ANTEC
LEYDEN

INTRO

Integrated Detector for Reduction and Oxidation

user manual

CE

Declaration of conformity

We Antec Leyden B.V., Zoeterwoude, The Netherlands, declare that the product

Electrochemical Detector INTRO (p.n. 130.0035)

to which this declaration relates, is in conformity with the following directives:

Safety (73/23/EEC)

Safety requirements for laboratory equipment (Class I, Installation cat. II, Pollution degree 2)	IEC 1010-1

Immunity (89/336/EEC)

Electromagnetic immunity	IEC 801-2/3/4 & ENV 50140
Radio frequency current injection	ENV 50141 & IEC 1000-4-6
Voltage dips and interruptions	IEC 1000-4-11

Emissions (89/336/EEC)

Electromagnetic radiation	EN 55022, Class B (CISPR 22)

Attention

Use shielded cable(s) to connect all I/O's with other devices. Thoroughly connect the shielding to common. Antec Leyden will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices which do not meet relevant safety standards.

February 21, 2007

Intended use

For research purposes only. While clinical applications may be shown, this instrument is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.

WEEE directive

All equipment of Antec Leyden which are subjected to the WEEE directive shipped after August 13, 2005 are compliant with the WEEE marking requirements. Such products are labelled with the “crossed out wheelie”, depicted on the left site.

The symbol on the product indicates that the product must not be disposed as unsorted municipality waste.

Collection & recycling information

Please ship the instrument back to the manufacturer (Antec Leyden, the Netherlands) at the end-of-life time of the product. The manufacturer will take care of the proper disposal and recycling of the instrument at its facilities.

Shipping address for the end-of-life products:

Antec Leyden
Industrieweg 12
2382NV Zoeterwoude
The Netherlands

In case of questions, or if further information is required about the collection & recycling procedure, please contact your local distributor.

ROHS directive

Our instruments are currently exempt from the RoHS directive because they fall under WEEE Annex IA categories 8 and 9, which includes medical devices and monitoring and control instruments. Nevertheless, we have taken steps to eliminate all restricted substances from our products.

Antec Leyden is an ISO 9001:2000 certified company.

Safety practices

The following safety practices are intended to insure safe operation of the equipment.

Electrical hazards

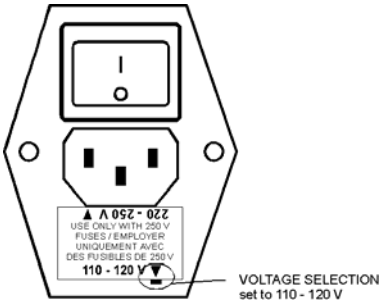
1. Disassembly exposes potentially dangerous voltages. Therefore, disconnect the instrument from all power sources before disassembly.

⚠ WARNING - RISK OF ELECTRIC SHOCK DISCONNECT POWER BEFORE SERVICING AVERTISSEMENT - RISQUE DE CHOC ELECTRIQUE COUPER L'ALIMENTATION AVANT LA MAINTENANCE

2. Replace blown fuses with size and rating stipulated on the rear panel, and in the manual where listed.

⚠ 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

3. Replace faulty or frayed power cords.
4. Check whether the voltage selector is in the correct position. If the triangle with the voltage range is pointing towards the small white block, the system is set to that line voltage. If not correct this insert has to be reversed. Also the fuses are included in the line connector. The correct values are given on the rear panel for the different line sources.



General precautions

1. Perform periodic leak checks on LC tubing and connections.

2. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.

Spare parts and service availability

Antec Leyden provides operational spare parts of the instrument and current accessories for a period of five years after shipment of the final production run of the instrument. Spare parts will be available after this five years period but on an ‘as available’ basis.
Antec Leyden provides a variety of services to support her customers after warranty expiration. Repair service can be provided on a time and material basis. Technical support and training can be provided by qualified chemists on both contractual or as-needed basis.

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INTRO

Congratulations with the purchase of your INTRO (INTegrated detector for Reduction and Oxidation). This analog electrochemical (EC) workstation warrants optimal EC conditions by means of a high degree of integration. Not only the flow cell is incorporated in the Faraday-shielded oven compartment, but also (optional) the LC column, a pulse dampener and injector.

The INTRO offers flexible but very stable working conditions even in ultra-trace analyses, combined with functional transparency and ease of use. Hence all crucial functions and values are ranked in logical order and can be monitored with high accuracy. Especially the cell or background current (I_{cell}), considered to be a crucial parameter for high-quality LC-EC, can be checked continuously with high, i.e. pico-ampere, resolution.

The INTRO has been designed for continuous use and therefore shows its maximum stability under such working conditions.

Fig. 1. The INTRO with Faraday-shielded oven compartment which accommodates flow cell, column, injector and pulse dampener.

C H A P T E R 2

Installation guide

Inspect the *transport box* for possible damage as it arrives. Immediately inform the transport company in case of damage, otherwise she may not accept any responsibility. Keep the transport box as it is designed for optimum protection during transport and it may be needed again. After unpacking the unit 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 inspected and tested to ensure the best possible performance. The results of the electronic tests are included in the shipkit.

