Varian Analytical Instruments
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ProStar 370 Electrochemical Detector

Operation Manual

QUALITY SYSTEM ISO 9001 CERTIFIED

03-914792-00:Rev. 1

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Introduction

General

Congratulations on your purchase of the ProStar 370. This advanced, integrated electrochemical detector enables you to perform all applications using electrochemical detection. The ProStar 370 includes a highly stable Faraday-shielded oven compartment accommodating column and flow cell. This flow cell has surprised researchers for its unsurpassed S/N ratio and therefore you now possess the best possible combination for extremely sensitive EC analyses.

The ProStar 370 covers the DC, pulse and scan mode. Important parameters in the DC and pulse mode can be changed on a time base by user-defined commands, which enables maximum control to fully automate the detection. In addition, crucial parameters can be controlled by either relays or TTL (open collector type).

Installation

Unpacking

Inspect the transport box for possible damage as it arrives. Immediately inform the transport company in case of damage, otherwise they may not accept any responsibility. Keep the transport box as it is designed for optimum protection during transport and it may be needed again. Carefully unpack the system and inspect it for completeness and for possible damage. Contact your supplier in case of damage or if not all marked items on the checklist are included.

Prior to shipment, your detector has been thoroughly inspected and tested to meet the highest possible demands. The results of all tests are included in the shipkit.

Please carefully follow the next steps for a successful installation and start-up.

1. To unpack the ProStar 370, lift it from its box by both hands (Figure 1). **Never lift the ProStar 370 at its front door**, but at its sides

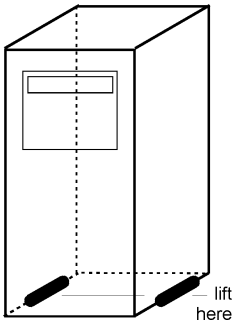
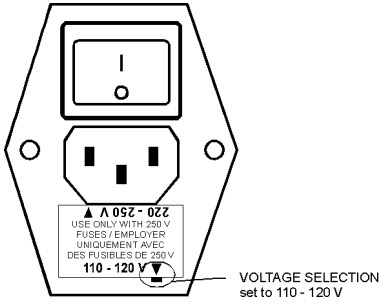


Figure 1 Lift instructions ProStar 370

2. Inspect the detector for possible damage and make sure that all marked (and ordered) items on the checklist are included.

3. Check whether the voltage selector is in the correct position, i.e., identical with the local mains voltage. If the triangle with the voltage range is pointing towards the small white block, the system is set to the correct mains voltage. If this is not the case, the voltage selector has to be reversed. Use only a supply appliance with protective grounding. The fuses are included in the line connector.

⚠ WARNING - RISK OF FIRE REPLACE FUSE AS MARKED		
	FUSE RATING	
INPUT VOLTS	UL / CSA	IEC 127
100-120 V 200-240 V	3.2A 250V TL 1.6A 250V TL	T 3.2A 250V T 1.6A 250V



The correct fuse values are given on the rear panel:

- For 110 V (AC) ± 10%, use two 3.2 AT fuses (slow, ¼" x 1¼ ", UL/CSA).
- For 220 V (AC) ± 10%, use two 1.6 AT-fuses (slow, 5 x 20 mm, IEC127).

4. If all the above is correct, switch ON the ProStar 370 by the mains switch on the rear panel.

HPLC connections

Use proper eye and skin protection when working with solvents.

The installation of the flow cell and column is shown in Figure 2.

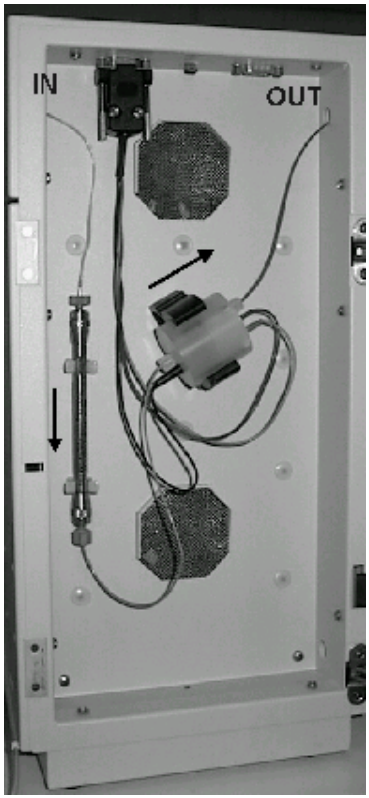


Figure 2 Installation of the ProStar 370

5. If the Rheodyne 7725i or another Rheodyne ‘i’ version injector is applied, the sensor cable must be connected to ‘manual valve’ on the rear panel to enable INJECT/LOAD functions.

6. If a VALCO (or VICI) electrically actuated valve is mounted, connect the ‘digital input/output cable’ to ‘electric valve’ on the rear panel to enable INJECT/LOAD functions.

7. Prior to connection of the HPLC system to the detector all metal parts should preferably be passivated with 15% nitric acid during 20 min. **Make sure that all parts that are not acid-resistant such as: nylon inlet filters, column and flow cell are *not* connected during this step.** The acid is flushed through the pump, the pump tubing, the dampener, the injector (in load and inject position) and to waste.

WARNING:
CHEMICAL HAZARD

8. After flushing with nitric acid, the system must be thoroughly flushed with demi water. Make sure that no traces of nitric acid are left in the tubing or pulse dampener (check with pH paper). Flush the system with HPLC buffer.
Make sure that the buffer contains 2 mmole/l chloride (KCl or NaCl) ions, which is required for the in situ Ag/AgCl reference electrode (ISAAC).

9. Before connecting a new column read the manufacturer’s instructions. Our experience is that thorough pre-conditioning of a column is always required. Only a pre-conditioned column is electrochemically clean. If not, the background current may be unacceptably high and substantial contamination of the working electrode occurs. For *reversed phase* columns flushing with 50% methanol in water for 3 days at a low flow rate is highly recommended. Before switching to mobile phase, flushing with water (10 column volumes) is recommended to prevent precipitation of buffer salts.

10. Passage of air bubbles through the flow cell will lead to unacceptable noise levels and ‘spikes’. Therefore, the use of an in-line degasser is strongly recommended. In our experience, a one-time degassing step of the HPLC buffer is almost never sufficient.

11. If the ProStar 370 is used for reductive ECD (at a negative working potential) additional steps should be taken to remove oxygen from the mobile phase. These include degassing with Helium and the use of stainless steel tubing (impermeable for oxygen).

12. The flow cell is assembled properly when it arrives. The force on the bolts is pre-set to 30 Ncm (“a little bit beyond fingertight”). Familiarize yourself with this force, since over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself. Also, be aware that the black marks on both blocks should be in line. This ensures the best performance.

NOTE: **If a flow cell is used different from the standard configuration with ISAAC reference electrode, please read information beginning on page 86.**

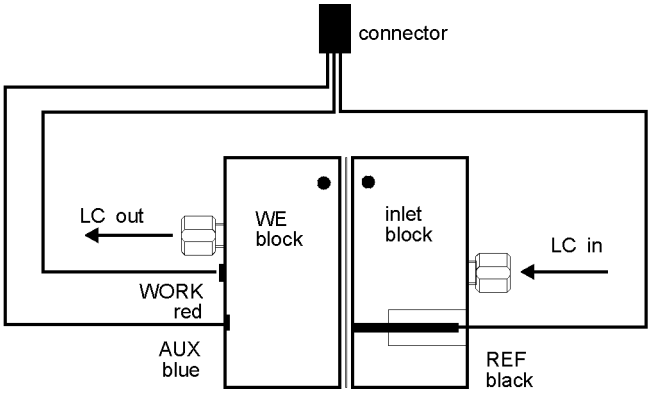
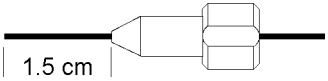


Figure 3 Installation of flow cell. WORK, AUX and REF are connected using the red, blue and black cell cable. LC out should be on top to prevent entrapment of bubbles.

NOTE:	**If an ISAAC reference electrode is applied:** **The ISAAC reference electrode requires 2 mmole/l chloride ions (KCl or NaCL) in the mobile phase. Add and equilibrate before installation of the ISAAC. See page 57 for optimization of working potential.**

13. Connect the column outlet to the flow cell inlet, using small-bore PEEK tubing (< 0.3 mm ID) and one of the fingertights supplied. Use only our factory supplied fingertights in the flow cell, others may cause serious damage! Let the tubing protrude for ca. 1.5 cm from the fingertight fitting and tighten it such that the tubing is not or slightly indented by the fitting.



14. Do not over-tighten the fingertight. Over-tightening affects the flow pattern through the tubing (turbulence) and may strongly decrease the flow cell performance.

15. Connect 0.5 mm ID PEEK tubing to the outlet of the flow cell. Use only our factory supplied fingertights in the flow cell, others may cause serious damage! Again (see above), do not over-tighten the fingertight.

16. Turn on the HPLC pump. Keep some tissues at hand as you probably will spill some mobile phase during this mounting procedure.

WARNING:
CHEMICAL HAZARD

17. Fill the flow cell, by keeping it in an angle of about 45° with the outlet (LC out) on top to force the air through the outlet.

18. Position the flow cell in its clamp in the controller with the REF at the lower side and the outlet at the upper side. This excludes trapping of air bubbles.

19. Connect the cell cable as illustrated in Figure 3.

Never switch ON the flow cell when:

- **the cell cable is not correctly connected**
- **the cell is only partly (or not at all) filled with buffer**
- **the outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection**

because substantial damage to the working electrode or electronics may occur.

20. Before switching on the flow cell, make sure that the buffer contains sufficient electrolyte (buffer ions). A stable baseline will never be obtained if the cell is switched on with only water or another non-conducting mobile phase. Also be sure that no air bubbles are trapped in the flow cell.

21. The outlet tubing from the flow cell should lead to a reservoir that is at a higher level than the flow cell. This ensures a minute back pressure which prevents air-bubble entrapment. The outlet tubing should be under the liquid level, to avoid 'dripping' noise.

22. Set the cell potential (see page 57 for optimization of the potential), switch on the flow cell (see page 13) and allow the system to stabilize for approximately 30 min. A 'good' stabilization curve shows a mono-exponential decline without jumps and/or spikes.

23. Connect the data system to the integrator (digital) or recorder (analogue) output (see page 24).

24. Your system is now ready for use.

25. The ProStar 370 has been developed for continuous operation. For maximum stability it is advised to leave the system ON continuously. If preferred, the flow cell may be switched off at night.

Chromatographic test

After stabilization the system is ready for operation. A sample and mobile phase which can be used for a first run is included in the performance kit. Table 2 lists performance criteria which the system should meet and these should be used as an indication of proper installation of the system.

1. Establish chromatographic conditions as described in Table 1 and allow approximately 30 minutes for stabilization.

2. Inject 20 µL of the standard and compare the results with Figure 4 and Table 2.

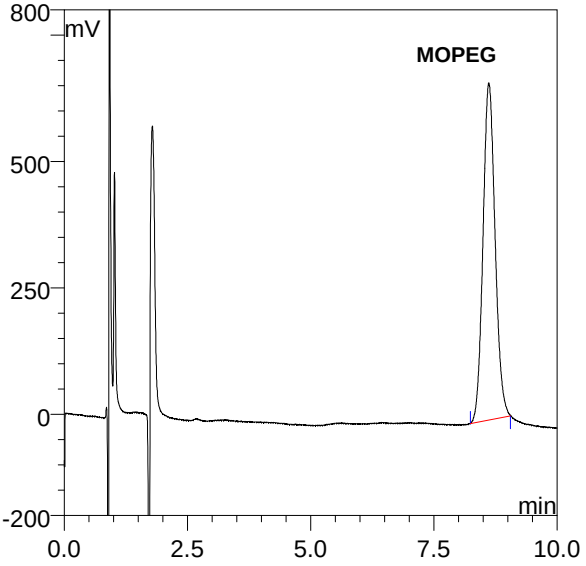


Figure 4 Analysis of 0.5 µmol/L MOPEG. Chromatogram is obtained using a 150 x 4.6 mm column and a flow rate of 1.5 ml/min.

Table 1 Chromatographic conditions.

Detector	ProStar 370
Output	INT, nA-range (100 nA full scale)
Column	Varian RES ELUT C18, 150 x 4.6 mm, 5 µm
Flow rate	1.5 mL/min
Mobile phase	0.1 mM EDTA + 50 mM acetic acid + 2 mM KCl, set at pH 4.5 using NaOH
Sample	20 µl injection of 0.5µmol/l MOPEG (dissolved in mobile phase)
Temperature	30 ^{o}C
Flowcell	Flow cell with 2 mm GC working electrode and 50 µm gasket
E-cell	+650 mV (vs. ISAAC 2 mM KCl in mobile phase) or +800 mV (vs. salt bridge Ag/AgCl in saturated KCl)

Table 2 LC-EC criteria.

Parameter	**Criterion**
background current (I-cell), after 1 hour stabilisation	< 10 nA
peak-to-peak noise of the baseline (pulse dampener installed)	< 20 pA
peak height MOPEG 0.5 µmol/l	> 1.5 nA

System Description

The ProStar 370 controller

The ProStar 370 has been designed for maximum functionality and ease of use. The control of ECD parameters is such that without reading this chapter, it should be possible to operate the detector. This chapter is intended as a reference guide in case questions arise during operation.

The information shown in the numerous screens is presented in alphabetical order. For each item an explanation is given, together with the item's nature and the screen(s) of appearance. The nature of an item can be:

- Control: parameters with a cursor box ('☐') can be attained via cursor buttons and changed by the value buttons.
- Status: without a cursor box a parameter reflects the current status.
- Functions: parameters in capitals are commands accessible via function buttons f1 - f5.

The 'Enter' button is only used to accept changes in cell potential. In the top right corner of each screen the name of the present screen is displayed. If available, the bottom left function button displays a previous screen, and the bottom right one the next screen.

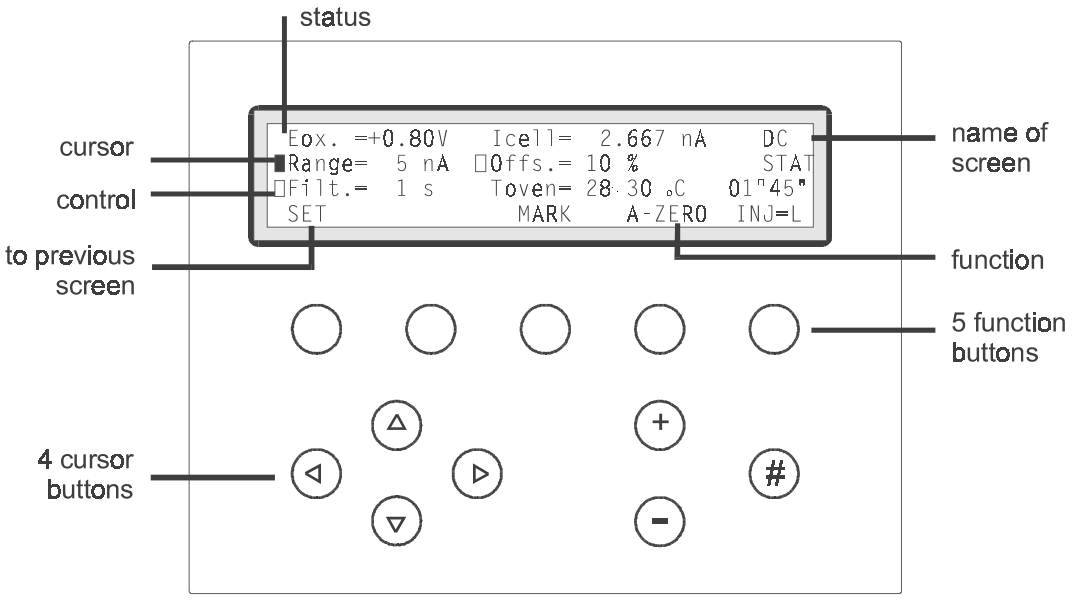
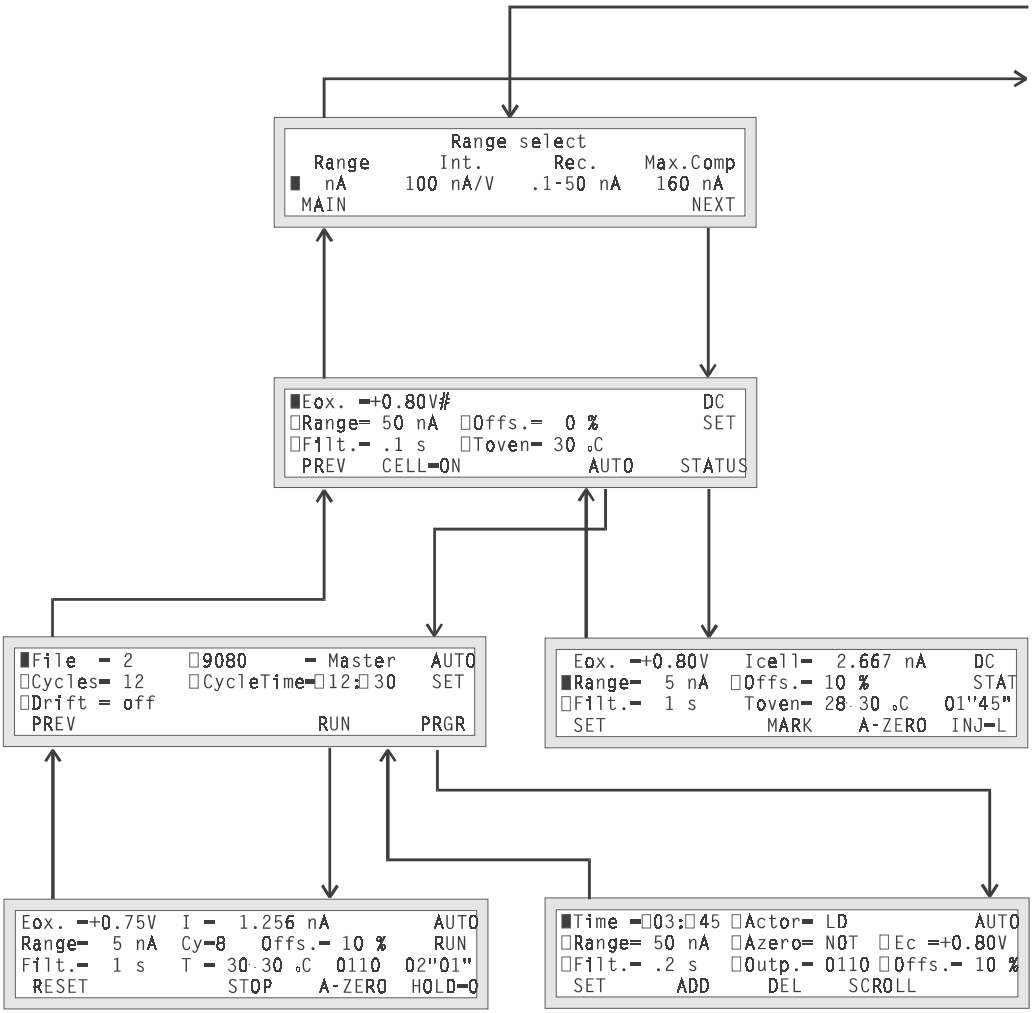
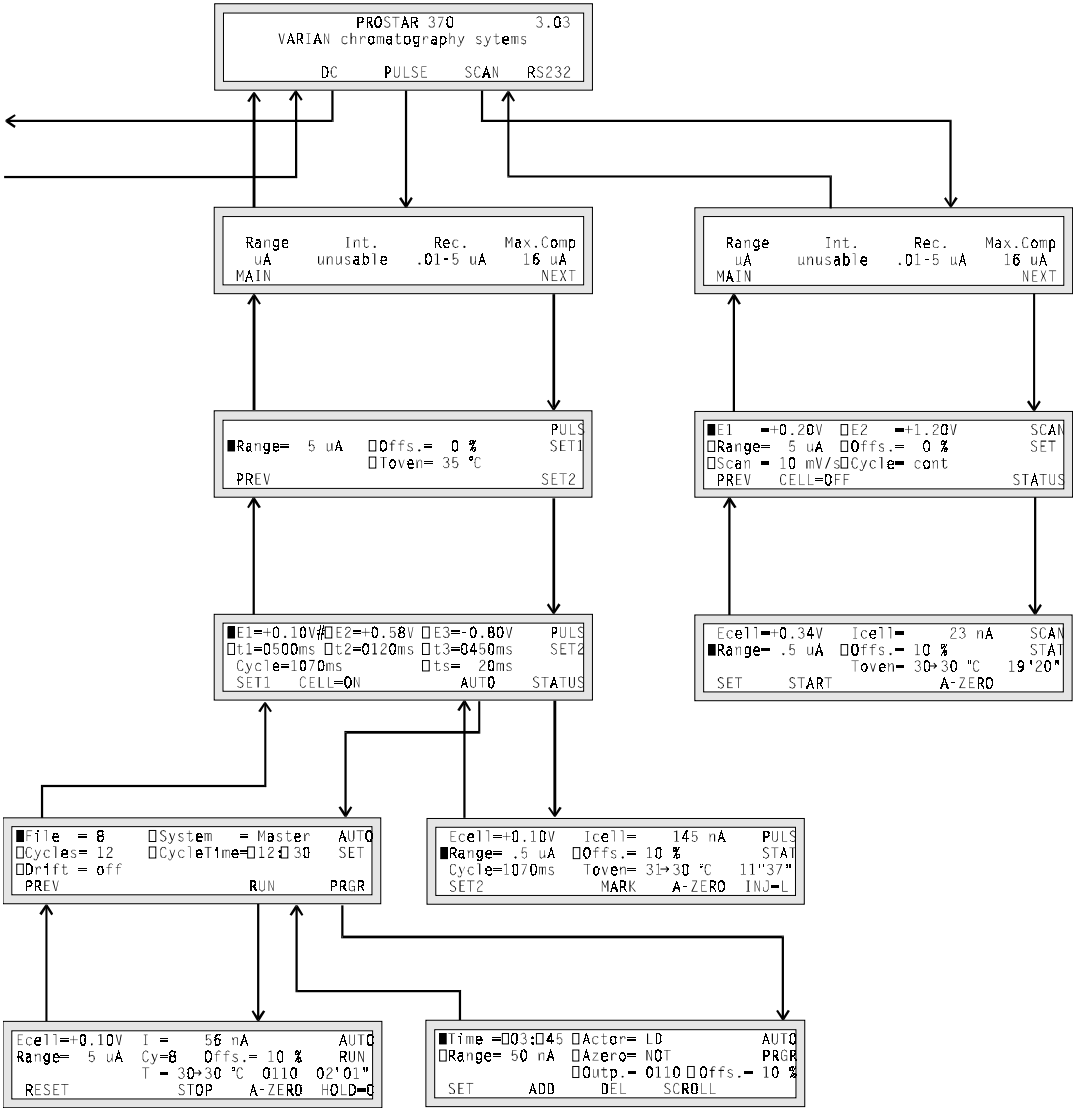


