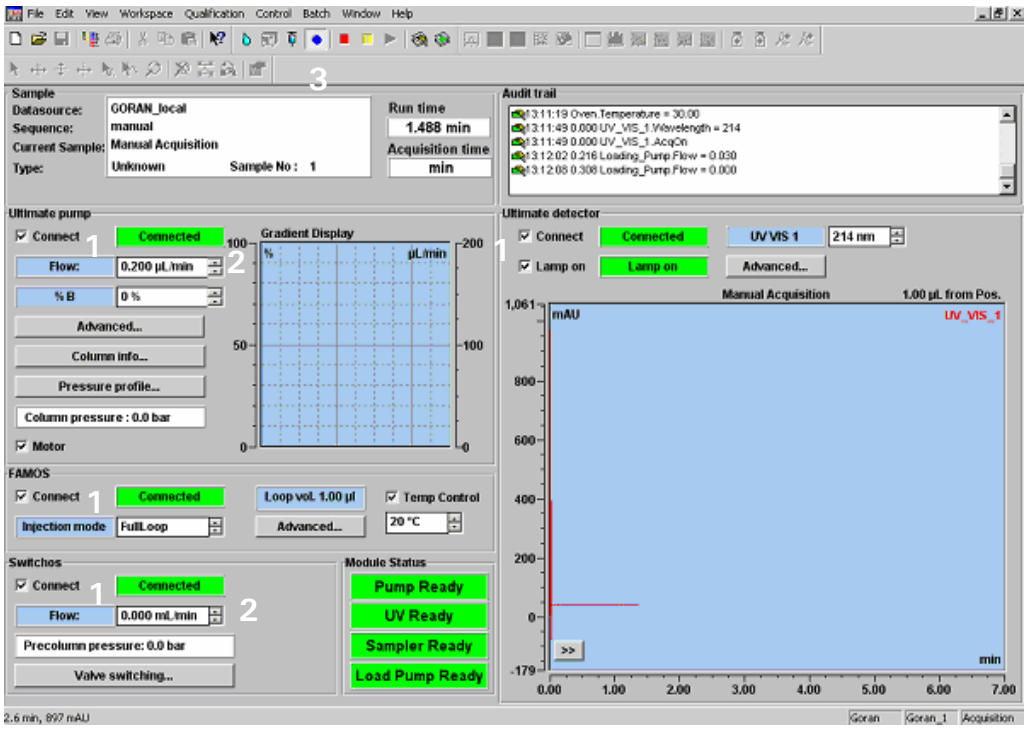


FIGURE 4-8 The Control Panel for UltiMate, FAMOS and Switchos

Flow delivery and baseline monitoring can now be started. Enter the column and the trap column flow rates in the **Flow** fields in the corresponding boxes (item 2; FIGURE 4-8) and click on the blue circle in the toolbar to start baseline monitoring (item 3; FIGURE 4-8).

4.3.5 Creating a Program File – Using the Wizard

CHROMELEON programs are text files and modifying a program is normally done by editing in the text window. When creating a new program file, a Program Wizard starts automatically and guides you through the programming to simplify the procedure. Enter the required information; in most cases the pre-defined values can be used.

The wizard provides a ready-to-use program based on your entries, without the need to type a special programming syntax. Press the F1 key for additional help and more detailed information. Section 4.3.6 provides a programming example.

4.3.6 Creating a Program – An Example

The following section provides information how to program the flow rate of the loading pump and how to use the switching valves.

For a column switching experiment (a pre-concentration step) the Switchos II Advanced Microcolumn Switching Unit, the UltiMate system (Nano configuration) and the FAMOS Microautosampler are used and need to be programmed.

The experiment has the following features:

- NanoLC separation on a 75 µm I.D., 15 cm PepMap column.
- Injection of 10 µl of cytochrom c sample (0.1 pmol/µL).
- Sample loading onto a trap column for 5 minutes at a flow rate of 30 µL/min (pre-concentration step).
- Solvent gradient from 0 - 50%B in 30 minutes.
- Analysis time of 60 minutes.
- Recording of the UV signal at 214 nm, the column pressure, the trap column pressure and the pump pressure.

CHROMELEON automatically provides a program wizard when a new program is to be created, which guides through creation of the program file.

To create a new program using the Program Wizard:

a) Select the **New** command on the **File** menu, and then select **Program File** from the *New* box. The Program Wizard starts automatically.

b) Select the Timebase from the *Select Timebase Options* box.

c) Setup the column oven temperature (if applicable) in the *Oven Options* box.

d) Setup the trap column pressure limits of the Switchos II Loading Pump (e.g. 0 bar and 200 bar) and fill in the time table in the *Loading_Pump Options* box.

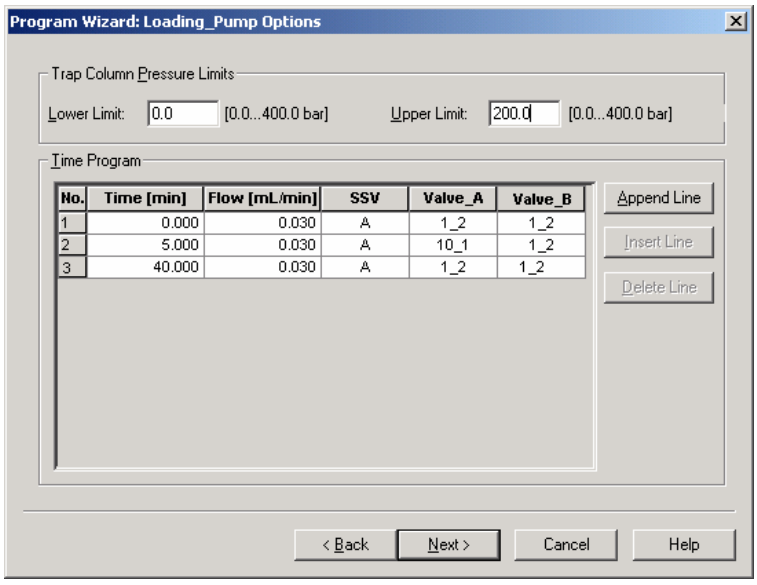


FIGURE 4-9 The Loading Pump Options Box

e) Setup the gradient conditions in the *Pump Options* box, use the 'Multi-step Gradient' option to run a gradient.

f) Define the gradient in the *Flow Gradient Options* box FIGURE 4-10).

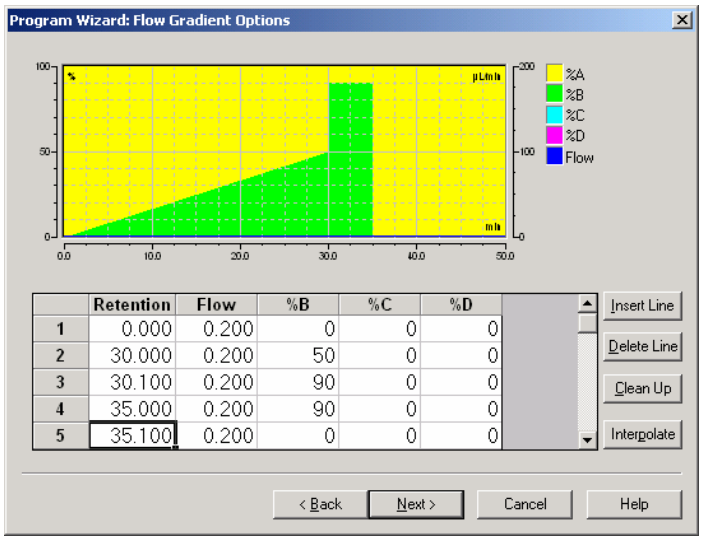


FIGURE 4-10 The Flow Gradient Options Box

Note: The *Flow Gradient* box is not present if the 'isocratic' option was selected in the *Pump Options* box in the previous step.

g) Setup the pressure limits for the separation column and the UltiMate pump, and define separation column parameters (e.g. I.D., length and stationary phase) in the *UltiMate Pump Options* box (FIGURE 4-11).