Installation

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

1. To unpack the INTRO, lift it from its transport box by both hands as indicated in Fig. 2A. In any other situation the INTRO should be lifted by both hands under the unit as indicated in Fig. 2B. **Never lift the INTRO by the cover.**

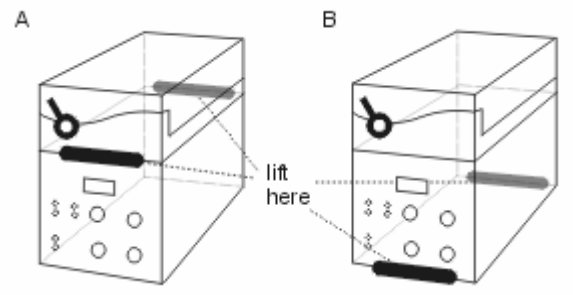
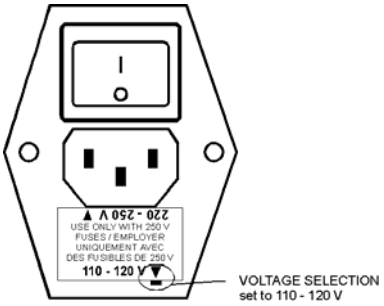


Fig. 2. Lift instructions INTRO.

2. Inspect the system for possible damage and make sure that all marked items on the checklist are included.
3. Position the INTRO preferably on the right-hand side of the HPLC system because the fluid lines can be hooked up then in the most direct way.
4. Check whether the voltage selector is in the correct position i.e. identical with the voltage of your local power supply. If the triangle with the voltage

range is pointing towards the small white block, the system is set to that line voltage. If not correct, this insert 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 values are given on the rear panel for the different line sources.
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).

5. Switch ON the INTRO 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, column and the injection valve (option) and pulse dampener (option) is schematically shown in Fig. 3.

6. In using PEEK tubing it is strongly advised to use an optional bulkhead union to enter the Faraday shield of the INTRO, irrespective of the presence or absence of a manual injector. Thus a proper grounding of the mobile phase is acquired excluding any possible interruption of the Faraday function.
7. If ordered, install the pulse dampener. Connect the mounting plate with four bolts to the pulse dampener and mount the pulse dampener with four bolts. For detailed instructions on the pulse dampener, see page 41.
8. If ordered, the Antec supplied Rheodyne valve is equipped with a Vespel rotor. In strongly alkaline solutions (pH > 10), this should be replaced by a Tefzel rotor. Consult your supplier for details.

9. For installation of a Rheodyne injector remove the handle assembly and place the body in the INTRO using the 2 factory supplied mounting screws. Re-assemble the handle assembly using the 2 set screws.
10. If the Rheodyne 7725i or another Rheodyne 'i' version injector is applied, it is only part of the Faraday shield if the yellow wire from the cell cable is clamped on a metal part of the injector. The other end of the yellow cable is connected to 'SHIELD' on the connection plate for the flow cell cable. The sensor cable must be connected to the phone jack (P1) to make the LOAD/INJECT output on the rear panel functional. If no injector is mounted a plug is installed. Use of this plug does not affect the Faraday function.

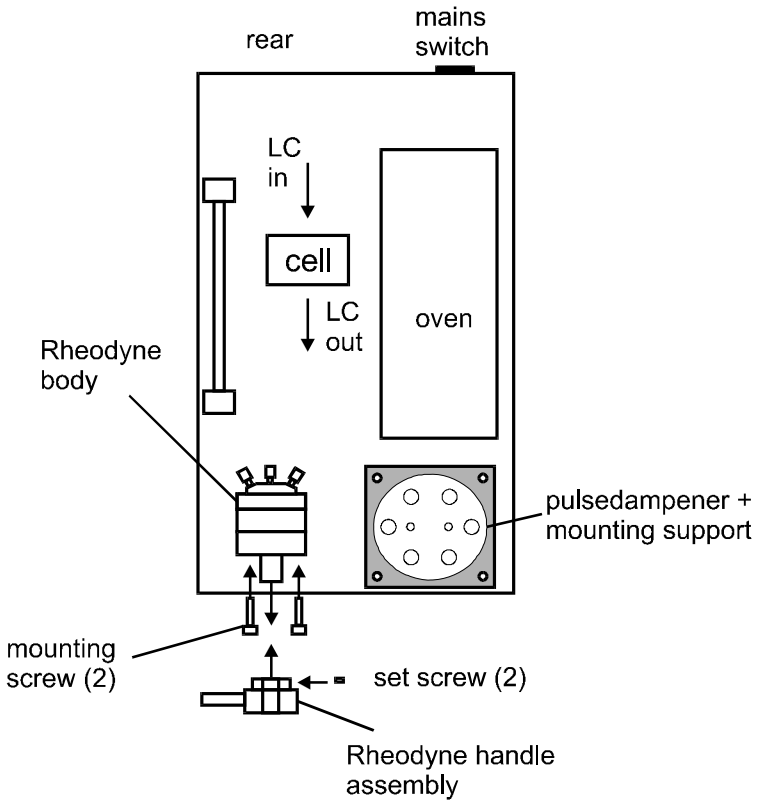


Fig. 3. Installation of the INTRO.

11. In case of leakage the drain ensures that the oven compartment is not flooded. The drain can be lead to waste by means of a wide bore tubing (6 mm diameter). **Do not use narrow bore tubing as this obstructs the drain. The waste should be placed *below* the drain level.** For proper function of the drain the detector must be placed level.
12. Prior to connection of the HPLC system to the detector all metal parts should preferably be passivated with 15% nitric acid during 20 min. The

acid is flushed through the pump, the pump tubing, the dampener, the injector (in load and inject position) and to waste. **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.**

13. 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.