Figure 5 ProStar 370 keyboard.

The cursor is on 'Range' which allows changes using the value buttons. The Enter button is only used to confirm changes in potential (Eox or Ered)

Overview of all ProStar 370 screens




PROSTAR 370 3.03
VARIAN chromatography sytems
DC PULSE SCAN RS232
Range Int. Rec. Max.Comp
uA unusable .01-5 uA 16 uA
MAIN NEXT
Range Int. Rec. Max.Comp
uA unusable .01-5 uA 16 uA
MAIN NEXT
PULS
Range= 5 uA Offs.= 0 % SET1
Toven= 35 °C
PREV SET2
E1 =+0.20V E2 =+1.20V SCAN
Range= 5 uA Offs.= 0 % SET
Scan = 10 mV/s Cycle= cont
PREV CELL=OFF STATUS
E1=+0.10V# E2=+0.58V E3=-0.80V PULS
t1=0500ms t2=0120ms t3=0450ms SET2
Cycle=1070ms ts= 20ms
SET1 CELL=ON AUTO STATUS
Ecell=+0.34V Icell= 23 nA SCAN
Range= .5 uA Offs.= 10 % STAT
Toven= 30→30 °C 19'20"
SET START A-ZERO
File = 8 System = Master AUTO
Cycles= 12 CycleTime=12:30 SET
Drift = off
PREV RUN PRGR
Ecell=+0.10V Icell= 145 nA PULS
Range= .5 uA Offs.= 10 % STAT
Cycle=1070ms Toven= 31→30 °C 11"37"
SET2 MARK A-ZERO INJ-L
Ecell=+0.10V I = 56 nA AUTO
Range= 5 uA Cy=8 Offs.= 10 % RUN
T = 30→30 °C 0110 02'01"
RESET STOP A-ZERO HOLD=0
Time =03:45 Actor= LD AUTO
Range= 50 nA Azero= NOT PRGR
Outp.= 0110 Offs.= 10 %
SET ADD DEL SCROLL

Parameters

Actor

(AUTO) PRGR	control

Controls the electrically actuated injector (VALCO option), during execution of a timefile. Forces this valve to load (‘LD’) or inject (‘INJ’) (see page 33).

ADD

(AUTO) PRGR	function

Adds the current data line to the timefile. Confirmation is asked for if an existing time is overwritten. As time 00’ 00” always exists, changing this time results in an overwrite warning (see page 33).

AUTO

DC SET, PULS SET2	function

Enters the auto mode (‘AUTO SET’ screen) for editing and running a timefile.

A-ZERO

DC STAT, AUTO RUN, PULS STAT, SCAN STAT	function

Sets the recorder and integrator output voltage to 0 V, or to the offset voltage (see page 24).

Azero

(AUTO) PRGR	control

Controls the autozero, which can be programmed in a timefile (see page 33).

CELL=ON/OFF

DC SET, PULS SET2, SCAN SET	function

Toggles between cell ‘ON’ and ‘OFF’. If the cell is switched ON in the pulse mode only E1 is applied, the pulsation starts when PULS STAT screen is entered (see page 47). In the DC or scan mode, Eox./Ered. or Ecell is applied.
Pulse mode: pulsation occurs as long as the cell is on, irrespective which screen is selected.

Cy

AUTO RUN	status

Displays the cycle counter. In the master mode, if a timefile has to be executed more than once (‘Cycles’>1), this is the number of times a timefile is started (see page 33).

Cycle

PULS SET2, PULS STAT	status

Displays the total duration of one pulse (t1 + t2 + t3). As from each cycle only one data point is sent to the recorder output, this duration determines the frequency of the recorder output (see page 47).

Cycle

SCAN SET	control

Controls the nature of the cycle: half, full and continuous. ‘Half’ means that the cell potential runs from E1 to E2 and stops at E2. ‘Full’ means that the cell potential runs from E1 to E2, and back to E1, and then stops. ‘Cont’ means that the cell potential runs from E1 to E2 and back to E1 continuously (see page 57).

Cycles

AUTO SET	control (master)

Controls the number of times a timefile has to be repeated. If the ProStar 370 is master this number can be 1 - 99 or continuous. If the ProStar 370 is slave the number of cycles is 1 (see page 33).

CycleTime

AUTO SET	control

Controls the duration of a timefile (max. 999’ 59”). When this time is reached the execution of the timefile stops. If programmed, the next run is started (see page 33).

DEL

(AUTO) PRGR	function

Deletes the current data line from the timefile. Confirmation is asked for. Time 00’ 00” can not be deleted (see page 33).

Drift

AUTO SET	control

Controls the driftmonitor in the auto mode, which intermits the execution of a timefile when the baseline is drifting. The available drift monitor settings depend on the range selected at time 00:00 in the timefile (see page 33). Can be set between 0.1 pA/min and 10 nA/min.

E1, E2, E3

PULS SET2	control

Controls the cell potential settings of the pulse. A change in E1 has to be confirmed with the ‘Enter’-button (see page 47).

Ec

(AUTO) PRGR (DC only)	control

Controls the cell potential in a timefile (see page 33).

Ecell

PULS STAT, AUTO RUN (Pulse only), SCAN STAT	status

Displays the cell potential during ‘t1’ in the pulse mode (see page 47).
Displays the actual cell potential in the scan mode (see page 57).

Eox. or Ered.

DC SET	control

Controls the oxidative or reductive cell potential. Can only be set or changed after confirmation with the ‘Enter’ button. Passing the 0.00 V value automatically changes the readout from ‘ox’ to ‘red’ and in reverse.

Eox. or Ered.

DC STAT, AUTO RUN (DC only)	status

Displays the oxidative or reductive cell potential.

File

AUTO SET	control

Controls the number of the timefile to be edited or activated. In the DC mode file numbers 1 - 5 are available, in the pulse mode file numbers 6 - 9 can be selected. The timefiles remain stored in RAM, also after switching off the ProStar 370 (see page 33).

Filt.

DC SET, DC STAT, (AUTO) PRGR (DC ONLY)	control

Controls the rise time filter settings for the recorder output, which are: 0.1, 0.2, 0.5, 1, 2 or 5 seconds (see page 24).

HOLD=0/1

AUTO RUN, SCAN STAT	function

Interrupts the execution of a timefile or scan. Toggles between 1 and 0. Pressing the hold button again continues the timefile or scan were it was stopped (see page 33).

Icell

DC STAT, PULS STAT, SCAN STAT	status

Displays the true, non-compensated cell current, i.e. unaffected by zero or offset. The cell current is one of the most important parameters in ECD.

INJ=I/L

DC STAT, PULS STAT	function/status

Displays or switches the position of the injection valve, toggles between inject (I) and load (L). If a manual injector with position sensor (e.g. the Rheodyne 7725i) is applied, it echoes the position of the injector. If an electrically actuated injector is used (optional) it is possible to switch the injector with this function button.

MAIN

RANGE screen	function

Returns to the start-up screen.

MARK

DC STAT, PULS STAT	function

Triggers a marker signal on the recorder output.

NEXT

RANGE screen	function

Enters the next (xx SET) screen

Offs.

DC SET, DC STAT, (AUTO) PRGR, PULS SET1, PULS STAT, SCAN SET, SCAN STAT	control

Controls the percentage offset, can be set between -50 and 50%. The offset % on *both* outputs relates to the recorder range setting (see page 24).

Offs.

AUTO RUN	status

Displays the percentage offset during execution of a timefile.

Outp.

(AUTO) PRGR	control

Controls the four output functions in the auto mode. The notation of the output '0000' corresponds to the four output controls at the rear panel of the controller (from left to right: relay 2, relay 1, aux 2, aux 1). The position indicated on rear panel refers to the '0000' setting (see page 33).

PREV

DC SET, AUTO SET, PULS SET1, SCAN SET	function

Returns to previous screen.

PRGR

AUTO SET	function

Enters the (AUTO) PRGR screen

Range

DC SET, DC STAT, (AUTO) PRGR, PULS SET1, PULS STAT, SCAN SET, SCAN STAT	control

Controls the recorder range setting, varying from 10 pA to 5 µA full scale, divided in 1, 2 and 5 steps. In the DC mode, selectable recorder ranges depend on the pre-selection of the integration range in the 'Range select' screen. In the pulse and scan mode only the µA range can be used (see page 24).

RESET

AUTO RUN	function

Aborts the timefile and returns to the 'AUTO SET' screen. The cycle counter ('Cy') is reset to 1 (see page 33).
The outputs Aux 1 and 2, and Relays 1 and 2 are deactivated (status: 0000).

RUN

AUTO SET	function

Starts the execution of a timefile (master/slave). If the ProStar 370 is slave, the system waits for the 'START' input trigger to start the next run (see page 33).
In the slave mode the RUN command initialises the AUTO RUN screen, the system waits for the 'START' input trigger to start a timefile.

Scan

SCAN SET	control

Controls the scan speed, can be set from 1 - 50 mV/s in 1, 2, 5 steps (see page 57).

SCROLL

(AUTO) PRGR	function

Scrolls through a timefile (see page 33).

SET

DC STAT, AUTO RUN, PULS STAT, SCAN STAT)	function

Returns to the previous (xx SET) screen.

SET1, SET2

PULS SET2, PULS STAT	function

Returns to PULSE SET screen 1 or 2

START

AUTO RUN, SCAN STAT	function

In the DC and pulse mode (master if cycles >1): toggles between STOP and START to control the execution of a timefile.
In the scan mode START starts a scan (see page 57).

STATUS

DC SET, PULS SET2, SCAN SET	function

Enters the next (xx STAT) screen.

STOP

AUTO RUN, SCAN STAT	function

In the scan mode, STOP aborts the scan and resets the cell potential to E1.
In the DC and pulse mode (master, cycles >1): toggles between STOP and START to control the execution of a timefile. Pressing 'STOP' aborts this run, cycle counter (Cy) is not reset. By pressing the same button ('START') again, the next cycle is started (Cy = Cy +1) (see page 33).
STOP also deactivates the outputs Aux 1 and 2, and Relays 1 and 2 (status: 0000). This toggle is also active in the slave mode.

System

AUTO SET	control

Controls the status of the system, toggles between 'Master' and 'Slave'. If 'Master' other parts of the HPLC system can be controlled by the ProStar 370 (see page 33).

t1, t2

PULS SET2	control

Controls the duration of a corresponding step in the pulse mode. The time can be set between 100 and 2000 ms in 10 ms increments (see page 47).

t3

PULS SET2	control

Controls the duration of a corresponding step in the pulse mode. The time can be set between 0 (=off) and 2000 ms in 10 ms increments (see page 47).

Time

(AUTO) PRGR	control

Controls the time to execute a data line in a timefile, can be set with one second resolution. Maximum time is 999 min and 59 s. The time to stop the execution of a timefile must be programmed by the CycleTime in the AUTO SET screen (see page 33).

Toven

DC STAT, PULS STAT	status

Displays the actual (left value) and the pre-set oven temperature (right value, set in 'xxSET').

Toven

DC SET, PULS SET1	control

Controls the temperature of the oven. Range: off, 15 - 45°C, selectable in 1°C steps. The oven is stable from 5 °C above ambient.

ts

PULS SET2	control

Controls the duration of the sampling time in the pulse mode. The time can be set between 20 and 100 ms in 20 ms increments (see page 47).

Data acquisition

Introduction

One of the characteristics of electrochemical detection is its tremendous dynamic range. In amperometric detection peak heights may vary from micro-amperes down to the pico-ampere range. The ProStar 370 covers such a wide range from 5 µA down to 10 pA full scale, without being limited by electronic noise.

The ProStar 370 is equipped with two output connections for data acquisition, the recorder ('REC') and the integrator output ('INT'). The integrator output has been designed to supply a non-manipulated, analog signal from the I/E converter which is as close as practically possible to the working electrode (WE). The recorder signal is manipulated by the CPU of the ProStar 370. The details on data acquisition in the various ranges will be discussed for both output channels.

Internal organization

At the working electrode (WE) in the electrochemical flow cell the electron transfer takes place due to an oxidation or reduction reaction. The resulting electrical current is amplified by the current-potential (I/E) converter (Figure 6).

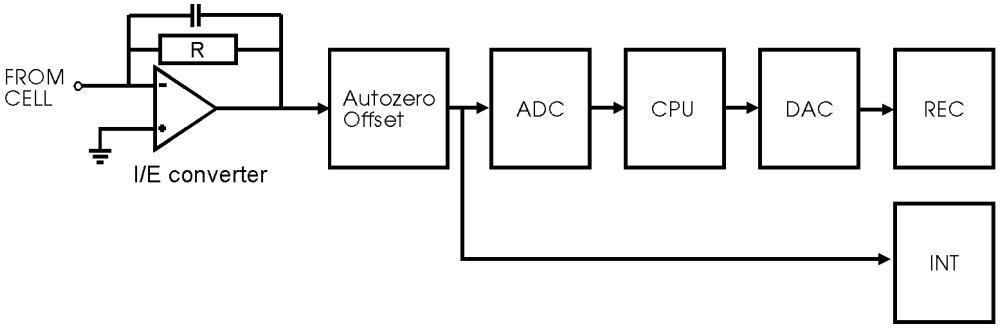


Figure 6 ProStar 370 signal processing from electrochemical flow cell to REC and INT output. R is a selectable resistor of 100, 10 or 0.1 MΩ, corresponding to the pA, nA and µA range respectively.

In the I/E converter a range of 10, 100 or 10000 nA full scale (FS) can be selected, which corresponds to the so-called pA, nA and the µA range, respectively. These are the three ProStar 370 integrator ranges that can be set in the 'Range select' screen.

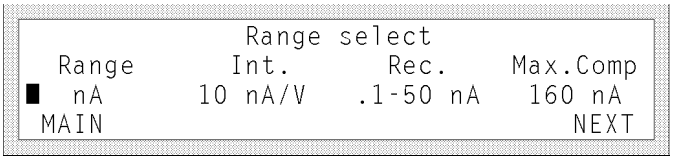


Figure 7 The ProStar 370 'Range select' screen. The 10 nA/V corresponds to 100 nA full scale on the integrator output (=10V).

The integrator range selection is the first step in the signal pathway (Figure 7). It affects both the integrator and the recorder output and has several important consequences for data acquisition (Table 3).

Table 3 Parameters affected by the integrator range setting.

Parameter	output	comment
signal output	INT/REC	gain increases with more sensitive range
Available REC ranges	REC	see Table 6
max. compensation	REC/INT	decreases with more sensitive range
I-cell display	screen	resolution increases with more sensitive range

The next step is the autozero and offset compensation. Then the signal is further manipulated for the recorder output or sent to the integrator output without manipulation.

Integrator output

The integrator output has been designed to supply a clean, purely analog signal from the I/E converter. Except for the autozero and offset compensation, no further manipulation of the signal occurs. If required, manipulation of a chromatogram (i.e. noise filtering) can be done by integration software. Thus a clean chromatogram will always be available for re-integration.

Table 4 Integrator range and maximum zero compensation.

Range	current, full scale	max. zero comp.	max. current
µA	10 µA	16 µA	26 µA
NA	100 nA	160 nA	260 nA
PA	10 nA	16 nA	26 nA

When data are obtained from the integrator output, it is obvious that the pA range allows the highest sensitivity. Each range has a different maximum zero compensation (Table 4). For example, in the nA range a current of 100 nA full scale can be managed. Together with a zero compensation of 160 nA, the maximum current that can be handled is 260 nA.

Due to the nature of data generation the integrator output is unusable in the pulse and scan mode.

Recorder output

Several steps occur before sending the signal to the recorder output. After analog-to-digital conversion (ADC), a number of recorder range settings can be selected in the central processing unit (CPU). This selection occurs in the ‘DC SET’ or ‘DC STAT’ screen.