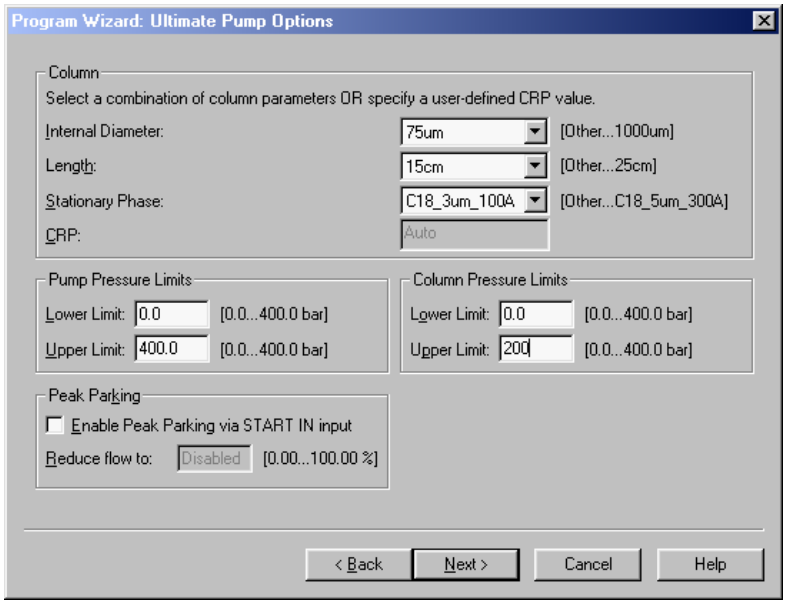


FIGURE 4-11 The UltiMate Pump Options Box

Note: If you do not specify the 'Column' parameters (e.g. the option 'other' is selected), you need to enter a CRP (Column Resistance Parameter) value. The CRP is required to calculate the (master) flow rate of the UltiMate Micropump.

h) Specify the detector settings (e.g. the data collection rate) in the *UV Options* box.

i) Define the type of injection you want to use and the corresponding injection parameters in the *Sampler Options* box.

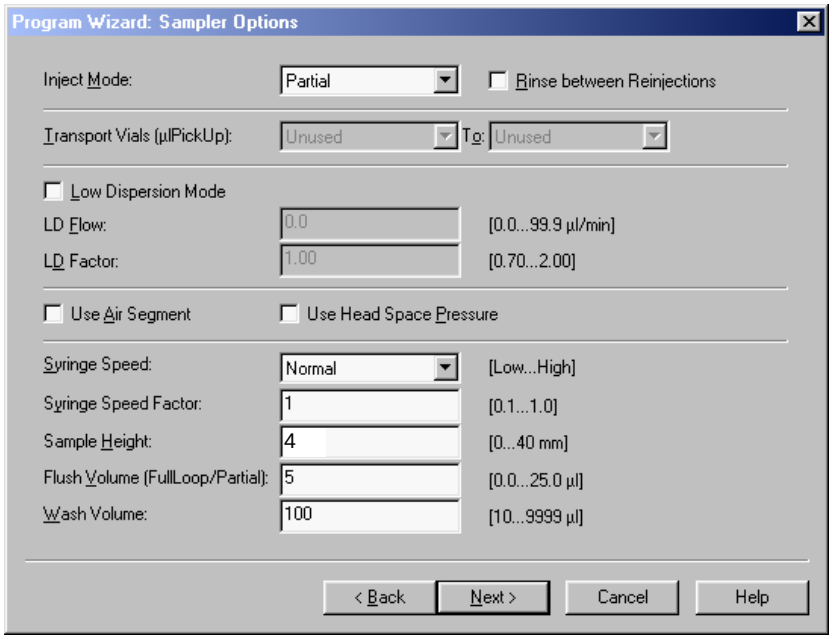


FIGURE 3-12 The Sampler Options Box

Note: If you want to create a 'User Defined Program', please refer to the CHROMELEON online (F1 key) help more details.

j) Select the number of UV channels used and the data acquisition time in the *Acquisition Options* box (FIGURE 4-13).

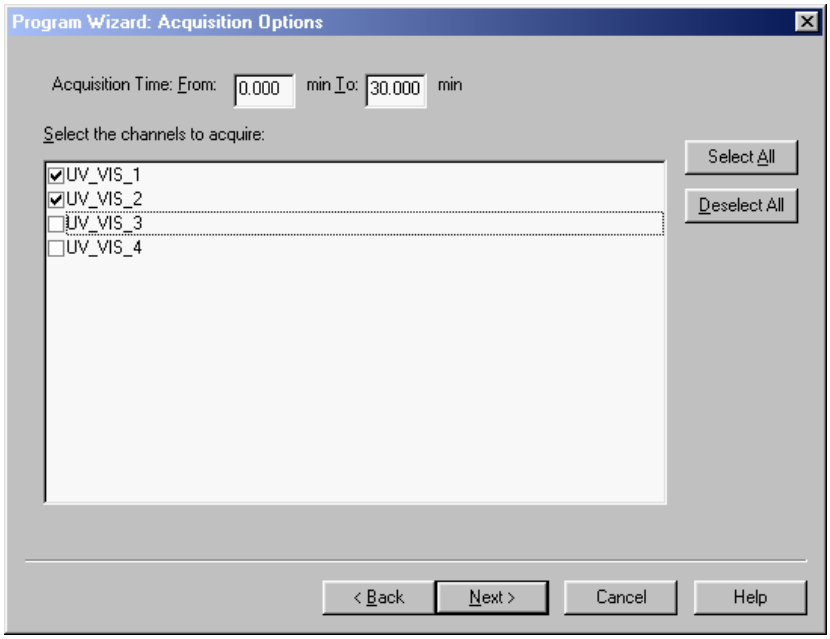


FIGURE 4-13 The Acquisition Option Box

The number of UV_VIS_n *Options* boxes to be filled in is dependent on the number of channels that you are monitoring (e.g. the different wavelengths (FIGURE 4-14).

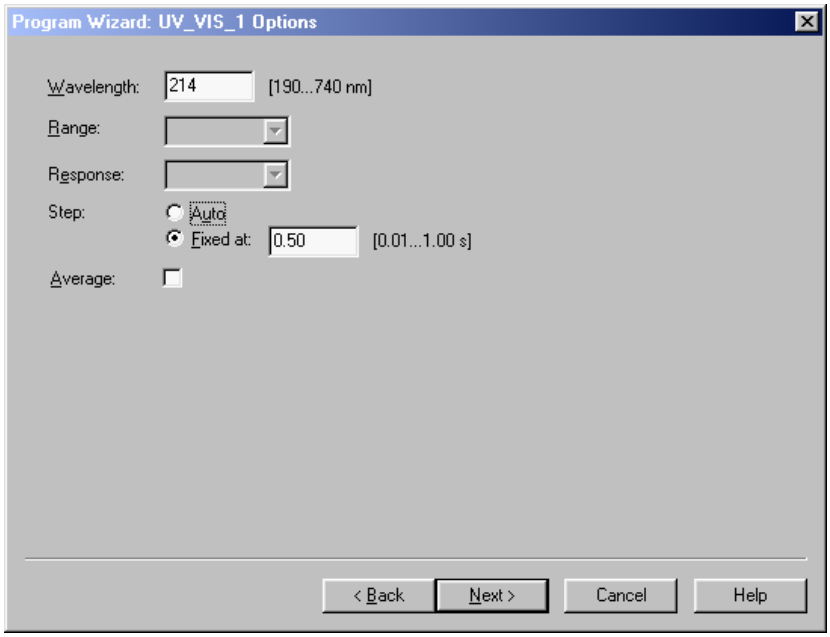


FIGURE 4-14 The UV_VIS_n Options Box

k) Specify the program name and path.

Note: The Appendix E of the user's manual of the UltiMate system presents 4 additional examples.

4.3.7 Changing a Program File

A CHROMELEON program can easily be changed by a double click on the program name in the CHROMELEON browser. The program appears in a text format. Program lines are inserted by describing the parameter or by using the F8 command.

CHAPTER 5

Testing the Switchos

5.1 Overview

This chapter describes a series of activities that can be used to check the operation of the system and to verify that your Switchos Advanced Microcolumn Switching Unit is operating in an acceptable manner.

This chapter includes:

- Testing the Solvent Bottle Caps and Degassing Unit (Section 5.2.1).
- Testing for the Solvent Selection Valve (Section 5.2.2).
- Testing of the fluid path for the Solvent Selection Valve and solvent lines (Section 5.2.3).
- Testing of the Switching Valves (Section 5.2.4).

Note: Unless otherwise noted, pure methanol should be used as the solvent and these tests should be performed at 25°C.

5.2 Checking System Components

5.2.1 Solvent Bottle Caps and Degassing Unit

The He lines, the regulating valve and connections can be tested by the following procedure:

a) Close the Shut-Off Valves (FIGURE 5-1) and apply 3 bar (45 PSI) of He pressure.