If the ISAAC reference electrode is used, add 2 mmole/l chloride ions (e.g. KCl or NaCL) to the mobile phase. Equilibrate HPLC and continue installation.

14. Before connecting a new column read the manufacturer's instructions. Pre-conditioning of the column may be necessary. A pre-conditioned column is electrochemically clean. If not, the background current may be unacceptably high. For *reversed phase* columns flushing with methanol for 3 days at a low flow rate is recommended.
15. Passage or entrapment of air bubbles in the flow cell will lead to unacceptable noise levels. Therefore, the use of an in-line degasser in the LC system is recommended. In our experience, a one-time degassing step of the HPLC buffer is usually not sufficient.
16. If the detector 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).
17. Install the flow cell as described in the flow cell manual.

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 cell is wet outside, particularly the part between the auxiliary and working electrode connection
because substantial damage to the working electrode or electronics may occur.

18. Before switching ON the flow cell, make sure that the buffer is containing electrolyte (buffer ions). If the cell is switched ON with only water or methanol in the mobile phase, a stable baseline will not be obtained. Also make sure that no air bubbles are trapped in the flow cell.
19. 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 small back pressure which

prevents air-bubble entrapment. The outlet tubing should be *under* the liquid level, to avoid noise due to dripping.

20. Select 'V_{cell}' with the DISPLAY knob and set the cell potential by pressing '^' or 'v'. Switch ON the flow cell and allow the system to stabilise for approximately 30 min.
21. Connect the data system to the integrator or recorder output (see page 21).

Your system is now ready for use.

The INTRO has been developed for continuous operation. For maximum stability it is advised to leave the power ON continuously. If preferred, the flow cell may be switched OFF at night.

C H A P T E R 3

The INTRO controller

Introduction

The INTRO 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 without problems. This chapter is intended as a reference guide in case questions arise during operation.

Front panel

Parameters accessible at the front panel are cell potential, temperature, zero setting, marker, rise time filter, attenuation and range. Using the DISPLAY knob enables selection of a parameter in the liquid crystal display (LCD).

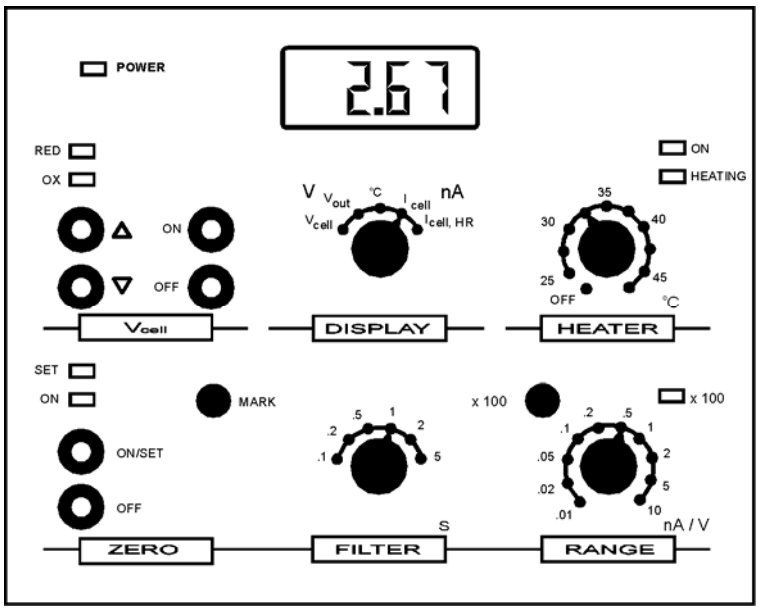


Fig. 4. The INTRO front panel is divided into 6 functional frames: V_{CELL}, DISPLAY, HEATER, ZERO, FILTER and RANGE.

V_{CELL}

In this frame the cell potential can be selected and the cell can be switched ON or OFF. To exclude erroneous cell potential settings, the up (^) and down (v) button is only accessible when 'V_{cell}' is selected with the DISPLAY knob.
Shortly pressing the up or down button results in a 1 mV change, continuously pressing allows faster changes. If the cell is ON, the OX (V_{cell} positive) or RED-LED (V_{cell} negative) lights up.
The flow cell can be switched ON and OFF by external equipment as well, via the I/O connector on the rear panel.

DISPLAY

The DISPLAY knob enables EC parameter selection in the liquid crystal display (LCD). A very important parameter is the cell current which is monitored immediately after the I/E converter. This means that it always reflects the real value, unaffected by offset, range, zero, rise time filter or dipswitch settings. In Table I a description of the DISPLAY parameters are given.

Table I. Description of DISPLAY parameters.