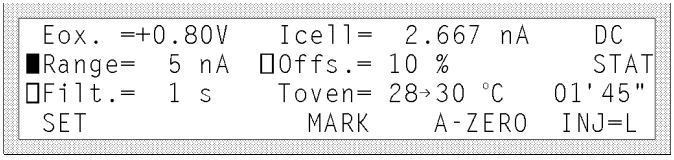


Figure 8 Selection of the recorder output range in the ‘DC SET’ or ‘DC STAT’ screen.

After digital to analog conversion (DAC) the signal is sent to the REC output. The recorder range selection has several consequences for the data acquisition (Table 5).

Table 5 Parameters affected by the recorder range setting.

Parameter	output	comment
Signal output	REC	gain increases with more sensitive range
Offset	REC/INT	see Table 7
Marker	REC	10% of full scale REC output

In the DC mode the signal can be smoothened by a rise time filter. In addition, more complex signal manipulation, needed for pulse and scan is possible. Although the name of this output suggests otherwise, it is often used in connection with an integrator.

The recorder output ranges between +1 and -1 V with 12 bits resolution, which means that the output resolution is 0.5 mV (2 V/212). The best way to exploit this output resolution is to set the range as sensitive as possible.

There is much overlap in the recorder range settings offering flexibility as to maximum zero compensation, Table 6. (For example, if a chromatogram is acquired at 10 nA, the nA or µA range can be selected (Table 6). With a high background current the µA is chosen (max. 16 µA compensation), at a low background the nA range is preferred (max. 160 nA compensation).

The resolution of the zero compensation is 16 bits per selected integrator range. At a high maximum compensation, the compensation circuit uses larger steps to reach a 0 V output, hence the resolution of the zero compensation decreases. Therefore, a compromise is made between maximum zero compensation and resolution. To maintain a high autozero resolution with accurate zero settings, the maximum compensation is decreased with the integrator range.

Table 6 Recorder ranges and maximum zero compensation.

Range			
PA	nA	µA	
10			
20			
50			
100	0.1		
200	0.2		
500	0.5		range
1000	1.0		overlap
2000	2.0		
5000	5.0		
	10.0	0.01	
	20.0	0.02	
	50.0	0.05	
		0.10	
		0.20	
		0.50	
		1.00	
		2.00	
		5.00	

Maximum zero compensation (nA)		
16	160	16000

In certain cases, when a small peak is ‘blown up’ with integration software, a typical stepwise (0.5 mV = 1 bit) peak profile is seen (Figure 9). In that case the recorder output resolution is not sufficient, and the detector should be set at a more sensitive recorder range.

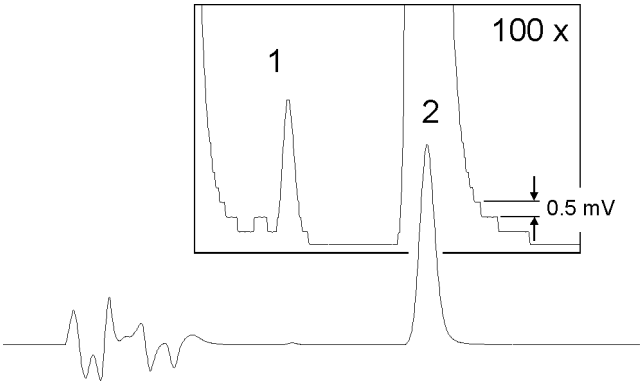


Figure 9 A 'stepwise' pattern after magnification of a chromatogram.

This phenomenon will not occur at the *integrator* output because this output is purely analog and therefore, in principle, with infinite resolution.

Integrator vs. recorder output

In the pulse and the scan mode, only the recorder output is suitable for data acquisition. In the DC mode both outputs can be used, in that case a choice has to be made.

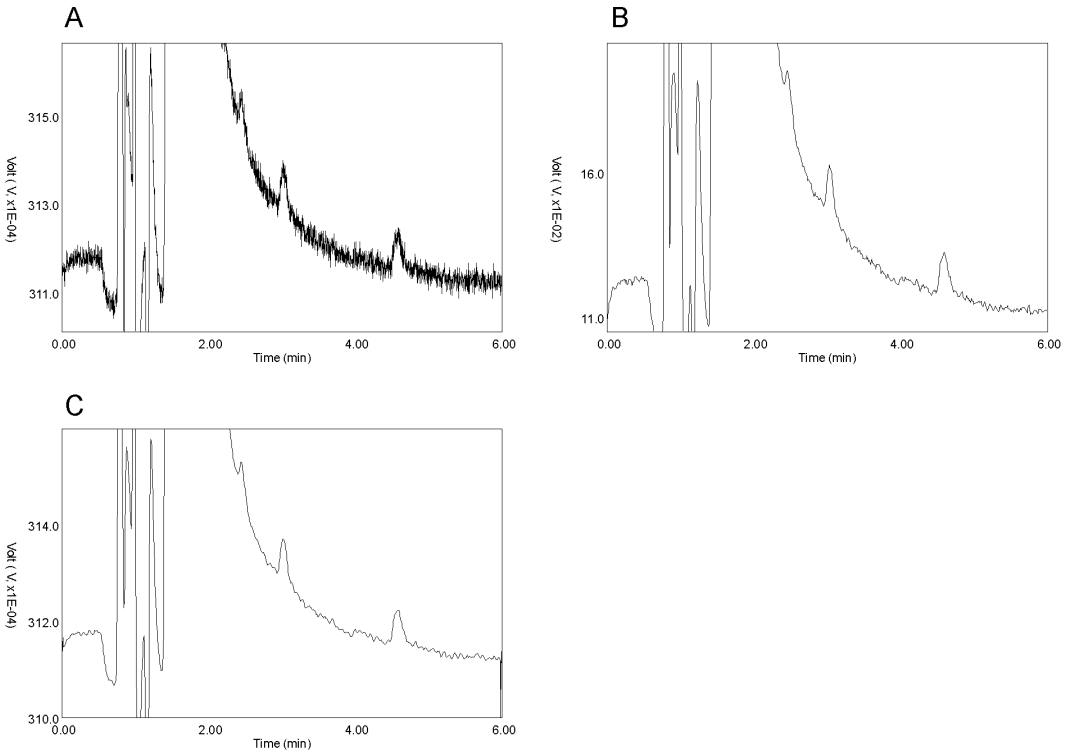


Figure 10 The clean signal from the integrator output (A) is smoothened by integrator software using a running average filtering (C). This results in a similar chromatogram as obtained from the recorder output using a rise time filter of 1 s (B).

A specific advantage of the integrator output is the large dynamic range and infinite resolution. It is an analog output which means that the resolution is only limited by the integrator that is used. In most integrators this is approximately 1 µV. The 12 bits recorder output has a maximum output resolution of 500 µV.

Often, the recorder output is chosen for data acquisition thus offering the possibility of smoothening by means of rise time filters (Figure 10). The advantage of having a clean signal from the integrator output is not always recognized, or the possibility for smoothening data is not implemented in the integration software.

Offset

A maximum offset of +50% and - 50% in 10% steps can be achieved, active on both the integrator and the recorder output. On the recorder output the offset is given as the percentage of the recorder range setting. For example, a 20% offset at 5.0 nA full scale setting will give a 1.0 nA offset. This is a 200 mV offset when the maximum recorder output is 1.0 Volt.

For the integrator output the situation is more complicated because the offset percentage relates to the recorder range setting. The integrator offset (in mV) is given Table 7 for a 10 V integrator output.

Table 7 Effect of recorder range on the offset of the 10 V integrator output.

Range			Offset (mV)				
PA	nA	µA	10%	20%	30%	40%	50%
10	0.1	0.01	1	2	3	4	5
20	0.2	0.02	2	4	6	8	10
50	0.5	0.05	5	10	15	20	25
100	1.0	0.10	10	20	30	40	50
200	2.0	0.20	20	40	60	80	100
500	5.0	0.50	50	100	150	200	250
1000	10.0	1.00	100	200	300	400	500
2000	20.0	2.00	200	400	600	800	1000
5000	50.0	5.00	500	1000	1500	2000	2500
1	10	1000	Integrator output (nA/V)				

Although this may seem a complex way of compensating the background current, in practice it gives the best possible flexibility and optimum resolution of the 16 bits compensation in each operating range. By simply changing the percentage offset or the recorder range, almost any offset compensation can be set between 1 and 2500 mV on the integrator output.

Operation

AUTO mode and timefiles

Introduction

The AUTO mode of the ProStar 370 enables a time-based, automated and full parametric control of electrochemical detection (ECD). This is particularly useful when during a run or between runs settings have to be changed such as the sensitivity, autozero or control of external equipment (i.e. trigger to start integration software etc.). A timefile contains a series of data lines in which the settings of the ProStar 370 can be changed with 1 second time resolution. A timefile is executed only in the AUTO mode.

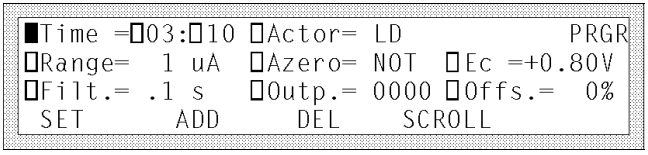


Figure 11 Programming a timefile using the '(AUTO) PRGR' screen.

The file is made using the ‘(AUTO) PRGR’ screen on the ProStar 370 (Figure 11). Programmable parameters comprise cell potential, range, autozero, offset, rise time, electrically actuated injector (if present) and the ProStar 370 output contacts to control the status of external equipment.

Range programming

In certain analyses large differences in analyte concentration (and peak height) occur. If the analyte with the highest

concentration is on scale, another analyte may be recorded at a low output resolution. When the small peak is magnified with integration software a typical stepwise peak profile is seen (Figure 9). In such a case a chromatogram can be obtained using a timefile to switch the sensitivity of the controller to another range during the run (Figure 12).

In an example is given of a timefile for sensitivity switching between 2 peaks. In this example the system is equipped with an automated VALCO valve, which means that programming the switching of the valve is possible (Figure 11, 'Actor'). At time 00:00 the valve is switched to 'inject', the trigger to start the integrator is connected to the 'INJECT MARKER'. At time 00:10 the valve is switched back to 'load' to enable continuous refilling of the injection loop. At time 1:26 the sensitivity is increased by a factor of 100 from 50 nA/V to 0.5 nA/V, together with an autozero command. After passage of the small peak, the range is switched back to 50 nA/V. At t=3:05 the end of the chromatographic run is programmed in the 'AUTO SET' screen, using the 'CycleTime' parameter.

If the integrator range is switched between µA, nA or pA, simultaneously with an autozero command this may result in an inappropriate baseline setting. Setting the range first is recommended in such case.

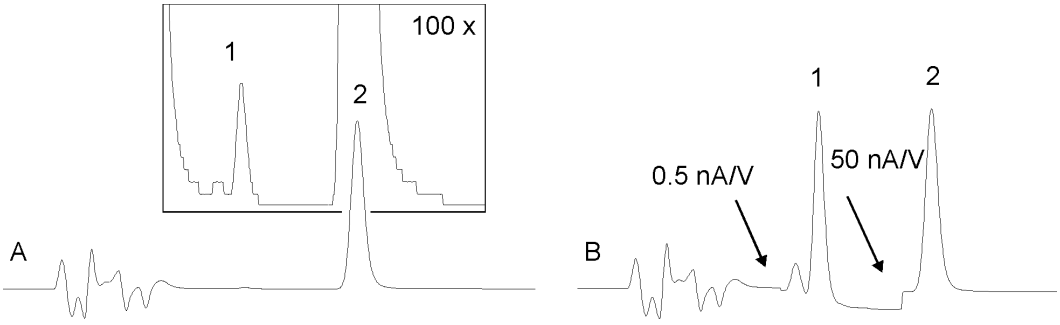


Figure 12 Example of range programming to maintain the recorder resolution. In chromatogram (A) a range setting of 50 nA/V is used, in chromatogram (B) the output range is automatically switched during the run resulting in a considerable improvement of the output resolution of peak 1.

Table 8 Timefile used for output resolution programming.

time	int. range	rec. range	rise time	valve	auto zero	offset	output	E cell
00:00	nA	50 nA	1 s	inject	set	00 %	0000	0.80 V
00:10	nA	50 nA	1 s	load	not	00 %	0000	0.80 V
01:26	nA	0.5 nA	1 s	load	set	00 %	0000	0.80 V
01:55	nA	50 nA	1 s	load	set	00 %	0000	0.80 V
03:05	Cycle Time (end of run)							

Drift monitor

The AUTO mode of the ProStar 370 is equipped with a drift monitor, which puts the detector on 'hold' when the baseline is drifting. The drift monitor is only active at the beginning of a timefile, both in the master and slave mode. After starting a timefile the baseline is monitored during one minute and the 'wait..' message is displayed. When the baseline is not within the pre-set limit, the drift monitor remains active. When the drifting baseline is within the pre-set limit the drift monitor is deactivated, and the run continues.

The drift monitor is useful when, for example, after each run a cleaning pulse is programmed to reduce contamination of the working electrode. A cleaning pulse consists of e.g. a 10 seconds potential step at -1V.

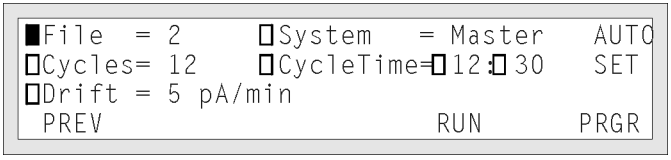


Figure 13 Programming the drift monitor in the 'AUTO SET' screen.

After this cleaning pulse it takes some time to get a stable baseline again. The drift monitor can be used to ensure a user-definable stable baseline before the start of the next run.

However, the duration of each run will always be at least one minute longer. In this minute the drift in the baseline is monitored.

Step by step programming of the AUTO mode (System = Master)

In this step-by-step guide the example from Table 8 will be programmed. In this example the system consists of an HPLC system, a ProStar 370 with VALCO option (automated injection valve), an integrator, and an on-line microdialysis set-up.

The ProStar 370 is master which means that the ProStar 370 starts a run, and controls the number of automated runs.

Hardware connections:

1. Connect the hardware trigger of the integrator to the 'Inject marker' on the rear panel of the ProStar 370 as indicated in in the figure below.

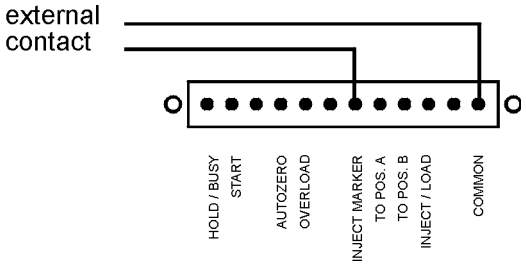


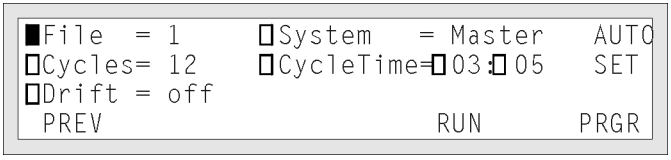
Figure 14 External contact to start the integrator by switching the automated VALCO valve.

2. Connect the dialysis tubing to the automated VALCO injection valve.
3. In this example the integrator is connected to the REC output at the rear panel of the ProStar 370.
4. Prepare the LC-EC system for analysis.

Programming the AUTO mode:

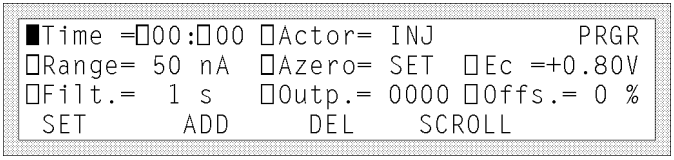
5. From the ProStar 370 start-up screen, choose DC, and in the ‘Range select’ choose NEXT. In the ‘DC SET’ screen, set the temperature at the desired value (or choose ‘off’) and choose AUTO. You are now in the ‘AUTO SET’ screen in the AUTO mode.

6. In the ‘AUTO SET’ screen select the timefile that you want to edit with ‘File’. When it is your first file, select nr. 1. There are 5 timefiles available in the DC mode (and 4 in the Pulse mode). The number of times you want to repeat this timefile is set with ‘Cycles’. Because the ProStar 370 will give the integrator and switching valve the starting signals, it is set at ‘Master’. The duration of one run is set with ‘CycleTime’. A step programmed at a time similar to or larger than the cycle time (i.e. t > 3:04), will not be executed!



Before the drift monitor can be set (if required), the first line in the timefile has to be programmed. The drift monitor is linked to the range setting of the first line in the timefile (at t = 0:00).

7. Press ‘PRGR’ to go into the edit mode of the timefile. The ‘PRGR’ screen appears:

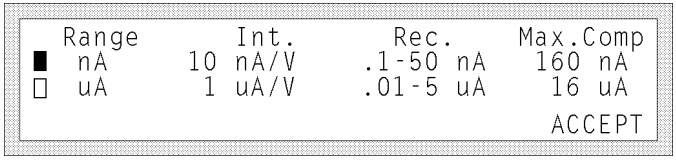


When all settings are correct, this line in the timefile can be saved by choosing ‘ADD’. When the time already exists, the question ‘Do you want to overwrite time?’ appears. As each

new timefile contains a t = 0:00, this question will always appear after editing this line.

In our example, after editing t=0:00 an additional screen will appear, asking you in which range you want to operate at the 50 nA/V setting. You may choose between the nA and the µA range. See ‘ Data acquisition’, page 24, for specific details on differences between both modes.

In our example the nA range is chosen, and confirmed with ‘ACCEPT’. The message ‘New time is saved’ is displayed.



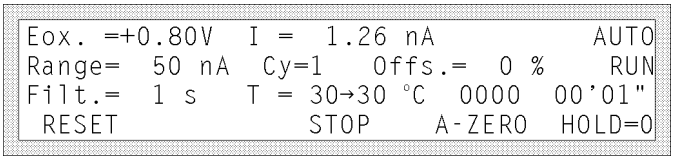
Choosing ‘SCROLL’ allows you to scroll through your timefile. With ‘DEL’ an existing line in your timefile can be deleted (after confirmation).

8. Step 7 is repeated for each line in the timefile. When a line is edited and saved, without changing the time, the original line is overwritten (after confirmation). When the time of an existing line is changed and saved, it is saved as a new line without overwriting the original line. Each timefile can contain a maximum of 20 data lines.

9. The last line in the timefile is at t = 1:55. By choosing ‘SET’ you may return to the ‘AUTO SET’ screen.

10. In the ‘AUTO SET’ screen ‘CycleTime’ is set at 3:05. If required, the drift monitor can be programmed. In the nA range a choice can be made between ‘off’, 1 pA/min - 0.1 nA/min in 1, 2, 5 steps.

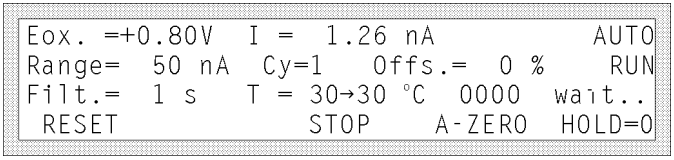
11. Your system is now ready for automated operation. A timefile is started by choosing ‘RUN’ in the ‘AUTO SET’ screen. The ‘AUTO RUN’ screen will appear.