CAUTION **Caution: Do not operate the He lines a higher pressure than 4 bar (60 PSI).**

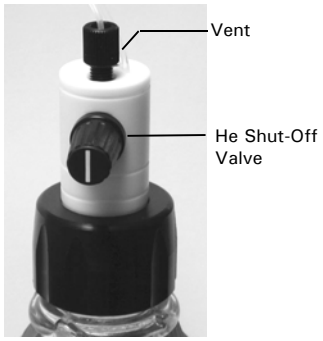


FIGURE 5-1. The He Shut-Off Valve (Opened Position)

b) Open the He Regulating Valve on the front panel. The regulating valve should be turned 90°. Check to see if there are any leaks (i.e. are He bubbles observed in the solvent bottle).

c) Open the shut-off valve on the bottle cap for solvent bottle A, by closing the He regulating valve verify that the bubbles of He exit on the side frit of the sparging/filter frit. Check to make sure that it is possible to regulate the He flow by opening the He regulating valves until the maximum is reached (90°).

d) Repeat steps (b) and (c) for each solvent bottle.

5.2.2 Solvent Selection Valve – Basic Test

To check the operation of the Solvent Selection Valve and the Valve Controller:

a) Set the Switchos in local mode by the **Remote/Local** switch on the back panel (the LEDs of the two push buttons on the front panel should be illuminated).

b) Select the next available solvent channel by the **SSV** button. The corresponding LED should be illuminated and the sound of the moving solvent selection valve should be heard for a few seconds.

c) Repeat step (a) for each solvent channel.

5.2.3 Solvent Selection Valve and Solvent Lines – Fluid Path Test

To determine if the fluidic path for solvent line is operating properly and if the resistance of the flow path is within the specifications:

a) Fill each solvent bottle (with methanol) until the fluid level corresponds to the top of the Switchos housing.

b) Open the four He shut-off valves on the bottle cap assemblies to open the solvent lines (the He supply line has to be shut off for this test).

c) The four solvent lines should be well flushed using the Purge function of the loading pump. If is not possible to flush the lines, check for clogged or dirty solvent filters (sparging/filter frits) or bent solvent lines.

d) Switch the flow delivery of the loading pump off, disconnect the solvent inlet line from the pump head and connect a 250 µL syringe (i.e. the plunger should be removed) using an adapter (P/N 160259) as shown in FIGURE 5-2.

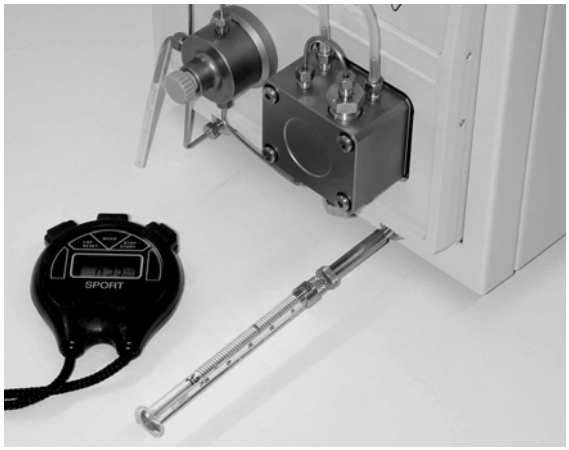


FIGURE 5-2. Placing the Syringe on the Solvent Inlet Line

e) Select solvent channel A by the **SSV** button on the rear of the Switchos. The LED corresponding to Valve A should be illuminated.

f) Measure the flow rate from the inlet tubing for one minute. The flow rate should be greater than 50 µL/min for a solvent line that is functioning properly.

g) Repeat steps (e) and (f) for each solvent line.

Note: If the flow is less than the specified value, repeat the test with the solvent filters removed. If the flow is within specifications after the filters have been removed, they are clogged, dirty or defective and must be replaced.

Note: As an alternative to using a syringe, this test can be performed by allowing the solvent to drip from the solvent inlet line and counting the number of drops per minute. At least 5 drops per minute should be observed if the proportioning valve opens properly. If the flow rate is less than 5 drops/min, repeat the test with the solvent filters removed. If the flow is within specifications after the filters have been removed, the filters are clogged, dirty or defective and must be replaced.

5.2.4 Switching Valves – Basic Test

To check the basic function of the Switching Valves and the Valve Controller:

a) Set the Switchos in local mode by the **Remote/Local** switch on the back panel. The LEDs of the two push buttons on the front panel should be illuminated.

b) Toggle valve A by the corresponding push button on the front panel. The LED corresponding to the current position (e.g. LED 1-2 or LED 10-1) should be illuminated and you should hear the sound of the moving valve for a short period of time.

c) Repeat step (b) for switching valve B.

CHAPTER 6

Maintenance and Troubleshooting

6.1 Overview

This chapter provides information to assist in optimizing the performance of the Switchos Advanced Microcolumn Switching Unit and maintaining it in your laboratory. It includes the following material:

- **Maintenance** - describes a series of activities that should be performed on a periodic basis to optimize the performance of the system and minimize down time (Section 6.2).
- **Replacing Components** – provides directions for replacing components, e.g. due to wear (Section 6.3).
- **Troubleshooting** - discusses a series of activities that should be used to determine the cause of a problem. (Section 6.4).
- **Spare Parts/Replacement Parts Lists** – Presents a listing of components that are used to maintain the unit or to change the configuration (Section 6.5).

6.2 Maintenance

Maintenance refers to a variety of activities that should be performed on a routine basis to optimize performance of the system. Many routine maintenance activities can be readily performed by the user. Detailed information about the maintenance of the Switchos Loading Pump is presented in the 'UltiMate Micropump User's Manual'.

In some cases (e.g. replacement of critical components), we recommend that a factory trained service engineer should be called to perform the operation. This will ensure optimal long term performance and maximum uptime. LC Packings provides a broad range of service support activities to ensure that the Switchos Advanced Microcolumn Switching Unit is functioning in a suitable manner. These activities can be customized to meet the specific needs of the customer. For further information, please contact your local LC Packings office or representative.

TABLE 6-1. Recommended Maintenance Schedule

Frequency	Operation
Every Day	Before operating, check for any air bubbles in the fluidic lines and degas the solvent.
	Check that the fluidics connections do not leak.
	Check that salts are not deposited by the fluidics joints.
	When using buffer solutions, flush the system thoroughly after use with a solvent that does not contain buffers/salts.
Every 3 months	Inspect the condition of all tubing (cracks, nicks, cuts, clogging).
Every year	Replace:
	Rotor seal of the switching valves
	Check:
	Stator of the switching valves
	Solvent Selection Valve for Leakage a)
	a) Contact your LC Packings representative.

Note: The frequency of the various activities described above is a reasonable starting point. As the user gains experience with the system, it will be found that some activities can be done less frequently and other need to be done more frequently. The frequency is dependent on a number of factors including the nature of the sample and the mobile phase.

6.3 Replacing Major Components

A variety of components on the Switchos Advanced Microcolumn Switching Unit can be readily changed by the user as required to ensure that the instrument is maintained in optimal condition.

This section provides information and procedures about how to replace user replaceable parts. In most cases, re-assembly of a component is identical to its disassembly, except that the steps are performed in the reverse order. If no comment is made, it should be assumed that the assembly of a component or installation of a component is identical to disassembly or removal, except that the actions are in the reverse order.

Sections 6.3.3 to 6.3.5 provide basic information about how to remove the pump head and how to replace check valves cartridges or piston seals. For more detailed information refer to the 'UltiMate Micropump User's Manual'.

6.3.1 Replacing the Sparging/Filter Frit

The Sparging/Filter Unit is connected via tubing which slides off the unit. Over time, the filter may become clogged by particulate matter and should be replaced. In some cases, the filter can be cleaned via an ultrasonic bath (depending on the nature of the particulate matter).

Note: A clogged filter may lead to poor analytical performance (Section 6.4 - Troubleshooting).

To replace the Sparging/Filter Frit:

a) Unscrew the Solvent Bottle Caps from the solvent bottles and take the tubing with the frit out.

b) Remove the old frit by pulling the tubing (item 1, FIGURE 6-1) off the filter body (item 2, FIGURE 6-1).

c) Insert the He tubing and the solvent tubing into a new Sparging/Filter Frit (P/N 160044). The He tubing should be placed in the hole closer to the He outlet.

d) Gently pull at each line to make sure that it is secure.

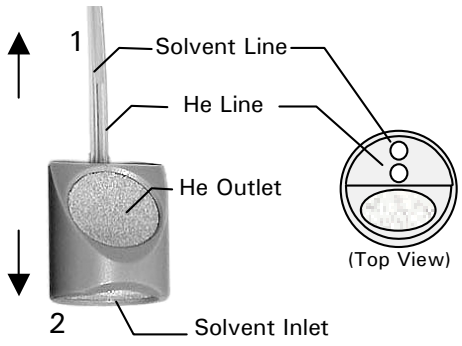


FIGURE 6-1. Sparging/Filter Frit

6.3.2 Replacing the High Pressure In-line Filter

The high pressure in-line filter serves to remove particulate matter from the mobile phase and is considered as a consumable item. If excessive pressure is required to deliver the mobile phase (indicated on the pump display), it is probable that the filter should be replaced.