Parameter	Description
V_{cell}	Shows working potential, allows changing cell potential (only in this position)
V_{out}	Reveals 10.0 V max. recorder output voltage, irrespective of dipswitch setting
^{o}C	Gives the actual oven temperature with 0.1^{o}C resolution
I_{cell}	Monitors cell current up to 19.99 or 1999 nA (att. 100)
I_{cell} HR	Monitors cell current at a high resolution (HR) up to 1.999 or 199.9 nA (att. 100)

HEATER

A number of different temperatures can be selected from 25°C to 47.5°C, with 2.5°C increments. If a temperature is selected the ON-LED is on, the HEATING-LED indicates heating of the oven. When the pre-set temperature is reached, the oven regulates the temperature, indicated by flashing of the HEATING-LED.
It is important to realise that flashing of the HEATING-LED indicates that the oven is stable at the chosen temperature. If a temperature at or below ambient is selected the oven does not heat up and is not effective (HEATING-LED is off), only the ON-LED will switch on.
At a temperature of at least 5 °C above ambient the accuracy of the oven is better than 0.5°C, the temperature stability which determines stable working conditions is better than 0.1°C. Please be aware that when the oven has

attained its pre-set value, it may still take several minutes before the baseline is stable, especially at high sensitivity settings. This is caused by the column and (if included) also the pulse dampener, which both heat up rather slowly. From the I/O connector on the rear panel the actual temperature can be monitored. If opened, the oven is switched off automatically by a micro switch (HEATING-LED: off), in order to prevent accidental overheating.

ZERO

The *recorder* output voltage can be compensated to 0 V or a pre-set offset voltage, by pressing the 'ON/SET' button or by activating ZERO ON/SET from the rear panel. The ON-LED indicates that the recorder output is compensated. If the 'ON/SET' button is pressed the SET-LED lights up and remains on until the compensation value has been attained. Switching OFF the zero, also inactivates the offset level. Once set, the offset value is not erased. Pressing ZERO ON/SET again, resets the output voltage to the offset level.
The maximum cell current that can be compensated in the oxidative mode is 3500 nA, in the reductive mode this is 12000 nA (see Table II). If these values are exceeded, the zero can be switched off and detection settings have to be optimised towards a lower background current.
The dynamic range (peak current **including** background current) amounts to 125 nA or 12.5 µA (att. 100). The zero compensation does not extend the dynamic range. For example, with a cell current of 1800 nA the maximum peak current that can be handled amounts to 12500 - 1800 = 11700 nA (att. 100).

Table II. Maximum zero compensation at an oxidative (OX) or reductive (RED) working potential.

	max. comp. OX (nA)		max. comp. RED (nA)	
dip 2	att. 1	att. 100	att. 1	att. 100
off	8.5	850	85	8500
on	35	3500	120	12000

The resolution of the zero compensation is 16 bits per selected range. If a high maximum compensation is selected, the compensation circuit uses larger steps to reach a 0 V output, hence the resolution of the zero compensation decreases. Consequently, at a sensitive range setting and a large maximum zero compensation, the zero value may differ somewhat from 0V. The maximum zero compensation should therefore be chosen as small as possible. In practise, a compromise must be made between maximum zero compensation and resolution.

To prevent an off-scale signal, the zero circuit has been designed in a way that if the zero is not precisely 0 V, the deviation will always be *positive* from zero.

MARK

Activation results in a 'spike' on the *recorder* output (1 or 0.1 V, dipswitch 4 off or on, respectively). The marker can be activated from the rear panel as well.

FILTER

The rise time filter smoothens the *recorder* output. It should be used with care, since for fast peaks it may change the peak shape as well. Filter settings of 0.1 - 5.0 s in 1, 2 and 5 steps are available. For most applications a risetime of 1 s can be used without problems (Fig. 5).

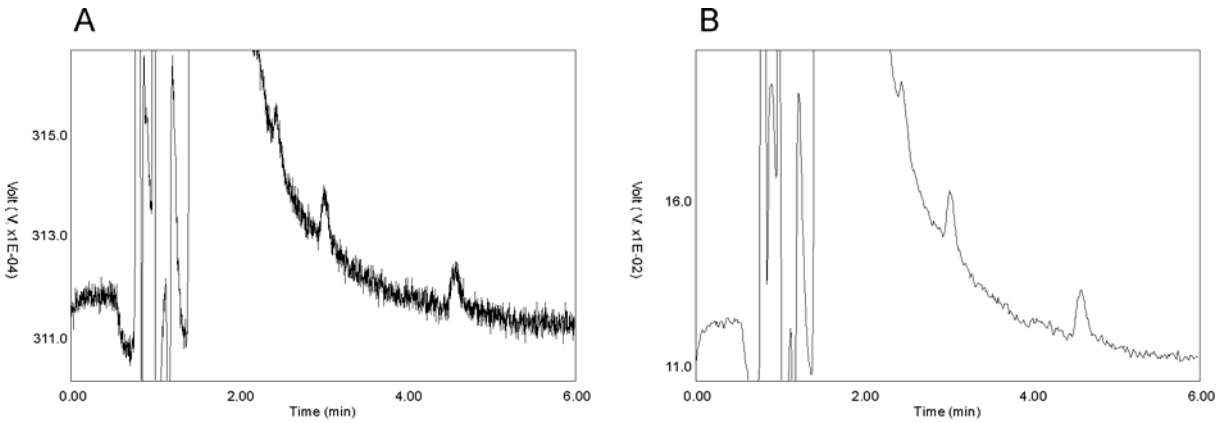


Fig. 5. Chromatogram obtained without filter (A) or with a rise time filter of 1 s (B).