This is the status screen of the AUTO mode. Current settings are displayed, including the actual cycle number ('Cy') that is executed.

The autozero command ('A-ZERO') is always accessible during operation in the AUTO mode.

When the drift monitor is programmed and the drift is beyond the pre-set value, the 'wait...' message is displayed in stead of the time.



NOTE: **If an injection or an external event is programmed at t=0:00, these events are executed immediately while the drift monitor is putting the system on hold. This can be prevented by programming these events at t=0:01.**
Furthermore, the duration of each run will always be at least one minute longer. In this minute the drift in the baseline is monitored

Interrupting a timefile:

12. When 'HOLD' is chosen, the timer is stopped, the message 'HOLD=1' appears in the corner of the 'AUTO RUN' screen. After pressing 'HOLD' again, the timer continues ('HOLD=0').

13. When 'STOP' is chosen, the execution of the timefile is interrupted. Starting the next run at t = 0:00 is possible by choosing 'START' (if cycles >1). The cycle counter ('Cy') is increased.
    STOP also deactivates the outputs Aux 1 and 2, and Relays 1 and 2 (status: 0000).

14. When ‘RESET’ is chosen, the execution of the timefile is interrupted and the ‘AUTO SET’ screen appears. The cycle counter is reset to 0 (‘Cy = 0’).
    RESET also deactivates the outputs Aux 1 and 2, and Relays 1 and 2 (status: 0000).

NOTE: In the master mode the HOLD, STOP, START and RESET functions are accessible only from the keyboard. In the slave mode these inputs are accessible via the rear panel of the ProStar 370. Accessible keyboard functions in the slave mode are: RUN, STOP, RESET and A-ZERO.

Step by step programming of the AUTO mode (System = Slave)

For the same example as given above, a timefile will be programmed. The system consists of an HPLC system with autosampler, a ProStar 370 and an integrator.

The ProStar 370 is *slave* which means that a run is started by an external contact (i.e. from autosampler). Also the number of automated runs is controlled externally.

Hardware connections:

1. Connect a hardware trigger of the autosampler to ‘START’ on the rear panel of the ProStar 370 as indicated in Figure 15. A contact closure is required between ‘COMMON’ and ‘START’ to start a timefile. Consult your autosampler manual for details on connecting the external contact to a relay.

2. Connect your integrator to the REC output at the rear panel of the ProStar 370.

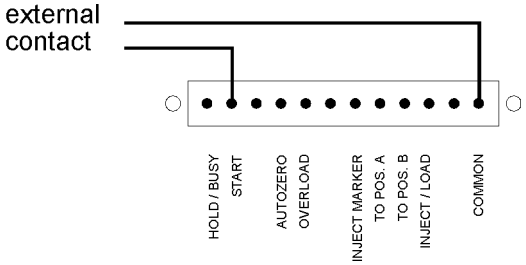


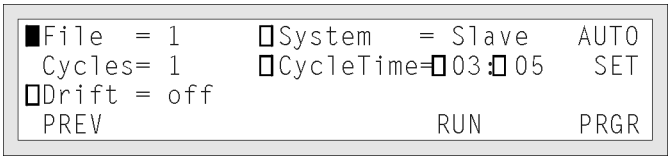
Figure 15 Input contacts needed for external control of the ProStar 370 (slave mode).

3. Prepare the LC-EC system for analysis.

Programming the AUTO mode:

The programming of the timefile is done in the same way as described for the master mode. In the following steps the differences with the master mode are given.

4. In the 'AUTO SET' screen choose 'ProStar 370 = Slave'. The number of cycles is controlled by the autosampler, in the ProStar 370 'Cy' is automatically set at 1 and can not be changed.



Choose 'PRGR' to go in the edit mode of the timefile and continue programming the timefile as described above.

5. By pressing RUN the system is ready for automated operation. The timefile is started by an external contact closure from the autosampler (Figure 15). The duration of this contact closure must be at least 100 ms. Each time a run is started the 'AUTO RUN' screen appears.

   The autozero command ('A-ZERO') is accessible during operation in the AUTO mode as long as 'I-Cell' is displayed.

   Accessible keyboard commands in the slave mode are: RUN, STOP, RESET and A-ZERO. This means that the timefile can also started (and stopped) from the keyboard.

Interrupting a timefile:

6. In the slave mode the ProStar 370 does not respond to a 'HOLD' command from the keyboard. The timer can be stopped by making an external contact closure between 'HOLD' and 'COMMON'. As soon as the contact closure is released, the timefile continues.

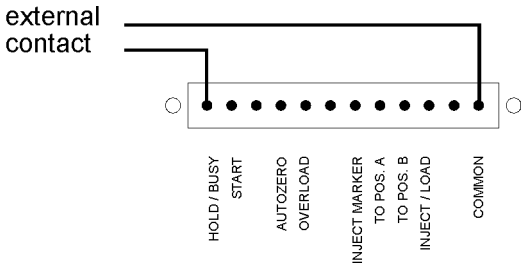


Figure 16 External contact to give a 'HOLD' command during execution of a timefile in the slave mode.

7. If 'STOP' is chosen from the keyboard, the execution of the timefile is interrupted. To start again, an external 'START' (or 'RUN' from keyboard) command is required.
   The STOP command toggles between STOP and START to control the execution of a timefile. STOP also deactivates the outputs Aux 1 and 2, and Relays 1 and 2 (status: 0000).

8. When 'RESET' is chosen from the keyboard, the execution of the timefile is interrupted and the 'AUTO SET' screen appears.
   RESET also deactivates the outputs Aux 1 and 2, and Relays 1 and 2 (status: 0000).

NOTE:	**In the *master* mode the HOLD, STOP, START and RESET commands are accessible only from the keyboard. In the *slave* mode these inputs are accessible via the rear panel of the ProStar 370. Accessible keyboard commands in the *slave* mode are: RUN, STOP, RESET and A-ZERO.**

Other events

Inject/load and inject marker:

A frequently used function is the inject/load (or inject marker) as a trigger for starting the integration software. This trigger is always accessible, not only in the AUTO mode.

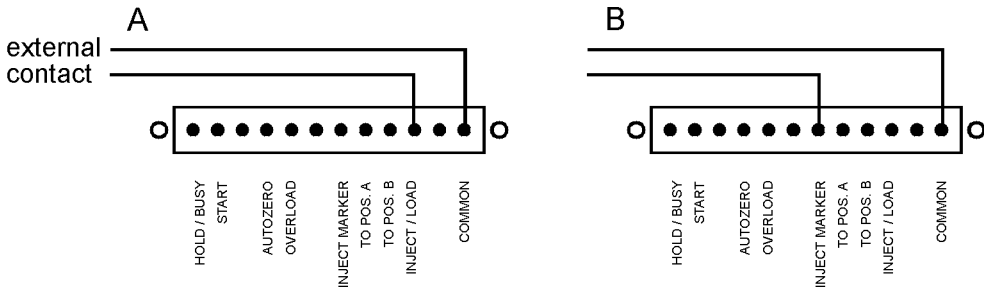


Figure 17 Output contact needed to start the integrator by switching the Rheodyne 7725i (A) or an automated VALCO valve (B).

The Rheodyne 7725i can be connected to ‘manual valve’ on the rear panel of the ProStar 370 which enables the inject/load contact of the rear panel. The contact is high when the valve is in ‘load’ position, and low in the ‘inject’ position.

The ‘INJECT MARKER’ is only active when an electrically actuated VALCO injector is present (VALCO option) and connected to ‘electric valve’ on the rear panel of the ProStar 370. This output is high when the injector is in ‘load’ position and low in the ‘inject’ position.

Hold/busy

The hold/busy is a double function.

Input: when a timefile is executed in the slave mode, the timer can be stopped externally by connecting ‘HOLD’ and ‘COMMON’ as described above (Figure 16). The timer continues after disconnection.

Output: the ‘BUSY’ output is active (low) when during operation in the master or slave mode the ProStar 370 is put on ‘hold’ by

the drift monitor. The ‘HOLD’ command from the keyboard does not affect the status of the ‘BUSY’ output!

Overload:

Activated when a recorder overload (RECOVLD) occurs, see also page 102 for details.

Autozero:

Enables external activation of the autozero command. This function is active only when the ‘I-Cell’ is displayed.

To pos A, B:

Forces the VALCO electrically actuated injector to position A (load) or B (inject).

Cell on (off)

Switches on (off) the flow cell. This input command can be used for example to switch on and stabilize the flow cell early in the morning by means of a timer.

Programming output functions:

Four output functions can be programmed at each time. The notation of the output ‘0000’ corresponds to the four output controls at the back panel of the controller. These are from left to right: relay 2, relay 1, AUX2, AUX1 (Figure 18).

For example, if AUX1 has to be activated, the output is set to ‘0001’. If a contact closure has to be made using relay 1, the output is set to ‘0100’. The contact is made between pin 3 and 5 at the 12 pins connector of the upper I/O connector. At the same time the contact between 4 and 5 is interrupted. The relay position indicated on the rear panel refers to the ‘0’ setting (see Figure 18).

In Table 9 the output commands and the corresponding external contacts are given. Combinations of commands enable control of multiple external contacts at the same time. For example, the command ‘0101’ activates AUX1 and relay 1 at the same time.

Table 9 Outputs and commands, combinations are possible.

Output	command

AUX1	0001
AUX2	0010
Relay 1	0100
Relay 2	1000

TTL contacts

The ProStar 370 uses TTL input contacts for START, AUTOZERO, TO POS. A and TO POS. B. These contacts require a minimum TTL-low pulse duration of 100 ms to be activated. If multiple activations are required the next pulse must be given after 100 ms TTL-high. If the input is kept low, only one activation will occur.

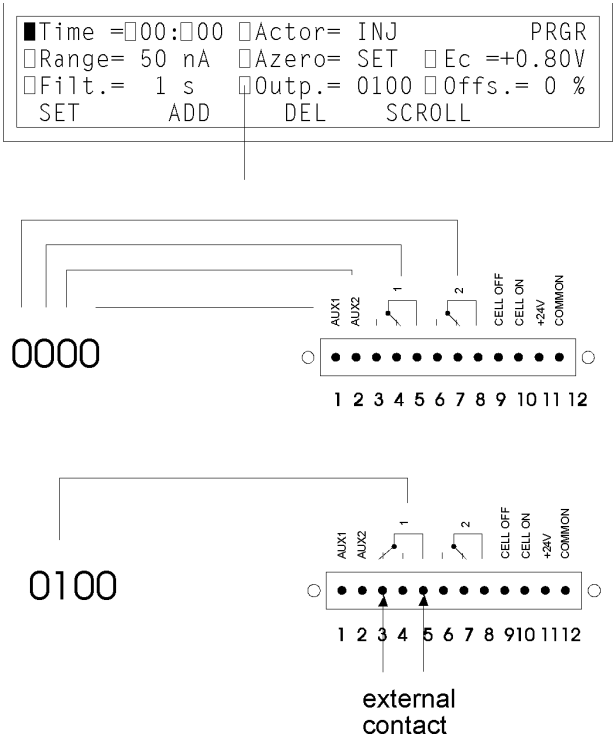


Figure 18 Programming an external contact closure using the 'Outp.' command in a timefile.

There are at least two ways to activate a contact by TTL-low. This can be done by making a contact closure of the input with common (see Figure 15 ‘START’ command) or by using an external TTL contact which is made low. In the latter case it is important that the status of the external contact is known, and that the apparatus is connected to the same ground (by the mains power supply) as the ProStar 370.

Table 10 I/O contacts, upper 12 pins connector. The connections 1-8 are only accessible in the AUTO mode (master and slave mode).

No.	Name	Function
1	AUX1	open collector output, I max. 250 mA, U max. 28 V
2	AUX2	open collector output, I max. 250 mA, U max. 28 V
3, 4, 5	relay 1	contact between 5 (common) and 4 (default) or 3, I max. 500 mA, U max. 28 V
6, 7, 8	relay 2	contact between 8 (common) and 7 (default) or 6, I max. 500 mA, U max. 28 V
9	Cell off	trigger to switch off the cell (level triggered)
10	Cell on	trigger to switch on the cell (level triggered)
11	+24 V	+24 V output, I max. 500 mA
12	common	ground

Table 11. I/O contacts, lower 12 pins connector. Default status is high (5 V).

No.	Name	Access	Activ(at)e	Function
1	Hold/Busy	Slave, Master	status: low	Output: active if drift monitor ‘holds’ the run (master + slave). Input: active as ‘HOLD’ command (slave mode).
2	Start	Slave	level triggered	Starts a timefile
3				
4	Autozero	Always	level triggered	Autozero command, always accessible when ‘I-Cell’ is in ProStar 370 display
5	Overload	Always	status: low	Active when recorder overload occurs (‘REC OVLD’)
6	not conn.			
7	Inject marker	Always	status: low	In combination with VALCO, high: ‘load’, low: ‘inject’.
8	to pos. A	Always	level triggered	In combination with VALCO, forces injector to ‘load’
9	to pos. B	Always	level triggered	In combination with VALCO, forces injector to

				'inject'
10	inject/load	Always	status: low	In combination with Rheodyne 7725i, high: 'load', low: 'inject'.
11				
12	common	Always		Ground

Some contacts are *level triggered*, they are activated by making an external contact closure for at least 100 ms with common (= low). Releasing the external contact does not affect the I/O contact function. Other contacts are *status sensitive*. They are active only when the status is low, releasing the external contact closure deactivates (status = high).

Lower contact 1 is only accessible in the AUTO mode (master and slave). Lower contact 2 is only accessible in the slave mode (AUTO mode). Lower contact 4 is accessible when 'I-Cell' is displayed. Other lower contacts are always accessible.

Pulsed amperometric detection

Introduction

Several advanced features are implemented in the ProStar 370. One of these features is the so-called pulse mode. In pulsed amperometric detection (PAD) the working electrode (WE) is regenerated at a frequency of 0.5 - 3 Hz by the application of a series of potential changes. This is particularly useful for certain applications where the working electrode is rapidly contaminated due to adsorption of insoluble reaction products. A well-known application area of PAD is the analysis of carbohydrates (Figure 19) [1].

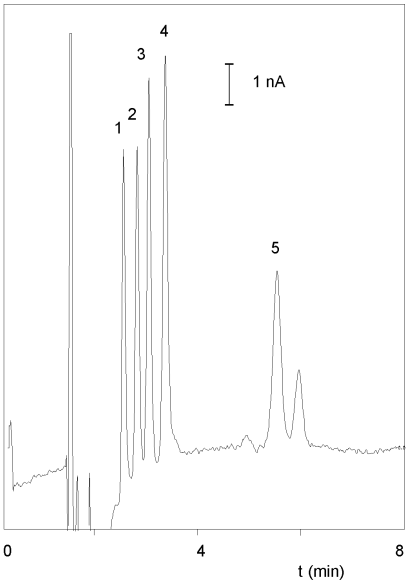


Figure 19 Pulsed amperometric detection of 100 nmol/l (2 pmol) carbohydrates. Peaks are: sucrose (1), galactose (2), glucose (3), α-lactose (4) and maltose (5).

The pulse mode is quite different from the DC mode:

- The output signal is sampled during a fraction of the total pulse cycle. This requires electronic manipulation of the signal. Therefore, only the recorder output should be used for detection.

- During the sampling time (ts) the signal generated at the WE is collected and this value is sent to the detector output. Further smoothening of the signal by means of rise time filters is not possible.

- When the frequency of the data acquisition system (integrator) is higher than the pulse frequency a typical stepwise pattern may appear in the chromatogram. This is normal and only visible after considerable magnification of the chromatogram.

- The background or cell current is usually considerably higher (100 - 1000 nA) than the maximum compensation in the nA range (160 nA). Therefore, only the µA range is available in the pulse mode.

- After prolonged use of the flow cell with a gold working electrode (WE) in the pulse mode, the gold oxide which is generated at the WE, precipitates on the auxiliary electrode (AUX). This gold oxide coating may electrically isolate the AUX and result in an increase of the noise. Simply polishing the working electrode on a regular basis (e.g. every month) prevents this.

- Reference electrodes of the Ag/AgCl type are less suitable for carbohydrate analysis. Due to silveroxide formation they require regular (monthly) maintenance. Hy-REF reference electrodes are maintenance free under these conditions and are therefore particularly suited.

- If a mobile phase is used with a high pH (pH>10, carbohydrate analysis), the standard Vespel rotors from the injection valve should be replaced by Tefzel rotors which are pH resistant.

- For carbohydrate analysis, only CO_2-free sodium hydroxide should be used since carbonate anions may disturb the ion exchange chromatography. The CO_2-free sodium hydroxide is available from several suppliers as a 50% solution (19.2 mol/l). NaOH pellets are not recommended because of their high CO_2 content.

- The accuracy of certain pH-electrodes is poor at high pH. For applications at high pH it is sometimes better to *calculate* the pH from the OH^- concentration.

- Organic modifiers (acetonitrile) strongly attenuate the signal of most carbohydrates in PAD and are therefore not recommended.

Some of these aspects will be discussed in detail.

Pulse settings

In PAD of carbohydrates the working potential is applied as a series of 3 potentials. During time interval t1 the detection potential is applied. The data collection occurs within t1, during time interval ts (sampling time). The time difference t1 - ts is the stabilisation time.

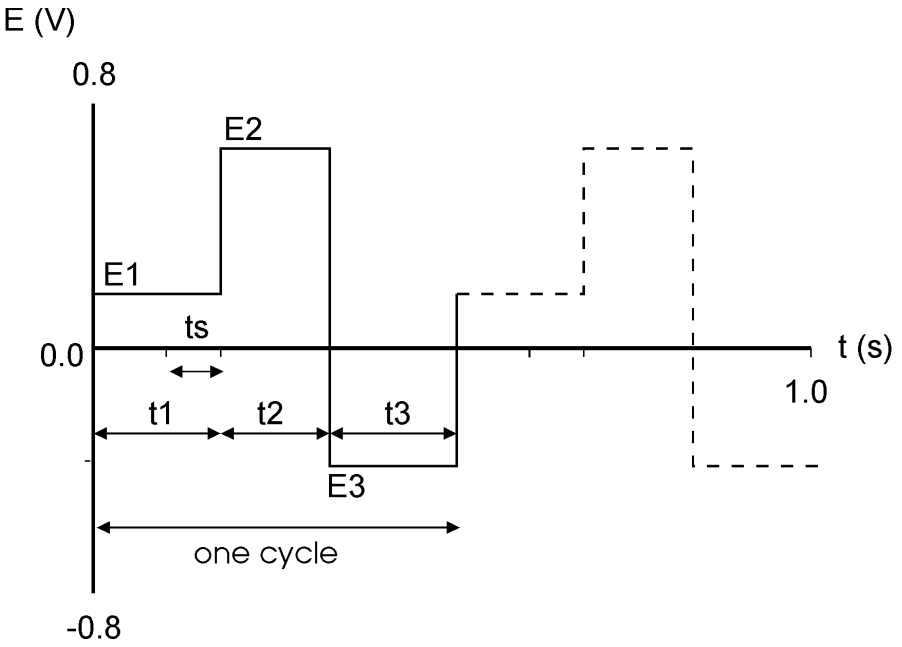


Figure 20 Potential steps in pulsed amperometric detection. A part of t1 is used for detection (ts). The metal oxide layer that is formed during t2, is removed during t3, resulting in a renewal of the electrode surface.