To replace the high pressure in-line filter:

e) Remove the capillary (item 1, FIGURE 6-2) between the purge valve and the filter unit.

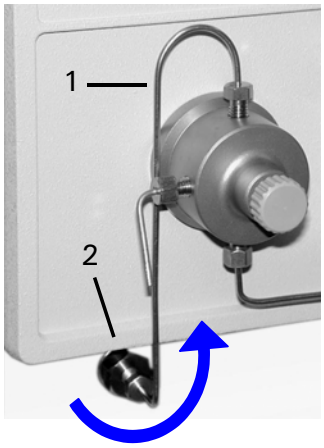
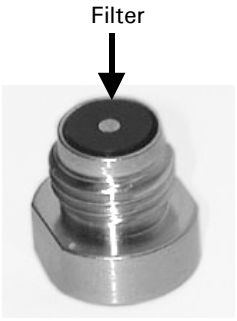


FIGURE 6-2. High Pressure Inline Filter

f) Use the socket driver (P/N 160073) or a wrench (11 mm) to remove the filter holder (item 2, FIGURE 6-2).

g) Remove the filter and place a new filter into the holder (FIGURE 6-3).



Description	P/N
Filter	
Standard (color: black)	160072
Inert (color: blue)	161107
Filter Frit Holder	
Standard (stainless steel)	163021
Inert (titanium)	162108

FIGURE 6-3. In-Line Filter in Filter Holder

h) Re-mount the filter holder.

CAUTION **Caution: Make certain that the filter remains in the filter holder when screwing the holder in and do not overtighten the filter holder. Overtightening could result in distortion of the filter which may lead to leakage and/or premature failure of the component.**

6.3.3 Removing the Pump Head

To remove the Pump Head:

a) Remove the inlet and the connecting capillary (FIGURE 6-4).

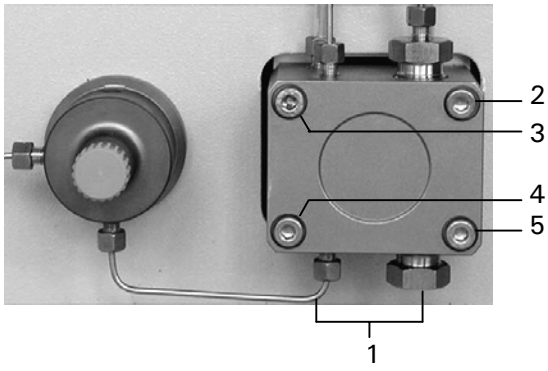


FIGURE 6-4. Disassembly of the Pump Head

b) Loosen two diagonally opposed screws (e.g. screws '2' and '4' in FIGURE 6-4 by a quarter turn at a time and remove the screws.

c) Carefully loosen the two remaining screws (e.g. screws '3' and '5' in FIGURE 6-4), alternating from one to the other, approximately half a turn at a time and remove the screws.

d) Remove the pump head.

Note: If the purpose of removing the pump head is to check the piston rods, there is no need to disassemble the pump head any further. Pull out each piston rod in a straight line using a pair of pliers.

To replace the Pump Head:

a) Position the pump head onto the housing and carefully line up the screw holes. Tighten all four set screws by hand.

b) Alternating from one to the next, tighten two diagonally opposed screws (e.g. screws '2' and '3') half a turn at a time until the pump head is correctly seated onto the housing.

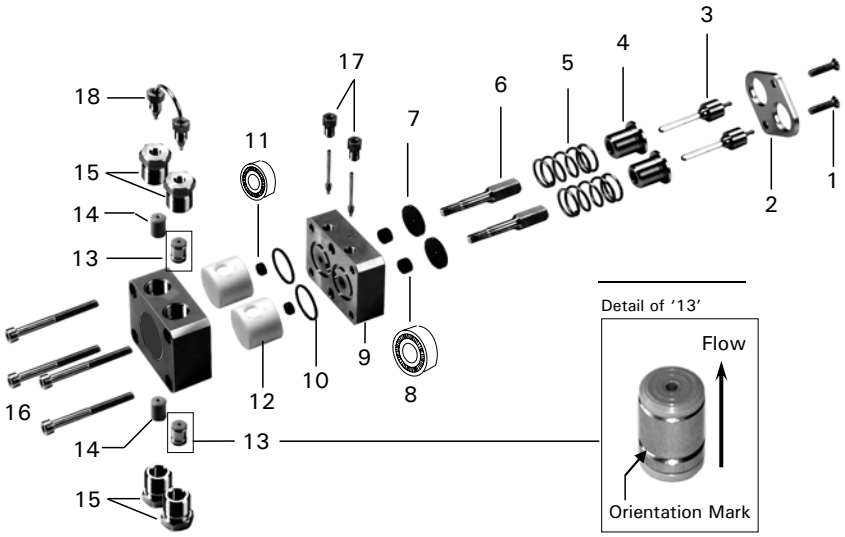
c) Tighten the two other screws.

d) Check that the screws that were tightened in step c) are well tightened.

6.3.4 Disassembling the Pump Head/Replacing Piston Seals

To disassemble the inert pump head (titanium inlays):

a) Remove the pump head and the piston rods (Section 6.3.3).



Item	Description	P/N	
-	Inert Pump Head	161399	
1	Recessed-Head Screw	-	
2	Retaining Plate	-	
3	Piston Rod	160047	
4	Guide for Spring	-	
5	Spring	-	
6	Spacing Bolt	-	
7	Support Washer	-	
8	Piston Seal (backflushing)	161020 [1]	
9	Pressure Plate	-	
10	O-Ring	160084	
11	Piston Seal (high pressure)	161020 [1]	
12	Titanium Inlay	161400	
13	Check Valve Cartridge	160049	
14	PEEK Adapter	162300	
15	Check Valve Bushing	161071	
16	Set Screw	-	
17	Backflushing Connection	-	
18	Connecting Tubing Pump Head	162010 [2]	Stainless steel
		161023 [3]	Titanium

Notes: 1) Set of 4 piston seals (includes two high pressure seals and two backflushing seals)
2) Used with the standard (stainless steel) version of the Switchos only.
3) Used with the inert version of the Switchos only.

FIGURE 6-5. The Inert Pump Head Assembly

b) Loosen the two screws that attach the retaining plate to the pump head (items 1, 2; FIGURE 6-5). As you remove the screws, alternate from one to the other as long as the springs are loaded to avoid damaging the retaining plate.

Note: Since the springs (item 5; FIGURE 6-5) exert a significant amount of force, care should be taken when the screws are loosened.

c) Remove the spring guides, the springs and the support washers (items 4, 5, 7; FIGURE 6-5).

d) You can remove/replace the piston seals (backflushing seals) now. Use an appropriate tool (e.g. a small screwdriver) to remove the seals (item 8; FIGURE 6-5).

e) Loosen the spacing bolts (item 6; FIGURE 6-5). Remove the two spacing bolts and then remove the pressure plate (item 9; FIGURE 6-5).

f) You can remove/replace the piston seals (high pressure seals) now. Use an appropriate tool (e.g. a small screwdriver) to remove the seals (item 11; FIGURE 6-5).

Note: The seals should only be replaced when the pump leaks. The useful lifetime depends on the mobile phase to be pumped and the back pressure of the system. The typical lifetime of the seals is in the order of 2500 h. It is recommended that both seals be replaced if one is defective.

g) To remove the titanium inlays, remove the four bushings, the check valves and the PEEK adapters (items 13, 14, 15; FIGURE 6-5).

h) Carefully insert new seals. The spring side should be placed as indicated in FIGURE 6-5.

To re-assemble the pump head:

a) Insert the titanium inlays, the two check valve cartridges (item 13; FIGURE 6-5) and the two PEEK adapters (item 14; FIGURE 6-5). As you replace the check valves, make certain that they are inserted in the right position.

CAUTION **Caution: Make certain that the orientation mark of the check valve cartridges is placed so that the mark is on the bottom. Installing the check valve cartridges in the wrong direction may lead to a damage of a cartridge or the pump head.**

b) Alternating from one to the next tighten the upper and the lower bushing half a turn at a time by hand.

c) Replace the pressure plate with the 2 o-rings (items 9, 10; FIGURE 6-5) and the two spacing bolts (items 9, 10; FIGURE 6-5). Alternating from one to the next tighten the two bolts by hand.

d) Tighten the two bolts, and then tighten the 4 bushings. Make certain that all screws are securely tightened.