RANGE

The range only affects the *recorder* output. Range settings of 0.01 - 10 nA/V in 1, 2 and 5 steps are available. The ranges refer to a 10.0 V maximum recorder output. For a 1.0 V maximum recorder output the nA value must be multiplied by 10 (see also page 26). The recorder ranges in combination with the attenuation show overlap. This offers additional flexibility as to the maximum zero compensation (see page 19).

x 100 attenuation

When activated (LED is on), the "x 100" attenuation switches to a factor 100 less sensitive range setting on the Recorder output. In other words, when the range button is set to 0.5 nA/V and "x 100" is switched on the actual range is 50 nA/V.
The integrator output has only 2 settings that are affected by the x100 only. The integrator range is 100 nA FS (x100 = off) or 10.0 µA FS (x100 = on).

The range is given as nA FS (full scale) which is referring to the 1.0 V or 10.0 V maximum output setting. If the maximum integrator output is set at 10.0 V (dipswitch 3 = off), the range is thus 100 nA/ 10.0 V = 10 nA/V.

Rear panel

The rear panel consists of a 10 pins I/O connector, four dipswitches, an integrator and a recorder output with adjustable offset, and the mains switch.

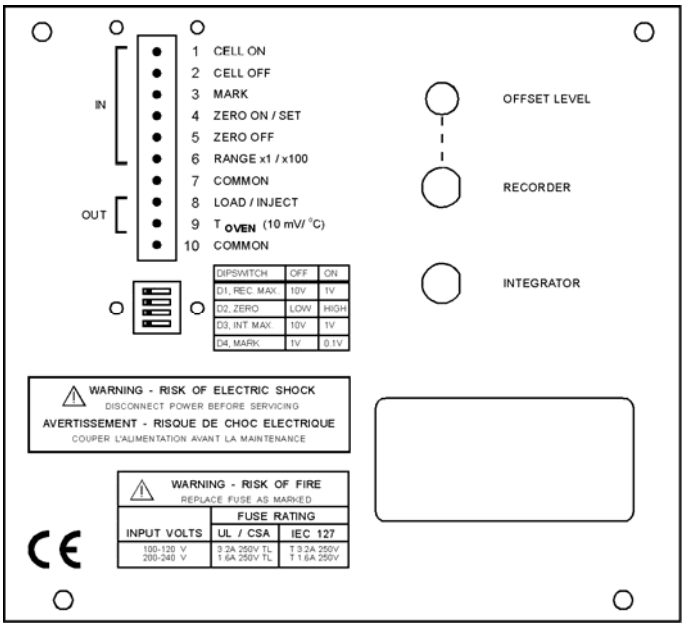


Fig. 6. INTRO rear panel.

Dipswitches

The dipswitches for the recorder and integrator output signal, are usually set at the same value as the data acquisition input voltage. For a 10.0 V input channel, the dipswitches 1 and 3 are set 'off', for a 1.0 V input channel the dipswitches are set 'on'. The DISPLAY value of V_{out} at the front panel is **not** changed by the dipswitch settings. If dipswitch 2 is set 'on', the maximum zero level is increased by a factor of 4 (see page 19).

Table III. Dipswitch settings, default is 'off'.

dip.	description	off	on
1	recorder output max. voltage	10.0 V	1.0 V
2*	max. zero compensation att. 1 att. 100	8.5 (85) nA 850 (8500) nA	35 (120) nA 3500 (12000) nA
3	integrator output max. voltage	10.0 V	1.0 V
4	marker output voltage	1.0 V	0.1 V

* Maximum zero compensation in the oxidative mode, between brackets the values for the reductive mode are given.

I/O connector strip

The I/O functions are TTL compatible, the default status is 'high' (not active). There are at least two ways to activate an input by a TTL-low pulse. This can be done by making a contact closure of the input with common 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 as the INTRO (connect common of both systems).
It should be realised that only a TTL *pulse* is required, after activation the output should be made high again. If for example the ZERO ON/SET is kept low, the recorder output will be continuously set to 0 V and detection will not be possible.

Table IV. I/O contacts 10 pins connector. Default status is 'high' (5 V).

No.	Name	Equiv. on front panel	Function
1	CELL ON	V_{cell} ON	Switches on the flow cell
2	CELL OFF	V_{cell} OFF	Switches off the flow cell
3	MARK	MARK	Triggers marker signal
4	ZERO ON/SET	ZERO ON/SET	Forces recorder output to 0 V, or to offset level
5	ZERO OFF	ZERO OFF	Inactivates zero compensation
6	RANGE x1/ x100	RANGE X 100	Attenuation: high: att. 100, low: att. 1
7	COMMON	-	To be used in combination with other inputs
8	LOAD/INJECT	-	Rheodyne 7725i, high: LOAD, low: INJECT
9	T_{OVEN}	DISPLAY: °C	Temperature read-out: 10 mV/°C
10	COMMON	-	To be used in combination with other inputs

Antec Leyden will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices which do not meet relevant safety standards.

A frequently used function is LOAD/INJECT as a trigger for starting integration software. The Rheodyne 7725i is equipped with an internal switch which is connected by a cable in the oven cabinet (P1) to the LOAD/INJECT of the rear panel. The status is 'high' if the valve is in 'load' position, and 'low' in 'inject'.
The oven temperature can be monitored between T_{OVEN} and common. For example, an output voltage of 301 mV reflects an actual temperature of 30.1 ^{o}C.

Offset

The offset is only active on the *recorder* output, and only when the zero is ON. It can be adjusted by a potentiometer at the rear panel, using the factory supplied screwdriver. **The effect on the baseline and in the DISPLAY (V_{out}) can be monitored during adjustment while keeping the ZERO 'ON/SET' button pressed**.