During the next time interval (t2) a monolayer of metal oxide is formed at the working electrode due to the high positive potential. This monolayer is electrochemically removed from the electrode surface during time interval t3, by applying a negative potential.

Optimization of wave forms

LaCourse and Johnson [2-4] have published several papers on optimization of wave forms in PAD. Several considerations are important for the choice of the pulse duration. Optimization is depending on the working electrode material, the sample constituents and the required detection frequency. The impression may arise that the number of variables, 3 potential steps and 4 time settings, may lead to a time-consuming optimization procedure. In practice, the pulse mode is more straightforward.

The potential for the cleaning steps, E2 and E3, are determined by the WE material. At alkaline pH gold oxide is already formed at E2 > +200 mV (*vs.* Ag/AgCl). At a higher potential the formation of a metal oxide layer is accelerated and a shorter time setting may be chosen. In practice an E2 value of +750 mV during 200 ms (t2) gives good results.

The choice of t3 is depending on the potential E3 and the t2 and E2 setting. It is essential that the duration of t3 and the magnitude of E3 is such that a complete removal of the metal oxide is achieved. Reductive dissolution already occurs at E3 < 0 mV, but a more negative voltage speeds up this process. An E3 value of -800 mV during 200 ms (Table 13), or -300 mV during 360 ms [4] can be used.

The measuring potential is compound dependent, usually literature data can be used as a starting point for further optimization. A sampling time ts can be chosen between 20 and 100 ms in 20 ms steps. These are multiples of the 50 Hz, to prevent noise due to oscillations of the AC power supply. Until a certain limit, increasing ts will result in an increase of signal. A limiting factor is the accumulation of adsorbed species at the working electrode that attenuate the signal. Another consideration, not only for ts but for all time settings, is that increasing the time will decrease the detection frequency.

Before sampling a stabilization time is applied, set by the duration of t1. In practice the stabilization time determines the level of the background current. When, for example, t1 = 100 ms and ts = 100 ms, there is no stabilization of the current before sampling (t1 - ts = 0 ms). Depending on the potential setting of

E2 and E3, a large positive or negative background current (micro amperes) may be detected which is seriously limiting the detection. In practice, often a 100 - 400 ms stabilization time is used.

Recorder output frequency

An important difference between the DC and the pulse mode is the frequency of the output signal on the recorder output. In the DC mode the signal has a 9 Hz frequency, in the pulse mode the frequency is determined by the duration of the pulse. Once every cycle, the ts signal is sent to the output. This can be visualized by magnification of a peak in the chromatogram (Figure 21).

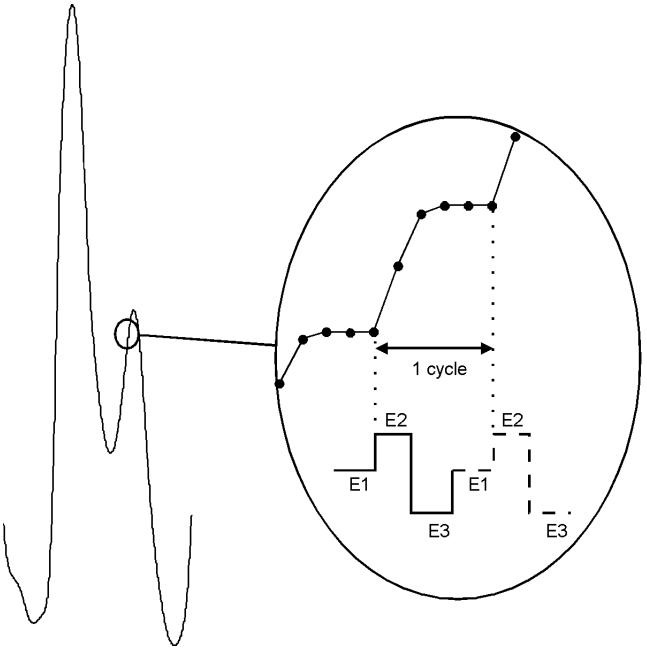


Figure 21 A magnified view of a chromatogram obtained with PAD. The integrator frequency is 5 times higher than the detector output frequency resulting in a typical stepwise signal.

A stepwise pattern in the chromatogram is only seen on an (analog) chart recorder, or on an integrator that has a higher sampling frequency than the output frequency of the detector. In fact, when this pattern is seen this means that the integrator has an unnecessarily high sampling frequency. This leads to large data files, but certainly not to a better chromatogram.

Peak width and integrator frequency

There are two important considerations with respect to integrator frequencies applied in HPLC. If the frequency is too low, data will be lost and artifacts may be introduced. If the frequency is too high, large data files are generated which take up an unnecessary large amount of disk space. As a rule of thumb, the sampling frequency of the integrator is set such that a chromatographic peak is build up of at least 10 data points. For a peak width of 10s this means that a sampling frequency of 1 Hz should be sufficient.

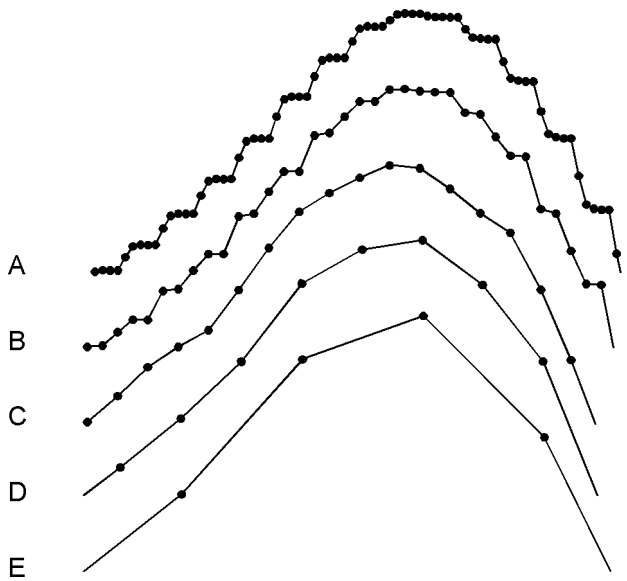


Figure 22 A detailed part of a chromatogram acquired at different integrator frequencies. The integrator frequency is (A) 5x, (B) 2.5x, (C) 1.2x, (D) 0.6x and (E) 0.3x the frequency of the pulse.

In case of PAD the duration of the pulse should also be taken in account. When the frequency of the pulse is 2 Hz, it makes no sense to acquire data on an integrator with a significantly higher frequency. This would result in acquisition of multiple data points containing the same output value (Figure 22 A and B). Matching the frequencies keeps the peak shape unchanged (Figure 22 C). Decreasing the integrator frequency to less than half the pulse frequency, changes the peak shape (Figure 22 E).

When the peak width is too small there are two options: either less data points are collected for such a peak, or the pulse and integrator frequency are both increased. In the latter case the pulse duration is decreased which will change other detection parameters as well. In practice, the pulse frequency almost never interferes with the HPLC analysis.

Working electrode material

Gold and platinum are used as working electrodes for PAD. Glassy carbon appears to be unsuitable due to the high electric capacitance of this material. Furthermore, resurfacing of the noble metal working electrode is based upon formation and removal of a (metal-) oxide layer. This is impossible with glassy carbon.

The change in cell current during the pulse mode is illustrated in Figure 23. When the potential is changed, a large charging current is detected (Figure 23, peak 1, 3 and 5), followed by a stabilization of the current (Figure 23, part 2, 4 and 6). The output signal is sampled during a fraction of part 2, depending on the pulse settings. The response of the glassy carbon material is considerably different from the noble metals. The capacitance of the electrode material is very high, such that the charging current is not stabilized before start of the next potential step. This pattern makes detection impossible.

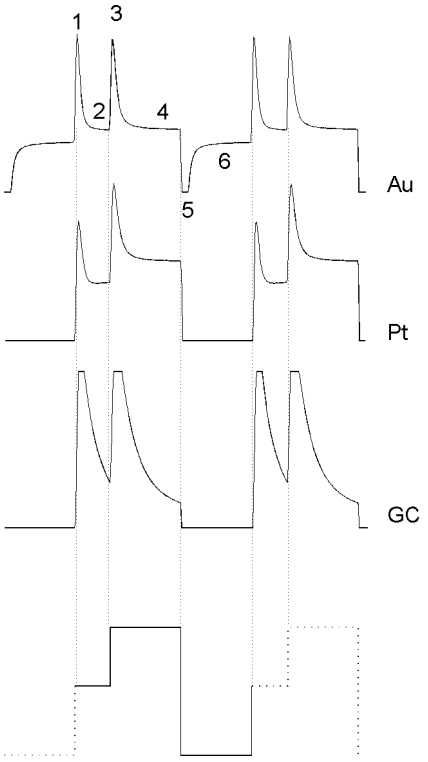


Figure 23 Change in cell current during PAD. The cell current of the noble metals gold (Au) and platinum (Pt) is stabilized faster than the cell current of glassy carbon (GC), due to a much lower capacitance of the noble metals. For Pt and GC the negative peaks run far off-scale, however the profile is similar to the mirror image of the positive peaks.

Examples of carbohydrate analyses are given in Figure 19. Typical PAD pulse settings are given in Table 12 and Table 13, these settings may be used as starting point for further optimization. Carbohydrates are oxidized at a pH of 12 or higher, which puts specific demands on the HPLC system used (see above).

Table 12 Potential settings for PAD of carbohydrates at a gold working electrode.

	s	1	2	3
t (ms)	100	400	200	200
E (mV)		+150	+750	-800

Table 13 Potential settings for PAD of glycols, alcohols, aldehydes at a platinum working electrode.

	s	1	2	3
t (ms)	20	300	100	100
E (mV)		+200	+1300	-100

Table 14 LC-EC conditions for PAD of carbohydrates.

Detector	ProStar 370
Column	CarboPac PA1, 4x250mm
Flow rate	1.0 ml/min
Mobile phase	200 mM NaOH
Sample	disaccharides 100 nmol/l, 20 µl injection
Temperature	30 ^{o}C
Flow cell	flow cell with 3.0 mm gold working electrode mounted with 50 µm spacer
REF	Hy-REF
I-cell	ca. 435 nA

References

1. D.C. Johnson, D. Dobberpuhl, R. Roberts and P. Vandeberg, *Review. Pulsed amperometric detection of carbohydrates, amines and sulphur species in ion chromatography - the current state of research*, J. Chromatogr. 640 (1993) 79-96
2. D.C. Johnson en W.R. LaCourse, *LC with pulsed ECD at gold and platinum electrodes*, Anal. Chem., 62 (1990) 589A-597A
3. W.R. LaCourse en D.C. Johnson, *Optimization of waveforms for pulsed amperometric detection of carbohydrates following separation by LC*, Carbohydrate Research, 215 (1991) 159-178
4. W.R. LaCourse en D.C. Johnson, *Optimization of waveforms for pulsed amperometric detection of carbohydrates based on pulsed voltammetry*, Anal. Chem. 65 (1993) 50-55

Optimization of working potential

Introduction

A current - voltage (I/E) relationship, or voltammogram, characterizes an analyte. It gives information on the optimum working potential, which can be used to improve detection sensitivity and selectivity.

There are several ways to obtain a voltammogram. A *hydrodynamic* voltammogram is obtained in the DC mode by running several chromatograms at different working potentials. Both peak height and background current are plotted against the working potential. A *scanning* voltammogram is obtained in the so-called scan mode of the ProStar 370: the voltage runs between two pre-set values and the current is measured.

Optimization of the working potential and the construction of a voltammogram is described.

Electrochemical reactions

In electrochemical detection (ECD) a reaction of the analyte at an electrode surface is monitored. This distinguishes ECD from most other detection techniques where detection is based on the physical properties of an analyte (i.e. mass spectrometry: molecular mass, absorbance detection: molar absorptivity). For electrochemically active compounds, the potential between reference electrode (REF) and working electrode (WE) determines the reactivity of the analyte at the WE. The potential difference supplies the energy level needed to initiate or enhance the electrochemical reaction. Different analytes may have different oxidation or reduction potentials, which determines the selectivity of ECD.

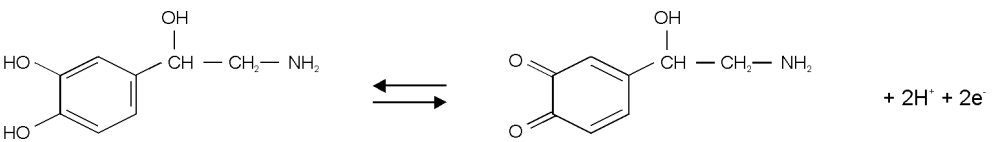


Figure 24 Oxidation/reduction reaction of norepinephrine.

An example of an electrochemical reaction is shown in Figure 24, norepinephrine is converted into a quinone by oxidation at the WE. Two electrons are transferred at the WE resulting in an electrical current that is amplified by the controller.

Hydrodynamic and scanning voltammogram

A hydrodynamic voltammogram is constructed when the pure analyte is not available and separation over an analytical column is required. Furthermore, under real chromatographic conditions reliable information about the S/N ratio is obtained

In case of metal working electrodes it is also advisable to use a hydrodynamic voltammogram. On the metal working electrode an oxide layer is formed which affects the electrochemical reaction and makes the interpretation of a scanning voltammogram diffcult.

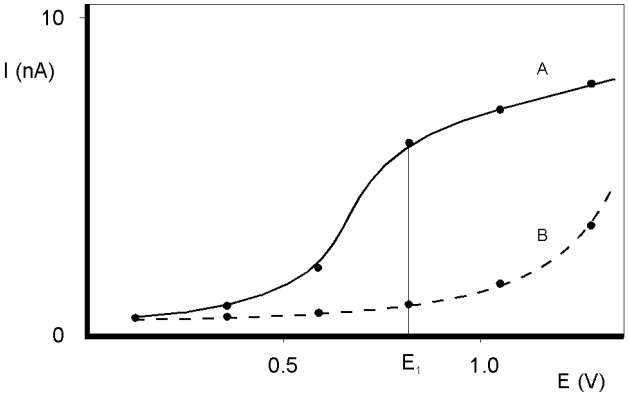


Figure 25 Hydrodynamic voltammogram of norepinephrine (A) at a glassy carbon working electrode, and the current of the baseline (B). At E_1 the electrochemical signal becomes diffusion limited.

An alternative for the chromatographic construction of an I/E relationship is the application of scanning voltammetry. The working potential runs between two pre-set values and the current is measured while the analyte is continuously flushed through the flow cell.

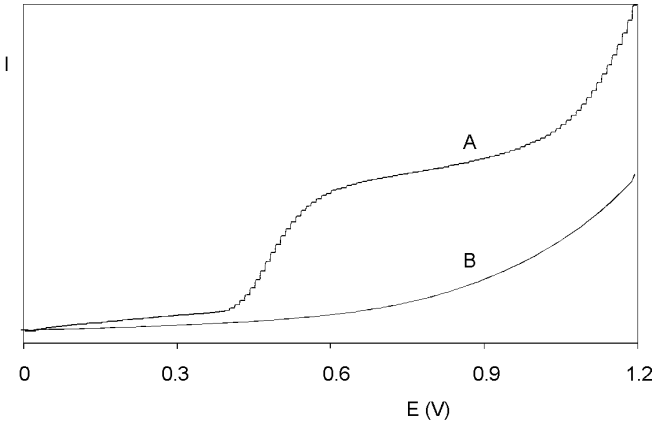


Figure 26 Scanning voltammetry of 1.0 µmol/l norepinephrine (A) at a glassy carbon working electrode, at a scan speed of 10 mV/s. Scan (B) is the blank solvent.

As peak heights are used, the signal in Figure 25, line A is only due to the analyte. The signal in Figure 26, line A is the sum of the analyte signal and the background signal. Subtracting both lines in Figure 26 results in a similar I/E relationship as in Figure 25, line A. It takes only a few minutes to construct a scanning voltammogram. This is an advantage, especially when a number of analytes have to be characterized. However, as the scan is obtained in flow injection analysis (FIA, without analytical column), it is a prerequisite to have the pure analyte dissolved in buffer. Any contamination may lead to artifacts. A blank scan of the buffer should be used to distinguish between solvent peaks and analyte peaks.

As can be seen in both Figure 25 and Figure 26, when the working potential is increased the electrochemical reaction is enhanced hence the signal increases. At a certain potential the I/E curve flattens. All analyte molecules that reach the working electrode are converted at such a high rate that the analyte supply becomes the limiting factor. At the working electrode surface a stagnant double layer exists, where molecular transport takes place by diffusion only. Therefore, the current at (and beyond) this potential is called the diffusion limited current.

With respect to sensitivity, a high working potential is important. However, at higher working potentials, more analytes are

detectable. So, as to selectivity, a low working potential will be favorable.

Working at a potential on the slope of the I/E curve will result in less reproducibility. Not only a small fluctuation in the applied potential, but any change in the system may result in a large difference in current.

In practice the choice of the working potential is a compromise between sensitivity, selectivity and reproducibility. In the example of Figure 25 a working potential (E1) of 0.8 V is chosen.

Optimization using a voltammogram

Sometimes, when interfering peaks appear in the chromatogram, it is possible to optimize the method with regard to selectivity. If the interfering compound has a higher oxidation potential, a working potential is chosen that gives the best selectivity, i.e. the largest difference in peak height. In the example of Figure 27 the selectivity for compound X is improved considerably by decreasing the potential to E2 or E1. Obviously, if compound Y is the compound of interest, optimization of selectivity in this way is not possible and the chromatography has to be optimized.

Electrochemical detection differs from most other LC detection methods in that a reaction takes place in the detection cell. Due to reaction kinetics an increased temperature speeds up the oxidation/reduction reaction. However, this not only holds for the analyte but also for the background current and possible interferences. An elevated temperature will therefore not automatically lead to a better detection. A constant temperature is of paramount importance for a stable baseline and reproducible detection conditions.

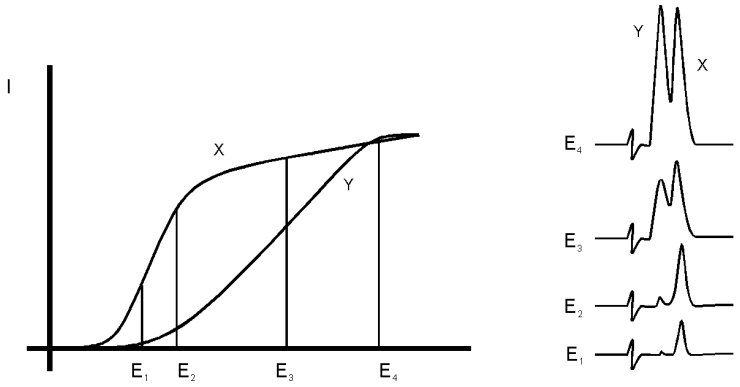


Figure 27 Selectivity in LC-EC of compound X and Y is optimised by choosing the working potential with the largest difference in peak height.

Electrochemical reactions are pH sensitive (Figure 28). For norepinephrine the I/E curve is shifted to a lower potential at higher pH. When the working potential is high (E_2), and the signal is diffusion limited, an increase in pH will result only in a small increase of the peak height. When the working potential is lower (E_1), and the signal is not diffusion limited, the signal will strongly increase at higher pH. In both cases the background current increases at a higher pH.