6.3.5 Replacing the Check Valve Cartridge

The inert pump head is equipped with two check valves (item 13; FIGURE 6-5), one on the inlet side of the main piston (lower right) and on the outlet of the main piston (upper right) while two PEEK adapters are used on the auxiliary piston (lower and upper left side).

To remove/clean the check valves cartridge(s):

a) Unscrew the two check valve bushings (items 15, FIGURE 6-5) and remove the check valve cartridges.

b) As you replace the check valves, make certain that they are inserted in the right position (item 13; FIGURE 6-5). Tighten the two check valve bushings by hand.

c) Alternating from one to the next, tighten the upper and the lower bushing half a turn at a time.

d) Tighten the two check valve bushings.

Caution: Make certain that the orientation mark on each check valve is placed so that the mark is on the bottom. Installing the check valves in the wrong direction may lead to a damage of the pump head.

6.3.6 Removing the Side Panels

The side panels must be removed to gain access to remove the loading pump and to access the Solvent Selection Valve.

To remove the panels:

a) Remove three screws on the back of each side panel (items 1-6, FIGURE 6-6).

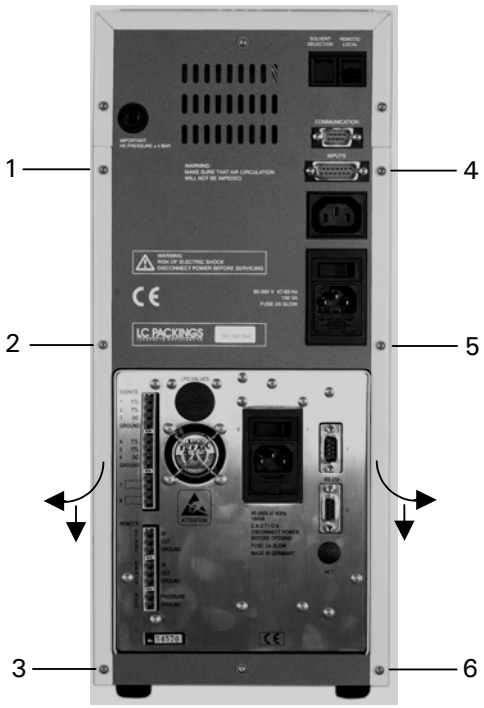


FIGURE 6-6. Rear View of Switchos

b) Remove the side panels.

6.3.7 Removing the Loading Pump

To remove the Micropump:

a) Disconnect all cables on the rear panel of loading pump.

b) Remove the capillary inlet and outlet connections from the loading pump.

c) Remove the side panels of the Switchos (Section 6.3.6).

d) Remove the two screws on the left side and the two screws on the right of the loading pump.

e) Remove the loading pump from the front of the Switchos.

Caution: When removing the loading pump from the Switchos, carefully pull the devices out of the housing. To avoid any damage to the component, make certain that it is not allowed to drop as it is being removed from the housing.

Note: When re-installing the instrument, take care that the mobile phase tubing is not bent or overly flexed.

6.3.8 Cleaning the 10-Port Switching Valve

Cleaning the 10-port valve can often be accomplished by flushing all the lines with an appropriate solvent(s). The selection of the solvent is dependent on the nature of the sample and the mobile phases that are used. Solvents such as methanol, acetonitrile, methanol/water (80/20) or acetonitrile/water (80/20) are normally used to clean the valve. See **Appendix A** for instructions about disassembling/assembling, cleaning and maintenance of the switching valve.

6.3.9 Replacing the Main Fuse

Danger: Disconnect the instrument from the mains before inspecting/changing the fuse.

To change the Fuse:

a) Pull out the fuse holder (item 1, FIGURE 6-7).

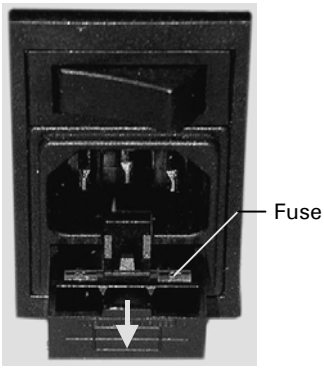


FIGURE 6-7. The Fuse Compartment

b) Replace the blown fuse by a fuse of identical rating (2 A, Slo Blo fuse).

c) Re-install the fuse holder.

6.4 Troubleshooting

Troubleshooting refers to the determination of the cause of a problem. Since the Switchos Advanced Microcolumn Switching Unit is incorporated into an HPLC system, the first step to determine if the problem is due to this instrument is to remove the unit from the system, then perform an injection and compare the results from the two runs. If the results are fine, the problem is due to the switching unit.

In addition, the typical system parameters (e.g. flow rates and pressure) of a pre-concentration setup with trap column and separation column are presented in TABLE 6-3

TABLE 6-1 Troubleshooting the Loading Pump

Problem	Probable Cause	Solution
Flow delivery stops after 1 minute during purging, error message "M**inimum pressure** ..." is displayed	System pressure dropped below the minimum pressure (Pmin) set in SETUP menu PRESSURE LIMITS	Set "min" value to 0 MPa to disable checking
Flow delivery stops during purging, error message „M**aximum pressure exceeded nn MPa**" is displayed	System pressure exceeded the maximum pressure (Pmax) set in SETUP menu PRESSURE LIMITS **or** in monitor screen	Open the purge valve knob
	Note: The pressure during purging is limited to 0.5 MPa.	
No solvent delivery, pump motor is running	Air in solvent/pump head	Purge pump and check degassing
	Clogged solvent inlet filter	Replace solvent inlet filter
	Leakage in the pump head	Check for any leakage
Leaking pump head	Loose inlet/ outlet fitting	Tighten the fittings
	Loose check valve(s)	Tighten the check valves
	Defective piston seals	Replace seals
Flow fluctuation	Flow restriction in the solvent inlet fluid path	Check tubing, filters, proportioning valves, etc.
	Air in solvent inlet fluid path	Purge pump, check degassing
	Defective valves	Clean/replace check valves
Flow rate too high or too low (deviation >10%)	Air in solvent inlet fluid path	Purge pump, check degassing
	Pump out of calibration	Contact LC Packings

TABLE 6-2 Troubleshooting the Switching Valves

The Switchos does not respond to any signal applied to the INPUTS connector	The Switchos is in LOCAL mode, e.g. the LEDs in the push buttons are illuminated	Change control mode to REMOTE (REMOTE/LOCAL switch on the rear panel)
The switching valves do not respond when pressing the controls (push buttons on front/rear panel)	The Switchos is in REMOTE control mode, e.g. the LEDs in the push buttons are not illuminated	Change control mode to LOCAL (REMOTE/LOCAL switch on the rear panel)
The Solvent Selection valve moves to the next solvent channel, and after a short moment it moves back to channel A (REMOTE control mode)	The signal applied to the SSV input has been active for longer 1.5 sec	Apply a shorter signal (< 1.5 seconds)
The valve A (B) switches to position 1-2 as soon as the instrument is set into REMOTE control mode	At the present time there is no active input signal and the valve is forced into position 1-2	Change the setting of the instrument controlling Switchos according to the needs of the application
LEDs 1-2 and 10-1 of Valve A (B) are switched off or switched on at the same time	The Switching Valve A (B) does not switch...	Switch OFF the instrument and ON again to reset the error
	due to valve sticking, ...	Clean Valve
	clamp ring slippage,..	Tighten clamp ring
	other positioning problems	Contact LC Packings
LED A-D illuminated at the same time	The Solvent Selection Valve does not switch...	Switch OFF the instrument and ON again to reset the error
	due to valve sticking, ...	Contact LC Packings
	clamp ring slippage,..	Contact LC Packings
	other positioning problem	Contact LC Packings

Note: It is important to note that many analytical problems are be caused by external influences such as the temperature and or the analysis of light sensitive samples and are not instrument related. When troubleshooting, make certain that the application was running in an acceptable fashion before the problems were observed and that nothing has been changed in the application. If you are making a change in the protocol, change a single parameter at a time and ensure that the effect of each change is well understood.

TABLE 6-3 Typical System Parameters of a Pre-concentration Setup

Parameter	Typical values (a)	
	CAP Configuration	NAN Configuration
Micropump Flow (Master flow)	200 µL/min	187.5 µL/min
Micropump Pressure (Master pressure)	160 – 200 bar	160 – 200 bar
CRP (b)	50	625
Column Flow	4 µL/min	300 nL/min
Column Pressure	60 – 80 bar	100 – 130 bar
Trap Column Flow	30 µL /min	30 µL /min
Trap Column Pressure	40 – 70 bar	40 – 70 bar
Connecting Tubing I.D. (c)	50 µm	20 µm
Response (delay) Time 'Injection to injection peak' (d)	2 -5 min	2 – 5 min
He pressure	1 bar	1 bar

Notes:
a) 100 % mobile phase A (start condition of the UltiMate standard system checkout procedure with the cytochrome c test sample).
b) No flow sensor for automatic CRP calibration used.
c) Used in the micro flow path.
d) If a trap column is used in conjunction with the Switchos, this is the time after switching the trap column into the micro flow.