A maximum of +/- 150 mV (10.0 V output) or +/- 15 mV (1.0 V output) can be set. The maximum current that can be handled is not affected by the offset. Turning OFF the zero also inactivates the offset level (see Fig. 7).

Cleaning

If the unit is stained wipe out dirt or dust with soft cloth or tissue. If necessary use a water-wetted cloth or tissue with a synthetic detergent. **Do not use organic solvents**.

Notes on service

The equipment should be serviced by qualified service personnel only when:
- Liquid has been spilled into the (lower) electronics compartment.
- The equipment does not operate normally and exhibits a marked change in performance.

Do not open the unit by unscrewing. There are no user serviceable parts inside. The user should not attempt to service the equipment beyond the scope that is described in the trouble shooting guide (page 33). All other servicing should be referred to qualified personnel.

C H A P T E R 4

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 INTRO covers such a wide range from 10 µA down to 10 pA full scale, without being limited by electronic noise or instability.
The INTRO is equipped with two output connections for data acquisition, the recorder and the integrator output. The details on data acquisition will be discussed for both output channels.

Internal organisation

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 measured by the current-potential (I/E) converter (Fig. 7). The I/E converter is equipped with a selectable resistor (R) for the x 100 attenuation (att. 100).

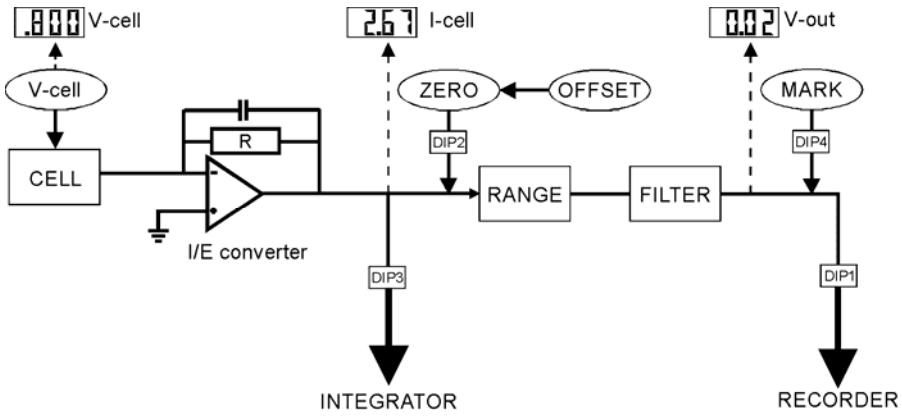


Fig. 7. Signal processing in the INTRO. A selectable resistor (R) in the I/E converter is used for the 'x 100' attenuation.

Immediately after the I/E converter the signal is sent to the integrator output. The recorder output differs from the integrator output in that range selection, zero, offset and rise time filters are possible. Both outputs are fully analog.

Integrator output

The integrator output on the rear panel receives its signal from the I/E converter. This output is only affected by the attenuation (i.e. **not** by zero, offset, range selection, rise time filter and marker). If the data acquisition system input is 10.0 V, dipswitch 3 on the rear panel should be 'off', for a 1.0 V input it should be 'on'. The maximum output voltage of the I/E converter is in both cases 25% higher, 12.5 and 1.25 V respectively. To avoid confusion, in this manual only the 1.0 or 10.0 V nominal output values will be dealt with.
If the 'x 100' attenuation is activated the 'x 100'-LED is ON. The maximum cell current that can be measured is 100 nA or 10.0 µA (att. 100).

Table V. Integrator output specifications.

attenuation	range (nA FS)
1	100
100	10 000

The range given in Table V as nA full scale (FS) is referring to both the 1.0 V and 10.0 V maximum output setting. If the maximum integrator output is set at 10.0 V (dipswitch 3 = off), the range is thus 100 nA/ 10.0 V = 10 nA/V. A measured voltage is related to a cell current by:

$$\mathrm{I\,(nA)} = \frac{0.1\ \text{attenuation}}{\mathrm{INT\,max.\,(V)}}\,\mathrm{V_{measured}\ (mV)} \qquad (1)$$

Equation 1 shows that the setting of dipswitch 3 affects the signal of the INT output by a factor of 10! For example, a measured peak height of 33 mV, att. 100, at a 10.0 V maximum integrator output corresponds to:

$$\mathrm{I} = \frac{0.1\,\mathrm{x}\,100}{10\,(\mathrm{V})}\ 33\,(\mathrm{mV}) = 33\,\mathrm{nA}$$

Recorder output

The recorder output can be affected by zero, offset, range selection, attenuation, rise time filter and marker. If the data acquisition system input is

10.0 V, dipswitch 1 on the rear panel should be 'off', for a 1.0 V input it should be 'on'. Also for this output the maximum output voltage of the I/E converter is 12.5 or 1.25 V respectively, but in this manual only the 1.0 or 10.0 V nominal output values will be dealt with. The maximum cell current measured is 100 nA or 10.0 µA (att. 100).
The nA/V ranges as indicated on the front panel of the INTRO refer to a 10.0 V maximum recorder output. For a 1.0 V maximum recorder output these nA/V values should be multiplied by 10. A measured voltage is related to cell current by:

$$\mathrm{I\,(nA)} = \frac{\mathrm{range\,(nA/V)} \cdot \mathrm{attenuation} \cdot 10\,\mathrm{(V)}}{\mathrm{REC\ max.\,(V)}} \cdot \mathrm{V_{measured}\,(V)} \qquad (2)$$