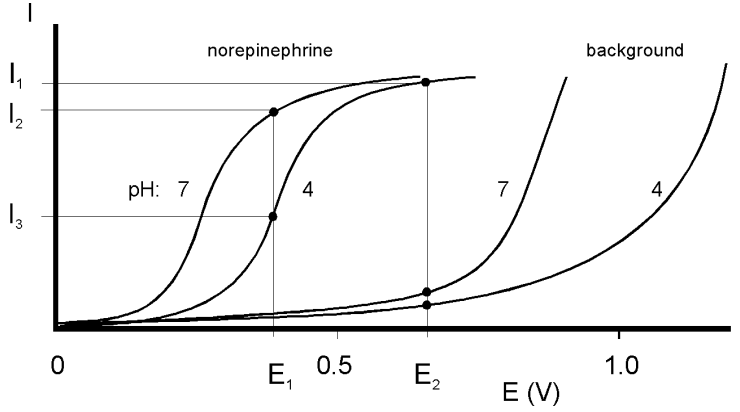


Figure 28 At a higher pH the I/E curve of norepinephrine is shifted to the left.

Reaction kinetics predict that electrochemical detection is mass flow dependent. When the LC flow is stopped in LC-EC, the analyte will be oxidized completely and the signal decreases rapidly. This means that the flow rate not only affects temporal peak width and analysis time but also peak height. Also the background signal is sensitive towards fluctuations in the flow rate. Therefore, it is important to use a pulse-free solvent delivery system.

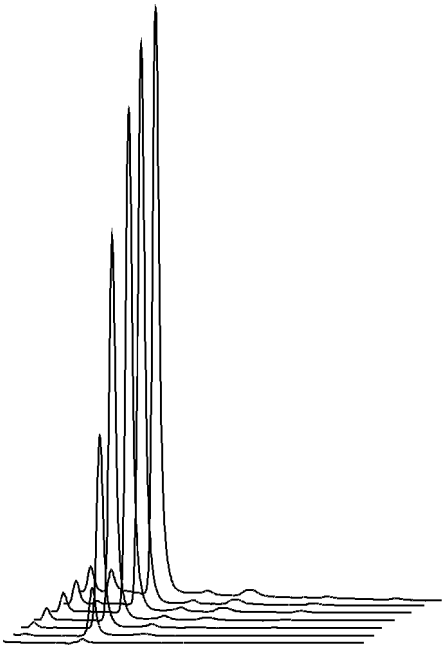


Figure 29 Construction of a hydrodynamic voltammogram for norepinephrine. Chromatograms are obtained at cell potentials ranging from 1.0 V (back) to 0.4 V (front), with 100 mV steps.

Construction of a hydrodynamic voltammogram

Before a hydrodynamic voltammogram can be obtained, the chromatographic conditions should be optimized. Then the following steps are taken:

1. A solution of the analyte at a concentration between 1 - 100 µmol/l, is prepared in mobile phase.

2. The electrochemical detector is stabilized in the DC mode at a high potential. After stabilization the background current is read from the display of the detector (I-cell) and the noise is measured.

3. The run is started by injecting the compound. When at the high working potential no signal is obtained, it may be concluded that the compound is not electrochemically active. In such a case derivatization of the compound may be an option.

4. If a peak is measured, the working potential is decreased by 50 or 100 mV and step 2 to 4 is repeated until the lowest potential setting (Figure 29).

5. The peak heights and the background currents are plotted against the working potential (Figure 25).

6. The working potential which gives the best sensitivity is obtained by plotting the signal-to-noise ratio against the working potential.

Construction of a scanning voltammogram

The scan mode is programmed in the ‘SCAN SET’ screen of the ProStar 370. Depending on the data acquisition software that is used and the experimental set-up, a full, half or continuous scan cycle can be chosen.

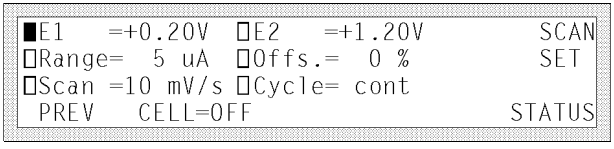


Figure 30 Programming the scan mode in the 'SCAN SET' screen.

In the example of Figure 26 and Figure 31 a ‘half’ scan is used, sweeping the potential from 0.2 V to 1.2 V. A full scan would include the reverse scan, i.e. from 0.2 V to 1.2 V and back to 0.2 V. In the continuous mode the voltage is swept up and down between both potentials.

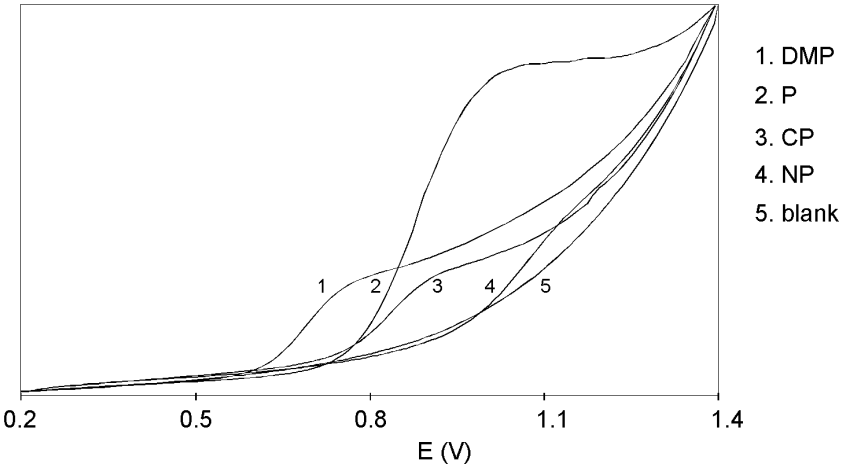


Figure 31 The scanning voltammograms of 2,4-dimethylphenol (DMP), phenol (P), 2-chlorophenol (2-CP) and 4-nitrophenol (NP).

The following procedure is used to obtain the voltammograms in Figure 26 and Figure 31.

1. The column is removed from the LC system. The voltammogram is recorded in the flow injection analysis (FIA) mode.

2. The pure compound is dissolved in (preferably) the HPLC buffer at a concentration of ca. 10-100 µmol/l. When the analyte is already in solution, it should be diluted in HPLC buffer until the desired concentration.

3. An injection loop of 100 µl is installed and the LC flow rate is set at 40 µl/min. The analyte plug will than be detected during approximately 2.5 minutes. The flow rate is lowered if more scanning time is needed.

4. An initial run is started in the *DC mode* at a high potential to estimate the required start and stop time of the scan after sample injection (Figure 32). In the scan mode, the scan is obtained at the flat top of an analyte plug. **The analyte delivery should be constant. Fluctuations result in unreliable results.**

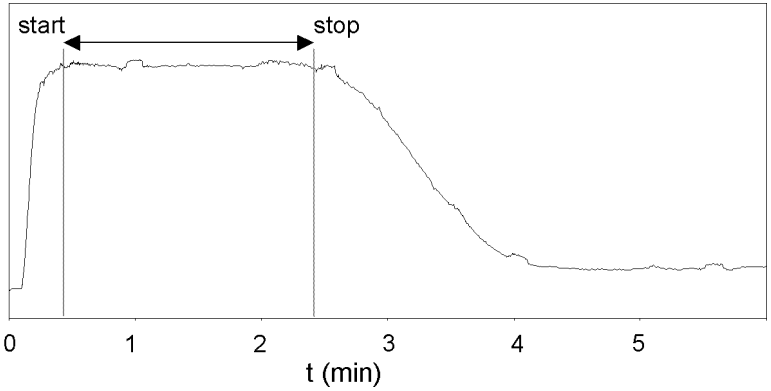


Figure 32 Chromatogram of the analyte plug obtained in the DC mode. Scanning takes place on top of the broad peak between 0.5 and 2.5 min after injection in FIA mode.

5. The sampling frequency of the integrator is set at 1 Hz. This is the same frequency as the voltage steps during the scan. If a higher sampling frequency is chosen a typical stepwise pattern may appear.

6. In the 'SCAN SET' screen an upper and a lower potential is chosen. The cycle is set at half. The range is set at 5 µA. A scan speed of 10 mV/s is selected. The integrator (or recorder) must be connected to the REC output of the ProStar 370.

7. The analyte is injected and the scan is started by pressing the 'START' in the 'SCAN STAT' screen of the ProStar 370 at the time the analyte plug enters the flow cell (see 4).

8. A background scan is obtained by scanning the HPLC buffer.

9. For reliable results it is recommended to repeat each scan three times.

Maintenance and Troubleshooting Guide

No detector response

Possible cause	Remedy
No power	Check line voltage setting, plug in power cord
Power switch off	Turn this switch ON (at the rear panel)
Faulty fuse	Replace fuse
Divergent mains voltage	Check line voltage, see page 3
Cell disconnected, or switched off	Check connection
Recorder/integrator disconnected	Check connection
Contaminated WE	Clean WE

High cell current

Possible cause	Remedy
Contaminated buffer	Replace buffer, do not recycle the buffer
High WE potential	Optimise potential, if possible: use smaller WE diameter
Salt bridge in REF not saturated	Refill with wetted KCl crystals
Retained peaks from previous runs	Wait for elution of these (very) broad peaks
Column is 'bleeding'	Replace column
High amount of Fe^{++} in buffer	Add EDTA to buffer, rinse metal parts with 15% HNO_3

Noisy baseline

Possible cause	Remedy
Salt bridge in REF not saturated	Refill with saturated KCl, add wetted KCl crystals
Air bubble in salt bridge REF or in cell	Remove air bubble, continuously degas the mobile phase
Slow temperature fluctuations	Isolate detector cell, set oven temperature
Contaminated WE	Clean WE
Leaking REF or cell	Tighten connections with care

Decreased sensitivity (low S/N ratio)

Possible cause	Remedy
Contaminated WE by dirty samples	Clean WE, if possible: dilute samples
Cell potential too low	Optimise potential
Contaminated buffer (high I_{cell})	Replace buffer, do not recycle the buffer

Baseline oscillations

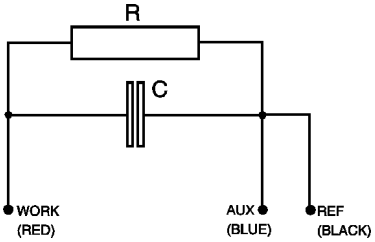
Possible cause	Remedy
Malfunctioning pump (regular pattern)	Check pump (seals, valves)
Over-tightened cell bolts	Adjust cell bolts, check pump pressure
Air bubbles in cell or REF	Maintenance REF
Temperature oscillations	Set oven temperature
Contaminated buffer (high I_{cell})	Replace buffer, do not recycle the buffer
Contaminated WE	Clean WE
Fe^{++} in buffer	Add EDTA, passivate metal parts with HNO_3

Saturation of output

Possible cause	Remedy
Damaged REF	Check with spare REF, replace if necessary
Damaged WE	Replace cell block
Cell incorrectly connected	Check connections (REF: black, WE: red, AUX: blue)
Cell potential too high	Optimize cell potential

Dummy cell

A successful dummy cell test confirms that the controller, including the cell cable, functions properly. If the result of the noise measurement with the dummy cell is within specs, the controller is excluded in a trouble shooting procedure.



The dummy consists of a resistor (R) of 300 MΩ and a capacitor (C) of 0.47 µF in parallel. The current is measured over the resistor according to Ohm's law (V = I x R), hence with a working potential of 800 mV the current drawn will be about 2.67 nA. Slight differences as to this (ideal) value are due to the tolerance of the resistor (± 1%). The capacitor functions as a 'noise generator' and in fact resembles the capacitance of a well-functioning flow cell in an ideal HPLC set-up.

The noise generated via the dummy should be less than 2 pA if the filter of the controller is set to 0.1 second, provided that the dummy is within the fully closed Faraday shield at the same position as the flow cell (see Table 15 for settings). With a 1 second risetime the noise should be better than 1 pA.

Table 15 Dummy cell test settings.

Parameter	Setting
Cell potential	800 mV
Cell current	2.67 nA (read-out)
Oven	30 °C, stable
Risetime filter	0.1 s (or as specified)
Range	nA range, 0.1 nA/V
Output	Recorder, data acquisition at 1 V

The results of the dummy test must be comparable with the test sheet supplied with your controller. If not, please consult your supplier.

Appendices

Specifications

General

Power	110-120/220-240 VAC, 50/60 Hz
Operating modes	DC, Pulse and Scan
Potential range	between +2.00 and - 2.00 V in 10 mV increments
Recorder output	between +0.1 and - 0.1 or between +1.0 and -1.0 V
Current ranges integrator	1 nA, 10 nA and 1 µA/V
Integrator output	between +10.0 and -10.0 V
Offset	between +50% and - 50% of recorder output, 10% steps
Drift monitor	0.1 - 10 nA/min (Pulse), 0.1 pA/min - 10 nA/min (DC)
Event marker	10% of recorder output
Autozero	range determined by integrator setting, in µA range: 16 µA, in nA range: 160 nA, in pA range: 16 nA; triggered by keyboard, rear panel contact closure, or RS232C control
RS232C	full parametric instrument control (option)
Injector sensor	starts system clock if Load → Inject
Oven	height 37 cm, from 5°C above ambient to 45°C, accuracy 0.5°C, stability 0.1°C; accommodates column and flow cell

DC mode

Current ranges recorder	10 pA - 5 µA in 1, 2, 5 steps
Filter (time constants)	0.1 - 5 s in 1, 2, 5 steps
Noise	< 2 pA with a dummy cell (load of 300 MΩ and 0.5 µF) with 0.1 s filter; with 2 s filter the noise is < 1 pA

PULSE mode

Range	10 nA - 5 µA in 1, 2, 5 steps
Pulse times	t1: 100 - 2000 ms; t2: 100 - 2000 ms; t3: 0 (off) - 2000 ms in 10 ms steps
Sample times	20, 40, 60, 80 and 100 ms

SCAN mode

Range	10 nA - 5 µA in 1, 2, 5 steps
Scan rate	1 - 50 mV/s in 1, 2, 5 steps
Cycle	half, full or continuous
	Start/Stop, Hold and Autozero, Starting potential (E1), End potential (E2)

AUTO mode

DC mode (5 files) and pulse mode (4 files), master/slave mode, cycle time, number of cycles, drift monitor
Time-based control of 20 time points as to sensitivity, filter settings, output contacts (2 open collector, 2 relays), autozero, offset, inject/load position of electrically actuated VALCO injector (option) and E-cell (DC only).

Rear panel I/O connections

Mains, Recorder (12 bits) and Integrator (analog), 2 open collector (AUX 1 and 2; I max. 250 mA, U max. 28V) and 2 relay outputs (I max. 500 mA, U max. 28 V), cell on, cell off, 24 V output (I max. 500 mA), Common, Hold/Busy, Start, Autozero, Overload, Inject marker, Electrically actuated injector control, Manual injector (phone jack), RS232C connector

Physical specifications

Dimensions	44 (D) x 22 (W) x 44 (H) cm = 17.3” (D) x 8.7” (W) x 17.3” (H)
Weight	16.6 kg (36.5 Lbs) without flow cell and column

Flow cells

Confined wall-jet design, working volume determined by spacer thickness and WE diameter

Spacers	25, 50 or 120 µm, stackable
WE diameters	0.5, 0.7, 2 and 3 mm
Cell volume	0.005 µl minimum
WE electrodes	glassy carbon, gold, platinum, silver and copper
Reference electrodes	salt-bridge Ag/AgCl; in-situ Ag/AgCl (ISAAC); Hy-REF
Auxiliary electrode	stainless steel
Wetted materials	PCTFE, FEP, 316-SS, Viton, Silver, Silver chloride and WE

The Electrochemical Flow Cell

Introduction

The flow cell is available with a glassy carbon, platinum, gold, silver and copper working electrode. In combination with the spacer set (25, 50 and 120 µm) a variety of detection volumes (down to 5 nl) can be attained. As a standard, the in situ Ag/AgCl reference electrode is advised (ISAAC). For special applications the Hy-REF reference electrode is available. A third reference electrode is the salt bridge Ag/AgCl.

The electrochemical flow cell has been developed for ultra-trace analysis in standard, microbore and capillary LC-EC. After extensive testing it was established that the confined wall-jet configuration gave the very best results. In addition it was found that the electrode materials quality and the finishing of the electrodes in the flow cell are decisive factors for the performance of an EC detector. While competitive designs usually deteriorate when in use, this flow cell, by design, improves in performance. The flow cell permits unusually short stabilisation times: trace analysis within half an hour after starting up may be expected. We have so much confidence in our flow cell that we warrant the glassy carbon flow cell for a period of 5 years.

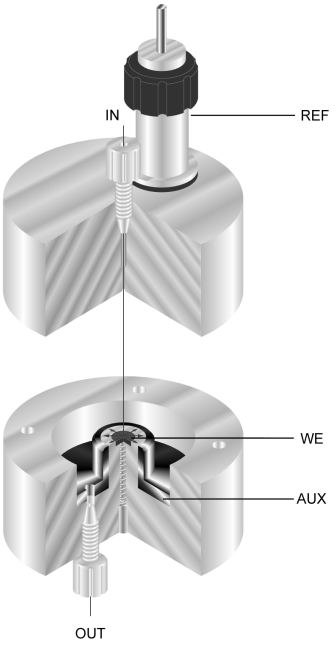


Figure 33 The electrochemical flow cell. The upper part, the inlet block, is separated from the working electrode block by means of a gasket (spacer, not shown).

Three-electrode configuration

In the flow cell a three-electrode configuration is used (Figure). The working potential is set between the working electrode (WE) and the auxiliary electrode (AUX). The AUX is kept at a precisely defined reference electrode (REF) potential by means of the so-called voltage clamp. This is an electronic feed back circuit that compensates for polarization effects at the electrodes.

At the WE, which is kept at virtual ground, the electrochemical reaction takes place, i.e. electrons are transferred at the WE. This results in an electrical current to the I/E converter, which is a special type of operational amplifier. The output voltage can be measured by an integrator or recorder.

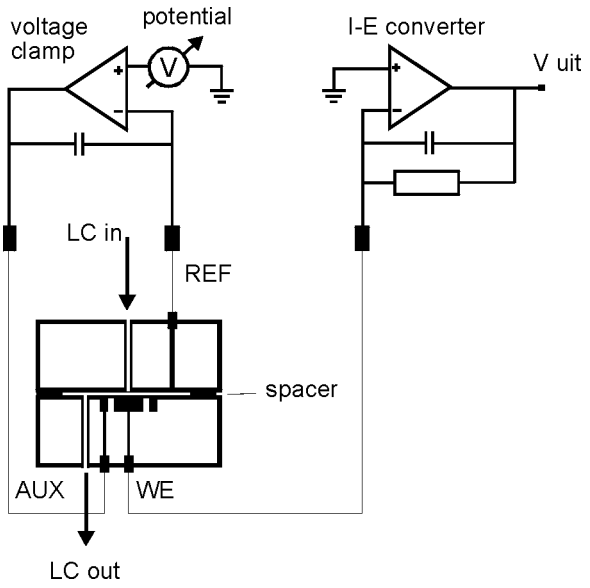


Figure 34 Schematic representation of an electrochemical cell with a three-electrode configuration.

Essentially, for the oxidation or reduction reaction it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration.

If the working potential would be applied only over an AUX versus the WE (without REF), the working potential would continuously change due to polarization effects at the electrodes, resulting in highly unstable working conditions.