6.5 Spare Parts Lists

Note: Please refer to the 'User's Manual UltiMate Micropump' for the lists of spare parts for the Micropump.

6.5.1 Major Items

Part Number	Description
163662 [a] 160170 [b]	Switchos™ Microcolumn Switching Module, consists of 1x loading pump, 2x 10-port valves and 1x solvent selection
163663 [a] 160559 [b]	Switchos™ Microcolumn Switching Module, INERT Version, consists of 1x loading pump, 2x 10-port valves and 1x solvent selection valve
Notes:	a) Instrument equipped with a blue LC Display b) Instrument equipped with a green/yellow LC Display

6.5.2 Tubing, Fittings and Bottles

Part Number	Description
160180	130 µm I.D. x 50 cm PEEK tubing, set of 2 pieces
160181	130 µm I.D. x 100 cm PEEK tubing, set of 2 pieces
160174	250 ml (safety) solvent bottle
160175	Caps for solvent bottles, set of 6 pieces
162015	Bottle cap assembly
160183	3 meter Helium supply tubing, 6 mm O.D.
161470	T-piece for Helium tubing
160483	Capillary cutter
160173	1/16" universal fingertight fitting, one-piece design, extra long body,4 pieces
160492	PEEK sleeves, precision cut and polished for connections with fused silica tubing (300 µm O.D.) set of 5 pieces
160266	Set of 20 nuts and ferrules for Valco switching valves
161000	PEEK Fingertight nuts and ferrules for inert injection/switching valves, set of 10
161007	PEEK Hex nuts and ferrules for inert injection/switching valves, set of 10
163915	PEEK ferrules with groove, for inert valves (20 pcs)
	Capillary LC – Standard Version
161479	50 µm I.D. Connecting tubing, Switchos to UltiMate injection valve, 70 cm length
161480	50 µm I.D. Connecting tubing, Switchos to UltiMate column compartment, length 50 cm, includes union
160182	30 µm I.D. PEEK shielded fused silica connection tubing µ-Precolumn™/Valve
	Nano LC – Standard Version
160178	20 µm I.D. Connecting tubing, Switchos to UltiMate injection valve, length 65 cm
160179	20 µm I.D. Connecting tubing, Switchos to UltiMate column compartment, length 50 cm, includes union
160182	30 µm I.D. PEEK shielded fused silica connection tubing µ-Precolumn™/Valve
	Capillary LC – Inert Version
161261	50 µm I.D. Connecting tubing, Switchos to UltiMate injection valve, 70 cm length
161262	50 µm I.D. Connecting tubing, Switchos to UltiMate column compartment, length 50 cm, includes union
160182	30 µm I.D. PEEK shielded fused silica connection tubing µ-

	Precolumn™/Valve
	Nano LC – Inert Version
161259	20 µm I.D. Connecting tubing, Switchos to UltiMate injection valve, length 20 cm – inert version
161260	20 µm I.D. Connecting tubing, Switchos to UltiMate column compartment, length 50 cm, includes union – inert version
160182	30 µm I.D. PEEK shielded fused silica connection tubing µ-Precolumn™/Valve

6.5.3 10-port valve

Part Number	Description
	Standard Version (Stainless Steel)
161472	Replacement rotor for VALCO C2, 10-Port
162016	Stator VALCO C2, 10-Port
	Inert Version (PAEK)
161005	Replacement rotor for VALCO C2, 10-Port valve – inert version
161006	PAEK Stator VALCO C2, 10-Port valve, inert version
Note:	**PAEK = Polyaryletherketone, PEEK = Polyetheretherketone**

6.5.4 Filters/Tools

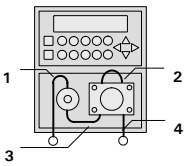
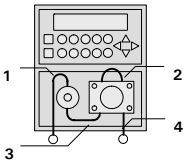
Part Number	Description
160044	He Sparging/Filter Frit
160073	Socket driver 11mm, for In-line filter replacement
160259	Syringe adapter for Valco switching valves
	Standard Version
160177	0.5 µm replacement in-line filter frit
161001	Frit Holder
	Inert Version
161107	0.5 µm replacement in-line filter frit – inert version
162014	Frit Holder, Titanium – inert version

6.5.5 Trap Columns and Column Accessories

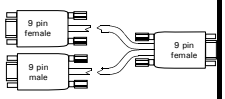
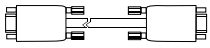
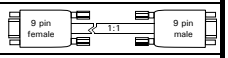
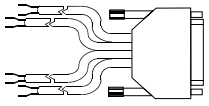
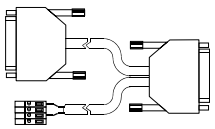
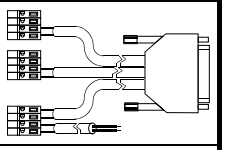
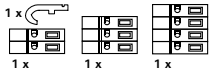
Part Number	Description
160461	Nano trap column holder, 1mm
160431	Micro trap column holder, 5mm
160432	Micro trap column holder, 15mm
	Micro Trap Columns
160454	300 µm I.D. x 5 mm, packed with 5 µm C18, 100Å, PepMap™, set of 5 cartridges
160446	500 µm I.D. x 5 mm, packed with 5 µm C18, 100Å, PepMap™, set of 5 cartridges
160450	500 µm I.D. x 15 mm, packed with 5 µm C18, 100Å, PepMap™, set of 5 cartridges
	Nano Trap Columns
160458	300 µm I.D. x 1 mm, packed with 5 µm C18 100Å PepMap™, set of 5 cartridges
Note:	Please refer to our web-site for more detailed information on stationary phases and column dimensions available (www.lcpackings.com).

6.5.6 Pump Head and Tubing - Inert Pump Head (Titanium Inlays)

Part Number	Description	
161399	Inert Pump Head (Titanium Inlays)	
160049	Check Valve Cartridge (1 piece)	
160050	Check Valve Cartridges (set of 5)	

161020	Piston Seals [Graphite Fibre Filled PTFE], 1/4" O.D. and 0.315" O.D. (set of 4)	
160047	Sapphire Piston	
161400	Titanium Inlay for the Inert Pump Head	
162300	PEEK Adapter for inert Pump Head	
160084	O-Ring, 17 mm x 1.5 mm	
161026	Torque Wrench	
	Connecting Tubing for the Switchos - Inert Version	
161024	Connecting Tubing Pressure Sensor to High Pressure Filter, Titanium [1]	
161023	Connecting Tubing for Pump Head, Titanium [2]	
161022	Connecting Tubing Pump Outlet to Pressure Sensor, Titanium [3]	
160053	PEEK Connecting Tubing Pump Inlet [4]	
	Connecting Tubing for the Switchos - Standard Version	
160056	Connecting Tubing Pressure Sensor to High Pressure Filter, Stainless Steel [1]	
162010	Connecting Tubing for Pump Head, Stainless Steel [2]	
162009	Connecting Tubing Pump Outlet to Pressure Sensor, Stainless Steel [3]	
160053	PEEK Connecting Tubing Pump Inlet [4]	
Note:	The 'Inert' and the 'Standard' system use the same type of pump head, but different connecting tubing.	

6.5.7 Control Cables

Part Number	Description	
160069	Y-Communication cable	
160070	Serial Communication Cable	
160071	Solvent Organizer Communication Cable	
160147	Contact Closure (4x) Cable for FAMOS P5	
160171	Cable for controlling *Switchos* by FAMOS P5 Auxiliaries	
160172	Cable for controlling *Switchos* by *UltiMate* Event Outputs	
160184	Set of Connectors (WAGO Type)	

CHAPTER 7

Specifications

7.1 Analytical Specifications

7.1.1 10-Port Switching Valves

Type	low dispersion 10-port valve, 2x		
Max. Pressure	350 bar (5000 psi)		
Wetted Surfaces		standard version	inert version
	stator:	stainless steel	PAEK
	rotor:	Valcon H	Valcon E
Flow Passage Diameter	0.15 mm		
Switching time	< 100 ms		
Operation	local control (push button), remote control (relay, open collector or TTL)		
Position Indicator	two LEDs per valve		

7.1.2 Solvent Selection Valve

Wetted Surfaces	stator: PPS (polyphenylene sulfide) rotor: Valcon E2
Switching Time	< 165 ms (channel to channel)
Position Indicator	four LEDs

7.1.3 Loading Pump

Pump Type	reciprocating double piston pump
Flow Rate Range	10 - 500 µL/min
Flow Rate Accuracy	< 0.5%
Pressure Range	0 - 400 bar
Pressure Monitoring	indication of µ-column pressure
Rapid Purge Valve	yes
Instrument Control	CHROMELEON® software or standalone

7.2 General

7.2.1 Physical

Dimensions (WxDxH)	185 mm (7.3 in) x 350 mm (13.8 in) x 430 mm (16.9 in).
Weight	16 kg (35 lbs.).