Equation 1 shows that the setting of dipswitch 1 affects the signal of the REC output by a factor of 10! For example, a measured peak height of 33 mV, on a 10.0 V maximum recorder output, att. 100 and range 2 nA/V, corresponds to:

$$\mathrm{I} = \frac{2\,\mathrm{(nA/V)} \cdot 100 \cdot 10\,\mathrm{(V)}}{10\,\mathrm{(V)}} \cdot 0.033\,\mathrm{(V)} = 6.6\ \mathrm{nA}$$

For the full scale signal, when $V_{measured}$ = REC max., eqn. (2) becomes:

$$I_{max}\,\mathrm{(nA)} = \mathrm{range\,(nA/V)} \cdot \mathrm{attenuation} \cdot 10\,\mathrm{(V)} \qquad (3)$$

A recorder range setting of 1 nA/V (att. 1), corresponds to 10 nA full scale for *both* a 1.0 and 10.0 V maximum recorder output. Eqn. (3) is perhaps the easiest way to relate the measured voltage with a cell current, as it holds for both the 1.0 V and 10.0 V maximum recorder output.

Examples

With a few examples the settings of the integrator and recorder output in combination with the maximum zero compensation setting will be illustrated.

Example 1

For a background current of 5 nA, at 800 mV, an expected minimum and maximum peak height of 80 pA and 80 nA respectively, the recommended settings are listed in Table VI.
For both stable and well-defined chromatographic and electrochemical conditions, it is recommended to use a pre-set temperature setting of at least 5 °C above ambient. Also the use of a risetime filter is recommended as it

improves the signal-to-noise ratio. The background current is compensated by switching on ZERO ON/SET.
Although it may be tempting to use always the maximum zero compensation (dipswitch 2 'on'), it is not recommended. The resolution of the zero circuit is better at a smaller maximum zero compensation setting. At peak heights above 10 nA, this will hardly be noticed, but at a more sensitive range setting this may result in a considerable (but always positive) offset when using ZERO ON/SET.

Table VI. Settings example 1.

Parameter	Setting
Cell potential	800 mV
Oven	5 °C above ambient, stable: HEATING-LED flashes
Zero	ON/SET
Risetime filter	1 s (or as preferred)
Attenuation	Not activated (att. 1)
Range	10 nA/V (=100 nA full scale, irrespective of dipswitch setting)
Dip. 2	Off, max. zero compensation 8.5 nA, high resolution
Output	Recorder 10.0 V (dip 1: off), data acquisition at 10 V Recorder 1.0 V (dip 1: on), data acquisition at 1 V

Also for the range setting it may be tempting to use always the maximum scale, i.e. 10 nA/V and att. 100. In combination with integration software, it may be possible to magnify the chromatogram in a way that even the smallest peaks are visible. However, it is not recommended, because in that case the integrator does the amplification of the signal. The best dynamic resolution is obtained when the powerful INTRO electronics are used for what they are designed for: to handle pico-ampere currents without being limited by electronic noise or instability.

Example 2

For the same example as above, but with a background current of 50 nA, the recommended settings are listed in Table VII.

Due to the high background current the attenuation must be ON. The expected maximum peak height of 80 nA on a background current of 50 nA would result in a cell current of 130 nA which exceeds the maximum current that can be handled with attenuation 1 (maximum current 100 nA full scale, see eqn. 3).
The dipswitch 2 setting is off, resulting in enough maximum zero compensation (850 nA) at a high resolution.

Table VII. Settings example 2.

Parameter	Setting
Cell potential	800 mV
Oven	5 °C above ambient, stable: HEATING-LED flashes
Zero	ON/SET
Risetime filter	1 s (or as preferred)
Attenuation	Activated (att. 100)
Range	0.1 nA/V (=100 nA full scale, irrespective of dipswitch setting)
Dip. 2	Off, max. zero compensation 850 nA, high resolution
Output	Recorder 10.0 V (dip 1: off), data acquisition at 10 V Recorder 1.0 V (dip 1: on), data acquisition at 1 V

C H A P T E R 5

Specifications INTRO

General specifications

Power: 100-120/220-240 VAC, 50/60 Hz, max. 150 W
Operating mode: DC
Cell potential: between -1.5 and +1.5 V
Integrator: max/min range 10 µA/100 nA, max 10 or 1 V output
Recorder: max/min range 10 µA/100 pA, max 10 or 1 V output
autozero, maximum compensation:
oxidative mode: 8.5 or 35 nA (att 1); 850 or 3500 nA (att 100)
reductive mode: 85 or 120 nA (att 1); 8500/12000 nA (att 100)
offset: continuously adjustable between -0.15 and +0.15 V
event marker: 1 or 0.1 V
Oven: 100 W, length 40 cm, stable from 5°C above ambient, max 47.5°C, accuracy better than 0.5°C, stability better than 0.1°C; accommodates flow cell, column and the following options: Rheodyne injector, SSI pulse dampener and bulkhead unions
Resolution display: cell potential (1 mV), output voltage (10 mV), oven temperature (0.1°C) cell current (1, 0.1, 0.01 or 0.001 nA)
Noise: better than 3 pA with load of 0.5 µF (+ 300 MOhm) and 0.1 s filter, with 1 s better than 1 pA