If the working potential would be applied only over the REF versus the WE (without AUX), the working potential would be very well defined. However, the potential of a REF is only well defined if the current drawn is extremely low (pico-amperes) resulting in a very limited dynamic range.

A three-electrode configuration, combines the best of both electrodes. The REF stabilizes the working potential and the AUX can supply high currents. This results in the tremendous dynamic range of a three-electrode system.

Working electrode

Electrochemical detection puts high demands on the WE material. The WE should be made of a (electro-)chemically inert material. Furthermore, to avoid an irregular flow profile over the electrode, it should have a very well defined surface. Finally, it is important that the analyte of interest can be oxidized (or reduced) with favorable I/E characteristics. This in fact means that a high signal must be obtained at a low working potential. For most applications glassy carbon will be the WE material of choice. Under certain circumstances other materials are favorable.

For example, for the analysis of iodide a silver WE can be used. At the silver WE the following oxidation reaction occurs for iodide:

$$Ag + I^- \rightarrow AgI + e^-$$

This reaction already takes place at a very low working potential (1 mV !), which results in an extremely high selectivity. This allows the determination of iodide in urine samples to take place almost without any sample pre-treatment.

Table 16 Working potential limits and application area for different WE materials.

WE material	potential limits (V)				major application
	alkaline		acidic		
Glassy carbon	-1.50	+0.60	-0.80	+1.30	catecholamines
Gold	-1.25	+0.75	-0.35	+1.10	carbohydrates
Platinum	-0.90	+0.65	-0.20	+1.30	alcohols, glycols
Silver	-1.20	+0.10	-0.55	+0.40	halides, cyanide
Copper	-	+0.60	-	-	amino acids, carbohydrates

Another consideration in choosing a WE is the oxidation or reduction of mobile phase constituents or WE material, that occurs when the potential exceeds the limits as given in Table 16. At high positive working potentials the water in the mobile

phase electrolyses and results in an strong increase of the background current and noise. Formation of metal oxides, resulting in an increase in background current is a limiting factor for metal electrodes. Glassy carbon and platinum have the highest positive potential limits and are therefore often used in oxidative ECD. For negative potentials the use of platinum electrodes is limited by the ease of reducing hydrogen ions to hydrogen gas.

Signal-to-noise ratio and concentration detection limit

One of the most important parameters used to characterize the performance of a detection system is the signal-to-noise ratio (S/N ratio) from which the concentration detection limit is derived. It enables objective comparison not only between different electrochemical detectors but also between complete analytical methods irrespective what detection system is used.

Table 17 LC-EC conditions for analysis of norepinephrine.

Column	ODS-2, 3 µm, 100 x 4.6 mm
Flow rate	1.0 ml/min
Mobile phase	H_3PO_4 50 mM, citric acid 50 mM, 20 mg/l EDTA, 100 mg/l octane sulphonic acid (OSA), pH=3.1 with KOH, 5% methanol
Sample	1.0 µmol/l norepinephrine, 20 µl injection
Temperature	30 ^{o}C
Flow cell	flow cell with 3 mm GC WE mounted with 50 µm spacer
E cell	800 mV (vs. Ag/AgCl, filled with saturated KCl)
I_{cell}	ca. 3 nA

In literature several ways are described to determined the detection limit. In principle, it does not matter which definition of detection limit is used, as long as the definition is precisely described.

In this manual the concentration detection limit (c_{LOD}) for a certain compound is defined as the analyte concentration that results in a signal that is 3 times the standard deviation of the noise:

$$c_{LOD} = \frac{3 \cdot \sigma_{noise}}{signal} c_A$$

where σ-noise is 0.2 x peak-to-peak noise and cA is the concentration of analyte injected.

In Figure 35 a typical S/N ratio of a glassy carbon flow cell with 2.74 mm WE is shown. In this example the concentration detection limit for norepinephrine based on three times the σ-noise is 11 pmol/l (see Table 17 for conditions).

Expressing the performance of a detection system by only the peak height makes no sense. A system can easily be changed in a way that a larger peak height is obtained. However, if the noise increases similarly, it has the same effect as switching a recorder to a higher sensitivity: peaks appear higher but the S/N ratio is the same.

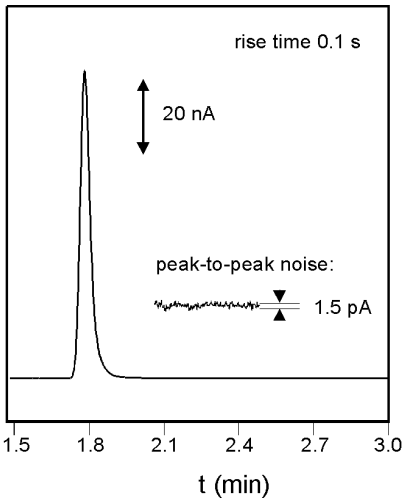


Figure 35 Typical S/N ratio for norepinephrine measured with a glassy carbon flow cell. The amount injected is 20 pmol (1.0 µmol/l). The concentration detection limit based on three times the σ-noise is 11 pmol/l.

Expressing the limit of detection in an absolute amount (i.e. in picomoles) without mentioning the injection volume, makes a good comparison between different systems difficult.

Working electrode diameter

The size of the WE is an important factor in LC-EC, it affects both the signal and the noise. For the flow cell several glassy carbon WE diameters are available (0.7, 2 and 3 mm). In a standard LC system the signal and the noise increases linearly with the WE diameter. This means that he S/N ratio remains more or less the same. In case of micro-LC an increase of the WE diameter will increase the noise more than the signal. Therefore, in micro-LC a decrease of the WE diameter will result in a better S/N ratio.

The choice for a flow cell is primarily based on the HPLC column diameter. This way the best possible detection limit for a standard, microbore or capillary column is warranted.

The following combinations are recommended:

Table 18 Flow cell recommendations.

Column diameter (mm)	Recommended flow cell
3 and higher	3 mm GC
3 - 1	2 mm GC
1 and below	0.7 mm µGC

The recommended combinations are giving the best S/N ratios. It should be kept in mind that other combinations are possible that still result in acceptable sensitivities for many applications. All flow cells are individually tested and meet our high standards of quality and detection sensitivity.

Spacer thickness

The thickness of the gasket affects the linear flow velocity in the cell. With a thinner spacer the cell volume is decreased (Table 18), resulting in a higher linear flow velocity. The signal increases with thinner spacers while the noise remains more or less constant (Figure 36).

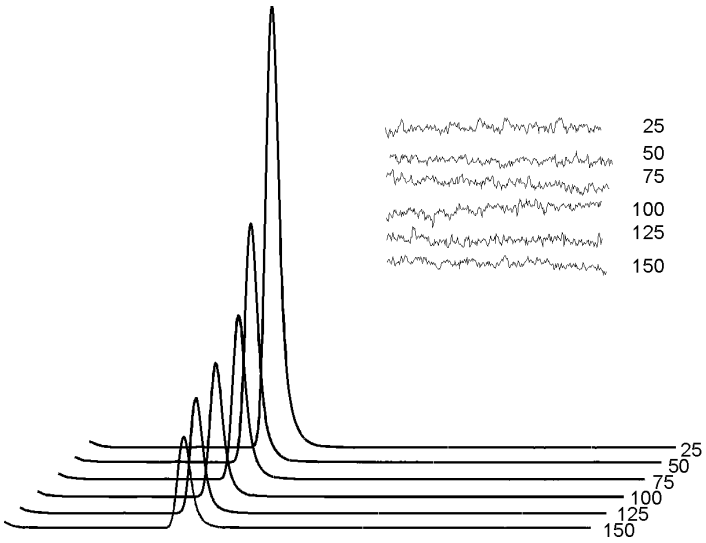


Figure 36 The signal and noise for 1.0 µmol/l norepinephrine measured at variable spacer thickness (given in µm). See Table 17 for other conditions.

Several authors have described the relation between the layer thickness (i.e. spacer thickness) in a thin layer flow cell and the measured current (S) as $S = k\ b^{-2/3}$ where b is the spacer thickness and k a constant. Also for the flow cell the relation between S and $b^{-2/3}$ results in a straight line (Figure 37).

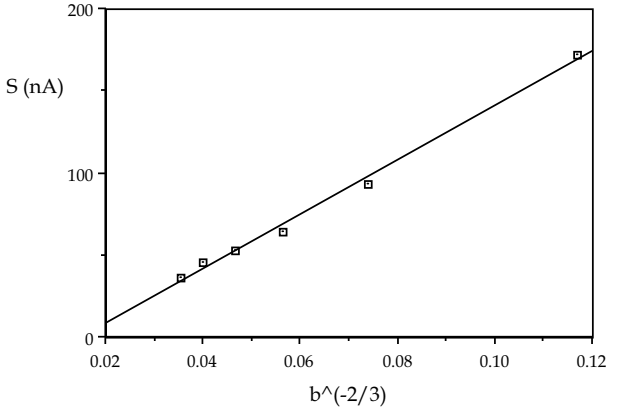


Figure 37 Peak height versus spacer thickness to the power -2/3.

Decreasing the spacer thickness is limited by an increased pressure drop over the flow cell which eventually will lead to an obstruction of the flow. The minimum spacer thickness available is 25 µm. Applying these small spacers should be done with care. Over-tightening of the bolts may cause an excessive pressure built up over the flow cell and increase the noise considerably.

Table 19 Flow cell volume

	WE diameter (mm)							
	3.00	2.74	2.54	2.00	1.90	1.00	0.75	0.50
spacer (µm)	cell volume (µl)							
25	0.18	0.15	0.13	0.08	0.07	0.020	0.011	0.005
50	0.35	0.29	0.25	0.16	0.14	0.039	0.022	0.010
120	0.85	0.71	0.61	0.38	0.34	0.094	0.053	0.024

Reference electrodes

The flow cell is available with a in situ Ag/AgCl reference electrode, a salt bridge Ag/AgCl reference electrode and the Hy-REF reference electrode.

In situ Ag/AgCl reference electrode

The ISAAC (in situ Ag/AgCl) reference electrode is in direct contact with the mobile phase which contains chloride ions. The chloride concentration determines the potential, therefore each time a fresh mobile phase is prepared it should contain exactly the same concentration of chloride ions.

The standard electrode potential of the Ag/AgCl electrode (in 1.0 mol/l Cl- solution) for the following half-reaction is defined as E0:

$$AgCl(s) + e^- \Leftrightarrow Ag(s) + Cl^- \qquad E^0 = 0.222\ V$$

The potential of the REF is dependent from the chloride concentration as described by the following equation:

$$E_{cell} = E^0_{AgCl} - \frac{RT}{F} \ln [Cl^-]$$

where R is the gas constant (8.314 Jmol-1K-1), T is the absolute temperature (293 K) and F is the Faraday constant (96485 Cmol-1).

The potential of the ISAAC at 2 mmol/l KCl is 379 mV (Table 20). The potential difference (dE) between the saturated KCl Ag/AgCl reference electrode and the ISAAC is 189 mV. If an application is running at 800 mV (vs. Ag/AgCl with sat'd KCl), the potential setting using the ISAAC should be 611 mV (vs. Ag/AgCl in 2mmol/l KCl).

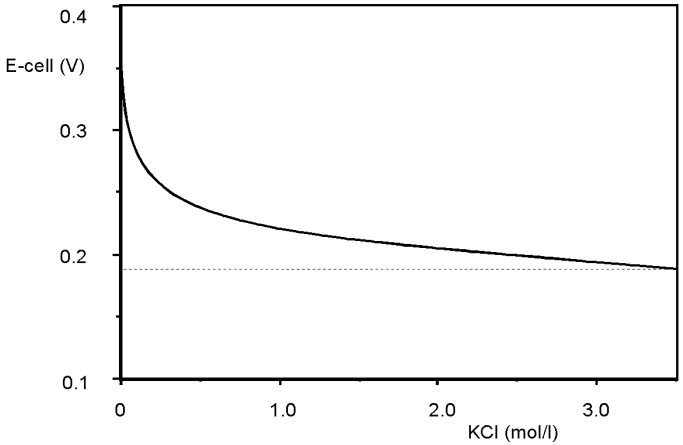


Figure 38 Dependence of the Ag/AgCl REF potential on the chloride concentration.

Table 20 Potential of the Ag/AgCl reference electrode.

Cl^- (mmol/l)	E Ag/AgCl (mV)	dE (mV)
3500	190	0
2500	199	8
1500	212	21
500	240	49
100	280	90
20	321	130
10	338	148
8.0	344	154
6.0	351	161
4.0	361	171
2.0	**379**	**189**
1.0	396	206
0.5	414	224

The addition of chloride to the mobile phase has a few restrictions. For example, the in situ Ag/AgCl is not recommended at a *high working potential* (> 1 V *vs.* Ag/AgCl in 2 mmol/l KCl) because Cl^- is oxidized and contributes to the background current. In *ion chromatography* the addition of Cl^- may lead to undesired chromatographic changes. In case of a *silver working electrode*, the addition of Cl^- to the mobile phase will cause formation of an AgCl coating on the working electrode leading to inactivation. At *high pH* or *high modifier concentrations* the ISAAC is less suitable and a Hy-REF is recommended.

Salt bridge Ag/AgCl reference electrode

The reference electrode of the Ag/AgCl type with salt bridge consists of a silver rod, coated with solid AgCl, immersed in a solution of saturated KCl, containing KCl crystals. Electrical contact with the other electrodes in the flow cell is made through a salt bridge consisting of a wetted cotton wool frit, which is electrically conducting and slows down leakage of KCl.

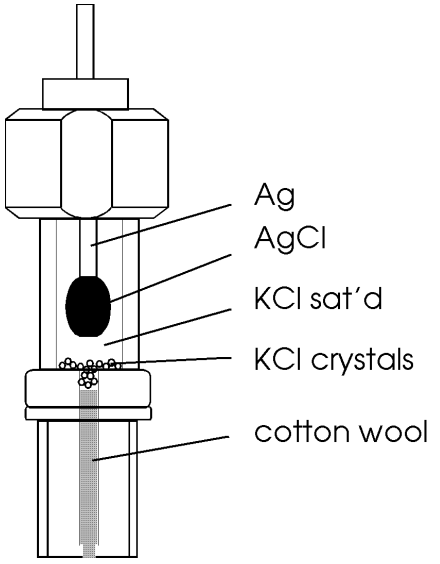


Figure 39 Schematic representation of the Ag/AgCl reference electrode.

This REF for the flow cell is factory filled with KCl. For certain applications another chloride salt is to be preferred. In case of perchlorate containing mobile phases, sodium chloride is mandatory, because potassium perchlorate precipitates and will clog the cotton wool frit. At high modifier percentages, the REF must be filled with lithium chloride for similar reasons.

Hy-REF reference electrode

The Hy-REF is a hydrogen reference electrode, its potential depends on the pH of the mobile phase. The Hy-REF is fully comparable with the standard Ag/AgCl REF as to baseline stability and S/N ratio. The Hy-REF is more user-friendly and in principle this REF is completely free of maintenance. Trapping of air bubbles like in the salt bridge Ag/AgCl type is impossible because of the absence of a salt bridge. Consequently, refilling the REF with saturated KCl is not longer required. Due to the absence of a salt bridge and its inertness, the Hy-REF is an excellent alternative for the Ag/AgCl REF, especially in case of high modifier concentrations (i.e. analysis of fat-soluble vitamins) or high pH (analysis of carbohydrates, PAD).

Depending on the pH of the mobile phase, the potential setting of the working electrode vs. the Hy-REF may differ significantly compared to Ag/AgCl.

I/E curves show a shift of more than 200 mV at pH 3.1 (e.g. catecholamines), no shift appears at pH 12 (e.g. PAD of carbohydrates). Therefore, it is advisable first to construct a hydrodynamic (or scanning) voltammogram when using the Hy-REF (see page 57). In Table 21 the potential of the Hy-REF is measured against the Ag/AgCl electrode at different pH values.

Table 21 Measured cell potential(Hy-REF - Ag/AgCl) versus pH.

PH	$E_{Hy\text{-}REF - Ag/AgCl}$ (mV)
3.3	232
6.2	130
7.5	90
11.8	0

So, if an Ag/AgCl REF is replaced by a Hy-REF, the pH effect must be taken into account (Figure 20). The pH vs. voltage relation is described by:

$$E_{Hy\text{-}REF} = E_{Ag/AgCl} - 328 + 29.9\ pH \qquad (1)$$

For example: a working potential of 800 mV (vs. Ag/AgCl with sat'd KCl) at pH 3, has to be changed to:

EHy-REF = 800 - 328 + 29.9*3 = 561.7 mV (vs. Hy-REF)

Installation and maintenance flow cell with Hy-REF or ISAAC

Installation flow cell with Hy-REF or ISAAC

The flow cell is assembled properly when it arrives. The force on the bolts is pre-set to 30 Ncm ("a little bit beyond fingertight"). Familiarise yourself with this force, since over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself. Also, be aware that the black marks on both blocks should be in line. This ensures the best performance.

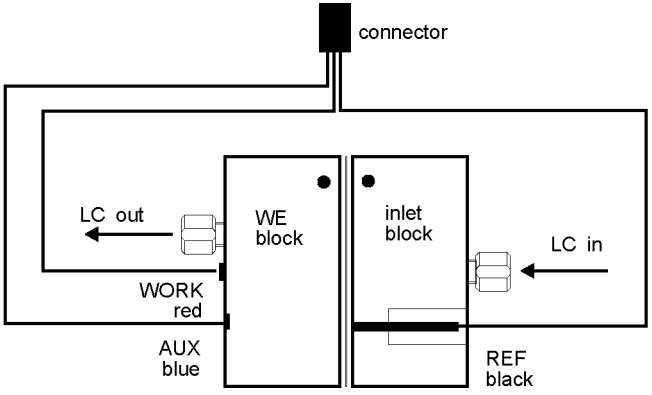


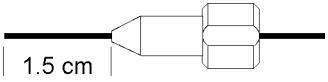
Figure 40 Installation of flow cell. WORK, AUX and REF are connected using the red, blue and black cell cable. LC out should be on top to prevent entrapment of bubbles.

The ISAAC reference electrode requires 2 mmole/l chloride ions (KCl or NaCL) in the mobile phase. Add and equilibrate before installation of the ISAAC. See page 57 for optimization of working potential

1. Connect the column outlet to the flow cell inlet, using small-bore PEEK tubing (<0.3 mm ID) and one of the fingertights supplied.

CAUTION	**Use only our factory supplied fingertights in the flow cell, others may cause serious damage!**

Let the tubing protrude for ca. 1.5 cm from the fingertight fitting and tighten it such that the tubing is not or slightly indented by the fitting.



2. Do not over-tighten the fingertight. Over-tightening affects the flow pattern through the tubing (turbulence) and may strongly decrease the flow cell performance.

3. Connect 0.5 mm ID PEEK tubing to the outlet of the flow cell. Use only our factory supplied fingertights in the flow cell, others may cause serious damage! Again (see above), do not over-tighten the fingertight.

4. Turn on the HPLC pump. Keep some tissues at hand as you probably will spill some mobile phase during this mounting procedure.

5. Fill the flow cell, by keeping it in an angle of about 45° with the outlet (LC out) on top to force the air through the outlet.

6. Position the flow cell in its clamp in the controller with the REF at the lower side and the outlet at the upper side. This excludes trapping of air bubbles.