7.2.2 Electrical

Power Requirements	90 - 260 V, 47 - 62 Hz, 100 VA
Fuse	2x Slow-Blow, 2A
INPUTS (VALVE A, VALVE B, SSV)	Input resistance: 10 kΩ max. Voltage 'Input inactive' 0.5 V min. Voltage 'Input' active 2.0 V absolute maximum ratings: min. voltage: -0.3 V max. voltage: 5,3 V
	Note: Maximum ratings are the extreme limits the input pin can be exposed to without causing permanent damage. Do NOT connect voltages lower/higher than those limits.

7.2.3 Communication

Switching Valve A and B	by RS-232 serial communication or by relay, open collector or TTL outputs (e.g., from FAMOS or *UltiMate* Micropump)
Solvent Selection valve	by RS-232 serial communication or by relay, open collector or TTL outputs (e.g., from FAMOS or *UltiMate* Micropump)
Loading Pump	by RS-232 serial communication network

7.3 Options

Inert Version (factory installed)	all fluidic pathways made out of inert materials, i.e. PAEK, PEEK, PTFE, ceramic, titanium
Flow Passage Diameter	0.25 mm

APPENDIX A

Switching Valves

A.1 Overview

The LC Packings Switchos II Advanced Microcolumn Switching Unit is equipped with two Cheminert® Model C2 10-Port Column Switching Valves manufactured by VICI™ Valco Instruments Co. Inc. These valves are designed to offer optimal results with the switching system as they are made from materials that are inert to materials used in liquid chromatography and are designed to provide exceedingly low carryover when switched.

Note: A detailed discussion on the Installation, Use and Maintenance of the valve is presented in Technical Note 801 from VICI Valco Instruments, Co. Inc. and can be obtained at the Valco website (www.Valco.com)

A.2 Maintenance

In most instances, the only maintenance that is required is cleaning of the valve. Cleaning can often be accomplished by flushing all the lines with an appropriate solvent(s). The selection of the solvent is dependent on the nature of the sample and the mobile phases that are used. Typically solvents such as methanol, acetonitrile, methanol/water (80/20) or acetonitrile/water (80/20) should be used.

A.3 Disassembly/Reassembly of the Valve

Note: Do not disassemble the valve unless system malfunction is definitely isolated to the valve.

A.3.1 Disassembly of the Valve

To disassemble the valve:

a) Use a 9/64" hex driver to remove the socket head screws which secure the cap to the valve (FIGURE A-1)

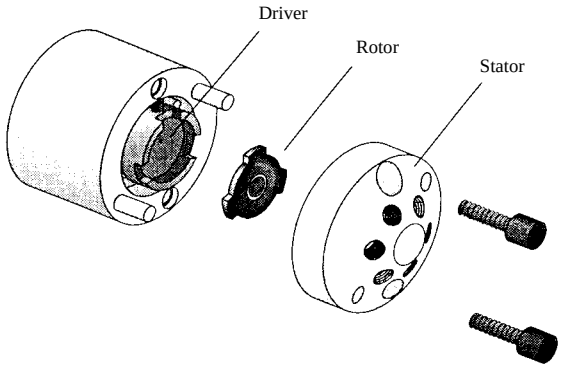


FIGURE A-1. Exploded View of the Valco Model C2 Valve (6-port Version)

b) To insure that the sealing surface of the cap is not damaged, rest it on its outer face. If the tubing is still attached, leave it suspended by the tubing.

c) Gently pry the rotor away from the driver with your fingers or a small screwdriver.

d) Examine the rotor sealing surface for scratches.

- If scratches are visible to the naked eye, the rotor must be replaced.
- If no scratches are visible, clean all parts thoroughly with an appropriate solvent. Take care that no surfaces get scratched while you are cleaning the components.

Note: The most common problem in the use of the valve with HPLC is the formation of buffer crystals, which are usually water-soluble. After cleaning, it is not necessary to dry the rotor.

A.3.2 Reassembly of the Valve

To reassemble the valve:

a) Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The pattern is asymmetrical to prevent improper placement.

b) Replace the cap. Insert the two socket head screws and tighten them gently until both are snug.

CAUTION **Caution: Do not overtighten the screws-they simply hold the assembly together and do not affect the sealing force, which is automatically set as the screws close the cap against the valve body.**

c) Test the valve by pressurizing the system. If the valve does not hold pressure it should be returned for repair.

Note: When re-installing the valve, make certain that the proper tubing is attached to the appropriate fitting.

A.4 Spare Parts

Part Number	Description	Figure
	Standard Version (Stainless Steel)	
161472	Replacement rotor for VALCO C2, 10-Port	
162016	Stator VALCO C2, 10-Port	
	Inert Version (PAEK)	
161005	Rotor for Switchos™, 10-port injection valve (VALCO C2), INERT	
161006	PAEK Stator for SWITCHOS™ 10-port valve (VALCO C2), INERT	
Note:	PAEK = Polyaryletherketone	

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APPENDIX B

Network Identification

B.1 Identifying the Switchos on the Network

The Device Identifier and Serial Number of the Switchos Advanced Microcolumn Switching Unit must be correctly set to ensure proper operation of the unit on the network.

The Device Identifier and Serial Number is factory set during the manufacturing process. It can be checked in the GLP Menu.

If it becomes necessary to reset the Device Identifier and/or the Serial Number, the following rules must be used:

- The Device Identifier for the Switchos must be 91.
- The serial number must contain six digits. If the serial number of the Switchos loading pump contains only 5 digits, add a leading zero (e.g. 72835 should be entered as 072835).

In Table B-1, we indicate the identification of various devices, including a Switchos, on a network.

TABLE B-1 Identification of a Various Devices on a Network

Device	Device Identifier		Serial Number (lower 6 digits)					
	8	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Switchos Loading Pump	**9**	**1**	0*	7	2	8	3	5
UltiMate Micropump	-	-	-	3	1	8	0	4
UltiMate UV Detector	-	-	-	4	8	1	9	0

"-" left blank
* " 0" in case the S/N has less than 6 digits

Note: If the Device Identifier or Serial Number for the Switchos is incorrectly set, the unit may not be able to communicate with other devices on the network (depending on the control software package).

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APPENDIX C

Cleanup of Proteins

C.1 Overview

This appendix describes a common application of the Switchos II™ Advanced Micro Switching Unit and is provided to give the user an understanding of the power and flexibility of the system. In this application the two 10-port switching valves are used for on-line sample clean-up, digestion of proteins and peptide separation.

An ion-exchange column and protein digestion column are connected to valve A, and a reversed phase trap column and the capillary/nano separation column are connected to valve B as shown in FIGURE C-1.

The following steps are involved in the process:

a) Proteins are injected and trapped onto the ion-exchange cartridge.

b) Valve A is placed in position 10-1 and non-charged compounds, e.g. detergents, are flushed to waste.

c) Valve A is switched back into position 1-2. The ion-exchange column is now in series with a protein digestion column and the reversed phase trap column, mounted on valve B (position 10-1). Proteins are eluted by injection of a salt solution. The peptides are concentrated onto the reversed phase trap column.

d) Valve B is switched back from position 10-1 the 1-2 to place the trap column and separation column in series. The gradient is started to elute the peptides from the trap column. The Peptides are separated on the capillary/nano column.

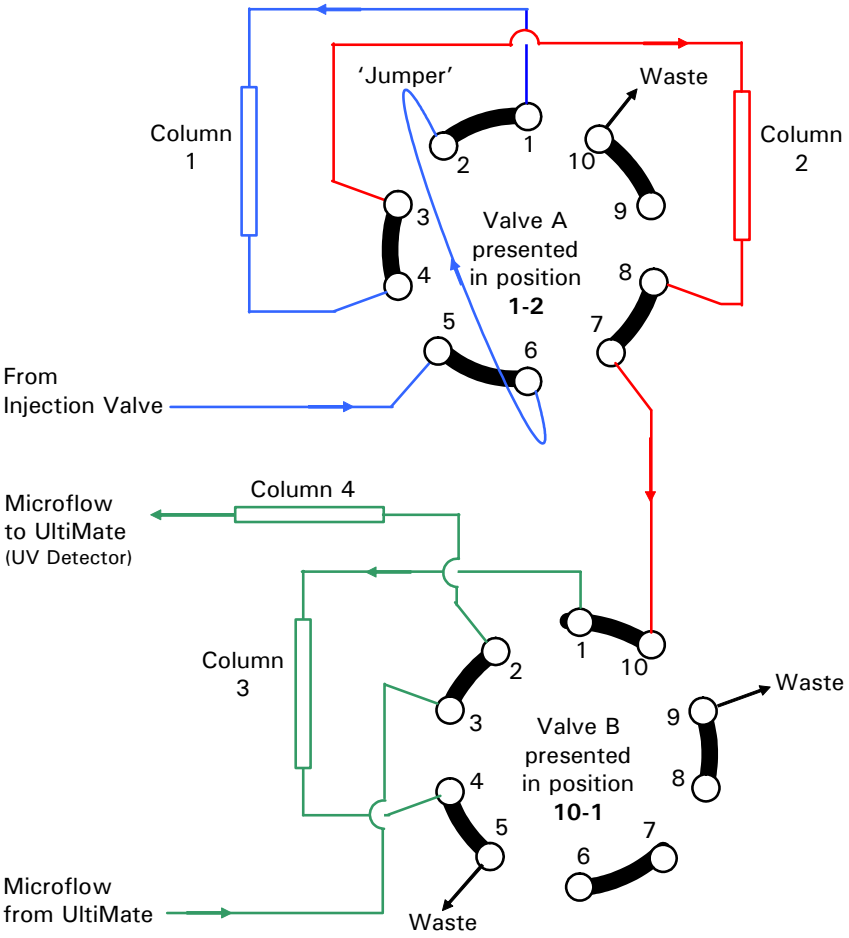


FIGURE C-1 Fluidic Setup of the two 10-Port Valves

TABLE 1 Description of the Columns

Column No.	Column Type
1	Ion-exchange
2	Protein Digestion
3	Trap Column
4	Separation Column

APPENDIX D

Connecting Fused Silica Capillary

D.1 Overview

This appendix provides instructions how to connect a NANO column or other fused silica capillary to the inert (PAEK) switching valves of the Switchos II Advanced Microcolumn Switching Unit. These directions should be used whenever a NANO column or a fused silica capillary is to be connected to the inert (PAEK) switching valves of the Switchos II.

CAUTION

Caution: Do not use a stainless steel nut and/or ferrule with inert (PAEK) injection/switching valves. The use of stainless steel nuts or ferrules may damage the valve. Use only the supplied fittings (PEEK) and follow the instructions below.

The PEEK sleeve connection is created using the components shown in FIGURE D-1:

- PEEK fingertight nut,
- PEEK ferrule,
- PEEK sleeve (with an appropriate I.D.),
- NANO column (or any other fused silica capillary)
- The female fitting port of the inert valve (FIGURE D-1 shows the assembly tool required for the assembling purpose only).

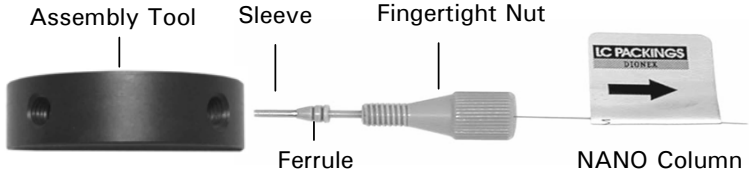


FIGURE D-1 Parts of a PEEK Sleeve Connection

D.2 Fitting Assembly

CAUTION

Caution: Never use the inert (PAEK) injection/switching valve to pre-assemble a fitting and never use any tool to tighten the fingertight nut. This may lead to damage of the valve.

To assemble the PEEK sleeve fitting (using the supplied stainless steel union):

a) Slide the fingertight nut and the ferrule onto the sleeve as shown in FIGURE D-1.

b) Insert this assembly into one of the fitting ports of the supplied assembly tool (P/N160142), screwing the nut in two or three turns by hand.

c) Push the sleeve and the Nano column (capillary) all the way forward into the fitting port that the sleeve and the capillary seats firmly.

Note: It is essential that the sleeve and the NANO column (capillary) are inserted all the way forward into the union for a proper zero dead volume connection!

d) Manually turn the nut into the union until it is finger tight.

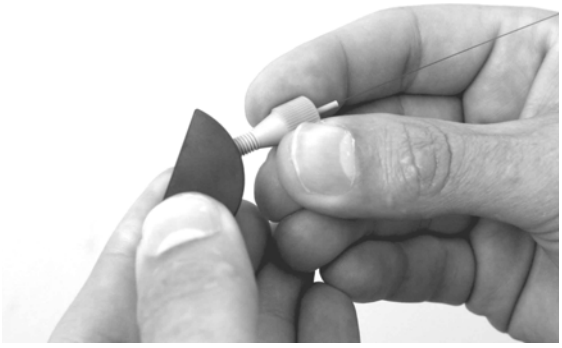


FIGURE D-2 Attaching a PEEK Sleeve Connector to a NANO Column

e) Carefully turn the nut 1/4 turn (90°) past the point where the ferrule starts to grab the sleeve (FIGURE D-2).

Note: The amount of force to turn the nut can vary considerably due to the friction between the nut and the threads and as well as the composition and wall thickness of the tubing used. Because of these variables, we do not provide a torque specification.

f) Remove the fitting and inspect it. When you pull gently at the NANO column (capillary), you should not be able to move the column (capillary) in the sleeve (e.g. it should be grabbed by the sleeve, FIGURE D-3). If the sleeve moves laterally, reinstall the fitting into the fitting port and tighten it another 1/8 turn past finger tight.

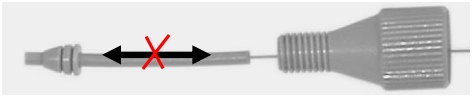


FIGURE D-3 PEEK Sleeve Connector attached to a Nano Column

g) Remove, re-inspect, and repeat, if necessary.

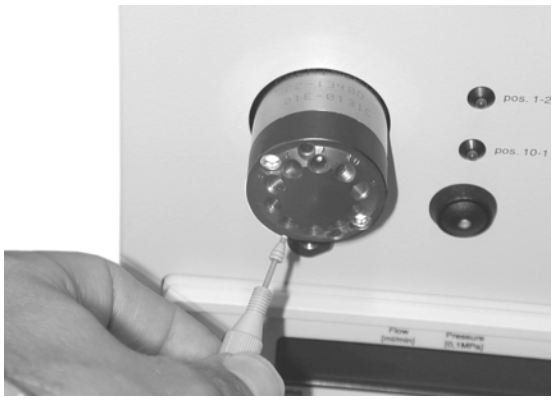


FIGURE D-4 Installing the Pre-assembled Fitting

h) Install the pre-assembled fitting in the inert (PAEK) injection/switching valve and manually turn the nut into the valve until it is finger tight (FIGURE D-4).

D.3 Using Long PEEK Hex Style Nuts

In case the supplied fingertight fittings cannot be used due to limited space, use the long PEEK hex style nuts (P/N 161007) supplied with the instruments instead (FIGURE D-5).

FIGURE D-5 The Long PEEK Hex Style Nut

To use the supplied long hex nuts:

a) Assemble the fitting as described in Section D.2.

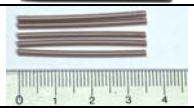
b) Install the pre-assembled fitting in the inert (PAEK) injection/switching valve and manually turn the nut into the inert (PAEK) valve until it is finger tight (FIGURE D-4).

c) Carefully tighten the hex style nut using a ¼" wrench.

CAUTION

Caution: Never use the inert (PAEK) injection/switching valves to pre-assemble a fitting and never use any tool to tighten the fingertight nut. This may lead to damage of the inert (PAEK) valve.

D.4 Spare Parts Lists

Part Number	Description	Figure
160142	Tool for pre-assembling of INERT fittings	
160492	PEEK sleeves, precision cut and polished for connections with fused silica tubing (300 µm O.D.), set of 5 (b)	
161007	PEEK 1/16" Universal Fitting for Switchos, INERT, 10 pc., long hex nut and ferrule with groove	
160693	1/16" Universal Fingertight Fitting with double ferrule for Inert valve, 1 piece	
161000	1/16" Universal Fingertight Fitting with double ferrule for Inert valve, 10 each	
163915	PEEK ferrules with groove, for inert valves (20 pcs)	
Notes:	a) PAEK = Polyaryletherketone b) PEEK = Poly-etheretherketone	

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