7. Connect the cell cable as illustrated in Figure 40.

Never switch ON the flow cell when:

- **the cell cable is not correctly connected**
- **the cell is only partly (or not at all) filled with buffer**
- **the outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection**

because substantial damage to the working electrode or electronics may occur.

The maximum detection stability is attained when not only the flow cell, but also the HPLC column is incorporated in the controller. The controller has an integrated Faraday cage and an accurately thermostatted oven compartment which ensures stable working conditions. Installing the flow cell and column within such a controlled environment is the minimum requirement for high-quality LC-EC trace analyses.

Maintenance of the Hy-REF reference electrode

The Hy-REF reference electrode is in principle maintenance free. If not in use it should be stored dry after disassembling the flow cell.

Maintenance of the ISAAC reference electrode

The ISAAC reference electrode requires maintenance, usually not more than once in 3 months. In practice this means that when the flow cell is opened to service the working electrode, the reference electrode should be serviced as well.

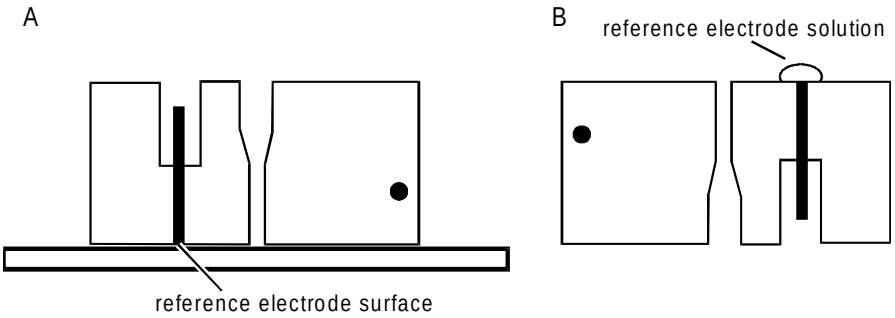


Figure 41 Servicing the ISAAC reference electrode, polishing (A) and coating (B).

Servicing the reference electrode is done by polishing the reference electrode surface until the shining metal appears (Figure 41 A). **Immediately after polishing** the electrode is coated by applying a few drops of the reference electrode solution on the reference surface (Figure 41 B). After 20 min the reference solution is flushed away with distilled water.

If not in use for longer period of time, disassemble the flow cell. The flow cell including the reference electrode should be cleaned with distilled water, dried with a tissue and stored dry.

Polishing

Polishing the reference electrode is done using the factory supplied polishing kit, containing diamond slurry and polishing disc.

1. Shake diamond slurry thoroughly before use!!

2. Rinse the polishing disc with demi water before applying the diamond slurry!

3. Apply a few drops of slurry on the wetted polishing disc, and polish the electrode with a ‘figure 8’ motion for about one minute. Apply only gentle pressure.

4. Clean the electrode with a wetted tissue and check the surface visually, repeat the procedure if necessary until the shining metal REF surface appears.

5. Clean the polishing disc with demi water.

6. Store the polishing disc dust free in its plastic bag.

Working electrode cleaning instructions

Cleaning of the working electrode block is necessary if the electrode surface has been electrochemically changed. This may be due to contamination by oxidation (reduction) reaction products. Excessively high currents also may change the electrode surface. This is noticed by a strongly decreased sensitivity after prolonged use.

As a rule of thumb: only polish if the surface of the working electrode lacks its mirror-like finish, which cannot be restored by wiping the electrode surface with a tissue wetted with ethanol or acetone.

Actions to take at decreased flow cell performance

Use proper eye and skin protection when working with solvents.

Avoid unnecessary polishing, take the next step only if the previous was not successful.

1. Electrochemical cleaning of glassy carbon WE: In the pulse mode let the potential jump between +1 and -1 V for 10 min. Settings: t1 = 1000 ms, t2 = 1000 ms, t3 = 0 ms, E1 = +1V, E2 = -1V.

2. Wiping the electrode surface with a tissue wetted with ethanol or acetone

3. Polishing the electrode surface

Polishing

1. Shake diamond slurry thoroughly before use!!
2. Rinse the polishing disc with demi water before applying the diamond slurry!
3. Apply a small amount, **a few drops** is sufficient, of slurry on the wetted polishing disc, and polish the electrode with a ‘figure 8’ motion for about one minute. Apply only gentle pressure.
4. Clean the electrode with an ethanol-wetted tissue and check the surface visually; repeat the procedure if necessary.
5. Reassemble the detector cell.
6. Clean the polishing disc with demi water.
7. Store the polishing disc dust free in its plastic bag.

Installation and maintenance of flow cell with saltbridge REF

Installation flow cell

The flow cell is assembled properly when it arrives. The force on the bolts is pre-set to 30 Ncm (“a little bit beyond fingertight”). Familiarise yourself with this force, since over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself. Also, be aware that the black marks on both blocks should be in line. This ensures the best performance.

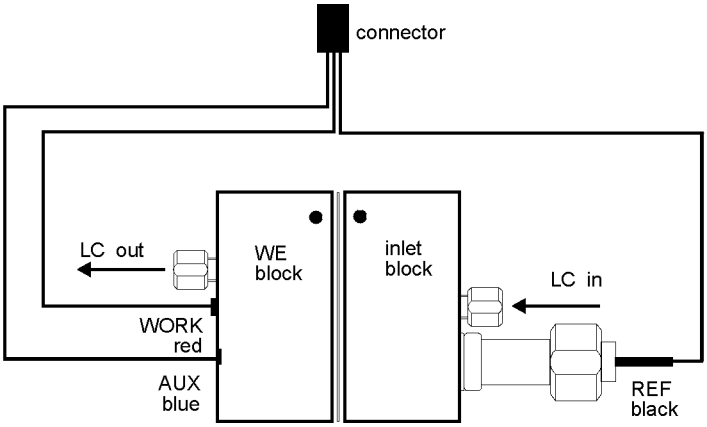


Figure 42 Installation of flow cell. WORK, AUX and REF are connected using the red, blue and black cell cable. The LC outlet is placed on top to prevent entrapment of bubbles.

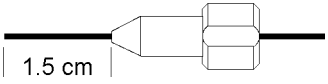
Use proper eye and skin protection when working with solvents.

1. Check the REF visually for air bubbles and saturation with KCl. Some KCl crystals should be visible. When no crystals

are visible or air bubbles are trapped the REF needs maintenance (see page 94). To prevent drying-out the REF is sealed with a cap on arrival. Remove the cap.

2. Tighten the black swivel of the REF, a small droplet should appear at the cotton-wool frit. Do not remove this droplet because it ensures proper contact of the REF with the mobile phase.

3. Turn on the HPLC pump. Place some tissues as some mobile phase may be spilled during this mounting procedure.
   Connect the column outlet to the flow cell inlet, using small-bore PEEK tubing (<0.3 mm ID) and one of the fingertights supplied. Use only our factory supplied fingertights in the flow cell, others may cause serious damage! Let the tubing protrude for ca. 1.5 cm from the fingertight and tighten it carefully.



   Over-tightening affects the flow through the tubing (turbulence) and decreases the flow cell performance.

4. Connect 0.5 mm ID PEEK tubing to the outlet of the flow cell. Use only our factory supplied fingertights in the flow cell, others may cause serious damage! Again (see above), do not over-tighten the fingertight.

5. Fill the flow cell, by keeping it in an angle of 45° with the REF fitting on top, is best done by blocking the outlet with a finger and letting the air escape via the REF fitting. Carefully check the thread of the fitting for trapped air bubbles.

6. When the REF fitting is completely filled with mobile phase, mount the REF while slowly releasing the outlet. Make sure not to include an air bubble!

7. Position the flow cell in its clamp in the controller with the REF at the lower side and the outlet at the upper side. This excludes trapping of air bubbles by the REF.

8. Connect the cell cable as illustrated in Figure 42.

Never switch ON the flow cell when:

- **the cell cable is not correctly connected**
- **the cell is only partly (or not at all) filled with buffer**
- **the outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection**

because substantial damage to the working electrode or electronics may occur.

The maximum detection stability is attained when not only the flow cell, but also the HPLC column is incorporated in the controller. The controller has an integrated Faraday cage and an accurately thermostatted oven compartment which ensures stable working conditions. Installing the flow cell and column within such a controlled environment is the minimum requirement for high-quality LC-EC trace analyses.

When not in use, please store the REF with the cotton wool frit immersed in a saturated KCl solution to prevent drying out.

Maintenance of the Ag/AgCl reference electrode

Three aspects determine the proper function of an Ag/AgCl reference electrode: *1.* The chloride concentration must be kept at a strictly fixed level. This is best guaranteed by using a saturated chloride salt solution at a constant temperature. *2.* The salt bridge must allow proper electrical contact with the mobile phase. The higher the leakage through the frit the better the conduction. This conflicts with the previous point. *3.* Air bubbles inside or close to the salt bridge will lead to instability of the three-electrode configuration. Because of their extreme compressibility, changes in conductivity and the ionic equilibrium of the REF occur. This increases the noise considerably.

The REF is factory filled with KCl unless specified otherwise. Other chloride salts should be used when the mobile phase contains perchlorate (use NaCl) or a high percentage of organic modifier (use LiCl).

Saturation and air bubbles

After prolonged use the salt bridge in the REF will not be saturated any more, which usually leads to a poor reproducibility in electrochemical detection. The potential of the REF is determined by the chloride concentration (see page 83). If the salt bridge is not saturated and the KCl concentration changes:

1. the noise in the system will slowly but continuously increase,
2. the background current will increase,
3. sensitivity for movements and pump noise will increase.

If an air bubble is trapped in the salt bridge or in the cotton plug that separates the salt bridge and the mobile phase the flow cell becomes extremely sensitive towards flow fluctuations and vibrations. This is caused by the high compressibility of the trapped air.

NOTE: **Check your REF regularly. If you do not see chloride salt crystals or if you see air bubbles, your REF needs maintenance.**

Material

1. An over-saturated and thoroughly degassed KCl solution.
2. A stainless steel rod of about 5 cm length and a diameter of 1 mm (e.g. a 1 mm drill).
3. Ordinary cotton wool.

Procedure

Use proper eye and skin protection when working with solvents.

1. Turn the cell off on the controller.
2. Stop the HPLC pump.
3. Disconnect the cell from the controller.
4. Remove the REF from the inlet block.
5. Disassemble the REF by unscrewing the black swivel (Figure 43).
6. Inspect the Vyton rings for wearing and especially the cotton wool frit, replace if required (see below).

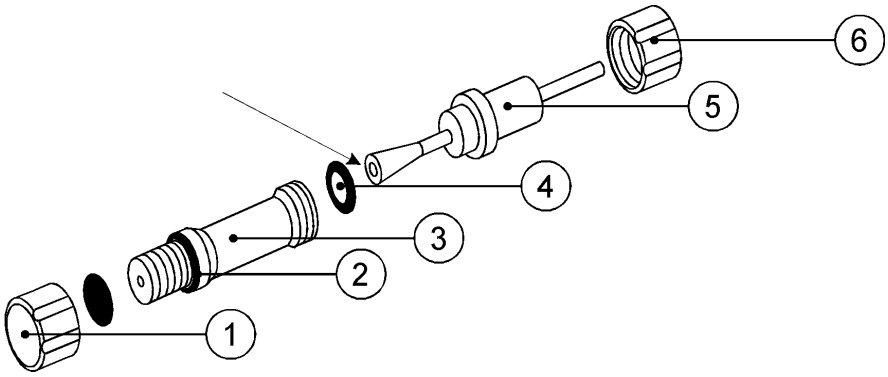


Figure 43 Exploded view of the reference electrode. The arrow indicates the tip of the AgCl coated silver rod. See Table 22for description and part numbers.

1. Remove the remaining KCl from the salt bridge.

2. Clean all parts with demi-water.

3. The Ag/AgCl electrode must be cleaned if the silver on the tip (Figure 43, arrow) has a non-metallic appearance by gently grinding it on sanding paper; also the AgCl can be gently resurfaced in this way.

4. The frit in the salt bridge ensures electrical contact with the buffer. If the frit is discoloured or dried out, it has to be renewed. In that case continue with 'Maintenance of the cotton wool frit' step 1. Otherwise, continue with step 7.

Table 22 REF parts and description.

Item	Description
1	REF cap for storage and shipment
2	Vyton ring (large)
3	Salt bridge
4	Vyton ring (small)
5	Ag/AgCl electrode + fitting
6	Swivel for REF

Maintenance of the cotton wool frit

Use proper eye and skin protection when working with solvents.

1. Use a drill of ± 1 mm (0.039 inch) to push out the frit from the outside (Figure 44). Be careful not to damage the frit constriction (first arrow).

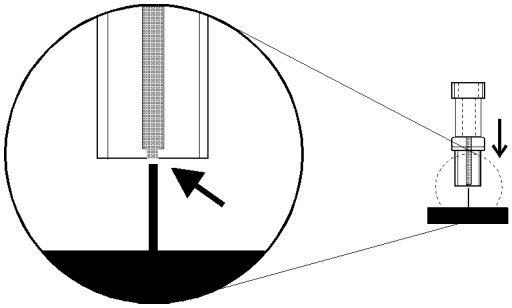


Figure 44 Pushing the cotton wool frit out.

2. Clean the salt bridge thoroughly by tap water and demi-water respectively.

3. Saturate a small piece of cotton-wool in saturated KCl to exclude trapping of air within the wool.

4. Plug the salt bridge with the REF cap and fill the salt bridge for ± 50%.

5. Use the drill to pack the wool from above through the KCl solution into the channel of the salt bridge, compress it firmly, but not too much, since electrical conduction is essential.

6. Remove the cap.

7. Fill the salt bridge completely, add some KCl crystals out of a saturated solution to ensure prolonged saturation.

8. Place the small Viton ring over the Ag/AgCl electrode and slowly insert it, in an angle of 45□ into the salt bridge. Make sure not to enclose an air bubble.

9. Tighten the black swivel such that a small droplet appears at the end of the salt bridge, but do not over-tighten the swivel.

10. Flush the complete, mounted REF with demi-water, dry it with a tissue, but keep the cotton wool frit soaked.

11. Carefully inspect the REF visually for trapped air bubbles, otherwise remove them (go back to step 7 or if necessary step 1).

NOTE: **When not in use, please store the REF with the cotton wool frit immersed in a saturated KCl solution to prevent drying out.**

For maintenance of the working electrode see page 90, ‘Working electrode cleaning instructions’.

Installation micro flow cell

The micro flow cell is assembled properly when it arrives. The force on the bolts is pre-set to 30 Ncm (“a little bit beyond fingertight”). Familiarize yourself with this force, since over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself. Also, be aware that the black marks on both blocks should be in line. This ensures the best performance.

Use proper eye and skin protection when working with solvents.

If the capillary connection has already been installed properly, continue with step 9. If not, start with step 1.

To prevent any damage to the flow cell the following steps should be carried out:

1. Insert the fused silica capillary into the tightly fitting Teflon sleeve supplied.

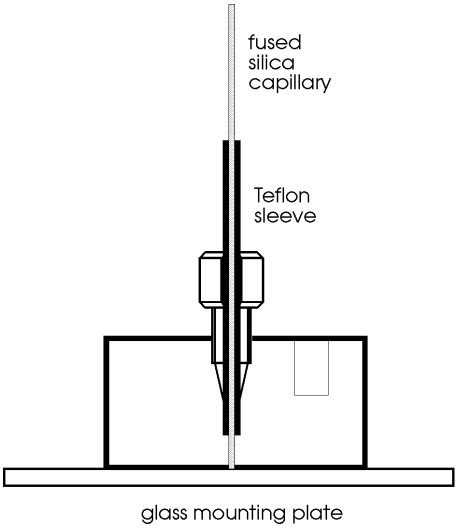


Figure 45 Mounting of the fused silica connector in the micro flow cell.

2. Protrude both through our factory supplied fingertight fitting.

CAUTION

Use only our factory supplied fingertights in the flow cell, others may cause serious damage!

3. Mount this combination carefully in the injection block.
4. Let the fused silica slightly (0.5-1 mm) protrude through the injection hole.
5. **Clean the factory supplied glass mounting plate from particles.**
6. Carefully push the block on the glass plate until the silica capillary is flush with the surface.
7. Fix the fused silica capillary firmly with the fingertight while keeping a slight pressure of the block on the glass plate.

8. Mount the two flow cell blocks by crosswise tightening of the bolts (max. 30 Ncm).

9. Continue the installation as described on page 86 (ISAAC REF) at point 3 or on page 92 (salt bridge REF) at point 1 (skip point 3), depending on the type of reference electrode used.

Never switch ON the flow cell when:

- **the cell cable is not correctly connected**
- **the cell is only partly (or not at all) filled with buffer**
- **the outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection**

because substantial damage to the working electrode or electronics may occur.

Error messages

Error	Message
Error 1	Current overload, maximum current + maximum compensation has been exceeded. Advise: use less sensitive range in ‘Range select’ screen.
Error 2	A ROM failure has been detected. Please contact supplier.
Error 3	A RAM failure has been detected. Please contact supplier.
Error 4	The system cannot detect the cell controller board. Please contact supplier.
Error 5	The system cannot detect the I/O board. Please contact supplier.
Error 6	The dipswitches on the I/O board have been wrongly set. Please contact supplier.
Error 7	A critical failure has been detected. False data have been written in RAM. Please contact supplier.
RECOVLD	Recorder output is either above +1.0 V or under -1.0 V. Pressing ‘A-ZERO’ may give an adequate read-out again. If the message ‘ERROR 1’ is displayed, immediately after pressing ‘A-ZERO’, the autozero function is unable to compensate for the offset and is out of the recorder range. Advise: use less sensitive range in ‘Range select’ screen (see page 69).
OVLD	In pulse mode: charging current out of range. Pressing ‘A-ZERO’ may give an adequate read-out again. If not, it is advisable to change the pulse settings (larger t1) or to use a less sensitive range.

Dipswitch settings I/O board

switch	comment	on	off
1-1	electrically actuated injector	present	absent
1-2	injector (e.g. Rheodyne 7725i)	present	absent
1-3	not used		
1-4	not used		

Rear panel

TTL inputs

The inputs on the lower I/O connector strip, ‘start’ and ‘autozero’ require a TTL-low pulse (minimal 100 ms) to be activated. One TTL-low pulse produces one activation, if more activations are required the next pulse must be given after 100 ms TTL-high. If the input is kept low, only one activation will result. See page 46 for detailed information on the I/O contacts.

3*99939

Figure 46 ProStar 370 rear panel.

RS232C (option)

The RS232 option consists of a ProStar 370 EPROM with R-extension. This interface provides full parametric control with a time resolution of 1 second. Programmable parameters comprise cell potential, range, autozero, offset, rise time, electrical injector and control of ProStar 370 output contacts for control of external equipment.

Electric valve

Connector for an external electrically actuated valve (e.g. Valco CC 0171) . Can be used together with INJ command on the keyboard (page 20), and the ‘to pos A’, ‘to pos B’ and ‘inject marker’ on the 12 pins connector (page 46).

Manual valve

Connector for manual valve (e.g. Rheodyne 7725i) . Can be used together with INJ command on the keyboard (page 20), and the ‘inject/load’ output on the 12 pins connector (page 46).

INT

Analogue output, see page 24 for details.

REC

Digital output, see page 24 for details.

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