Front panel

Frames
V_{cell}: cell on/off, ox/red indication, cell potential up and down
Display: V_{cell}, V_{out}, °C, I_{cell}, $I_{cell,HR}$
Heater: off, 25 - 47.5°C in 2.5°C increments, on/heating indication
Zero: on/set/off indication, mark
Filter: 0.1 - 5 s in 1, 2, 5 increments
Range: 0.01 - 10 nA/V, or 1 - 1000 nA/V in 1, 2, 5 increments, att 100 indication

Rear panel

Mains
Recorder (adjustable offset)
Integrator
I/O connector
Cell on, cell off, mark, zero on/set, zero off, range x1/x100, common, load/inject, T_{oven} (10 mV/°C), common
Dip switches
Recorder: max 10 or 1 V
Zero: low or high
Integrator: max 10 or 1 V
Mark: 1 or 0.1 V

VT-03 flow cell

Confined wall-jet design, working volume determined by spacer thickness and working electrode (WE) diameter
Spacers: 25, 50 or 120 µm, stackable
WE diameters: 0.5, 0.75, 1.00, 1.90, 2.00, 2.54, **2.74** and 3.00 mm
Cell volumes: 0.005 µl minimum
WE materials: glassy carbon, Pt, Au, Ag and Cu
Reference electrode: long-life Ag/AgCl, fully serviceable
Auxiliary electrode: stainless steel
Wetted materials/parts: Kel-F, FEP, Viton, working, auxiliary and reference electrode

Physical specifications

Dimensions: 44 (L) x 19 (W) x 26 (H) cm = 17.3" x 7.5" x 10.2"
Weight: 10.9 kg (24.0 lbs.)

C H A P T E R 6

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 Chpt. CHAPTER 2 Installation guide, pg. 11
Cell disconnected, or switched OFF	Check connection
Recorder/integrator disconnected	Check connection
Fouled WE	Clean WE (see flow cell manual)

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

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	Optimise cell potential

Noisy baseline

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

Decreased sensitivity (low S/N ratio)

Possible cause	Remedy
Fouled WE by dirty samples	Clean WE (see flow cell manual), 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)
Overtightened cell bolts	Adjust cell bolts, check pump pressure
Air bubbles in cell or REF	Maintenance REF (see flow cell manual)
Temperature oscillations	Set oven temperature
Contaminated buffer (high I_{cell})	Replace buffer, do not recycle the buffer
Fouled WE	Clean WE (see flow cell manual)
Fe^{++} in buffer	Add EDTA, passivate metal parts with HNO_3

C H A P T E R 7

Optimisation of working potential

Introduction

A current - voltage (I/E) relationship, or voltammogram, characterises an analyte. It gives information on the optimum working potential, which can be used to improve detection sensitivity and selectivity.
A *hydrodynamic* voltammogram is obtained by running several chromatograms at different working potentials. Both peak height and background current are plotted against the working potential. In this chapter optimisation 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.

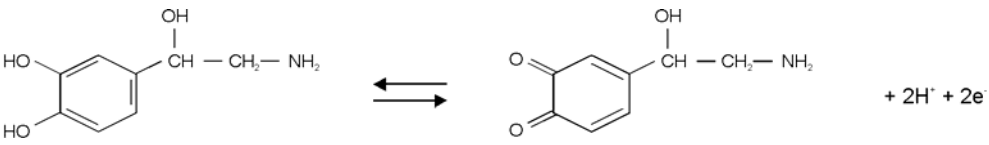


Fig. 8. Oxidation/reduction reaction of norepinephrine.

An example of an electrochemical reaction is shown in Fig. 8, 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 voltammogram

Increasing the working potentials enhances the electrochemical reaction, as can be seen in the hydrodynamic voltammogram of Fig. 7. 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*.

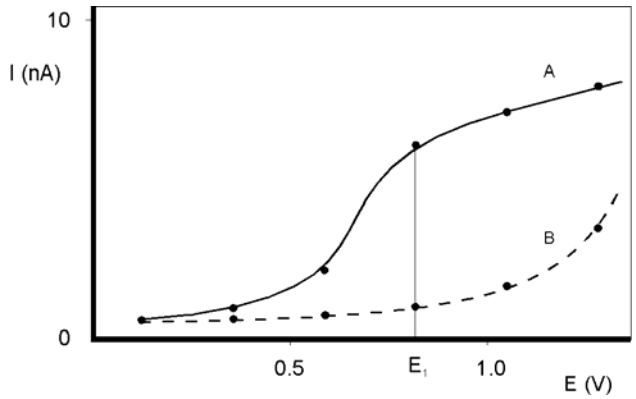


Fig. 9. Hydrodynamic voltammogram of norepinephrine (A) at a glassy carbon working electrode, and the current of the baseline (B). At E_1 the electrochemical signal becomes diffusion limited.

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 is favourable.
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 Fig. 9 a working potential (E_1) of 0.8 V is chosen.

Optimisation using a voltammogram

Sometimes, when interfering peaks appear in the chromatogram, it is possible to optimise the method with regard to selectivity.
When 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 Fig. 10 the selectivity for compound X is improved considerably by decreasing the potential to E_2 or E_1. Obviously,

when compound Y is the compound of interest, optimisation of selectivity in this way is not possible and the chromatography has to be optimised. 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.

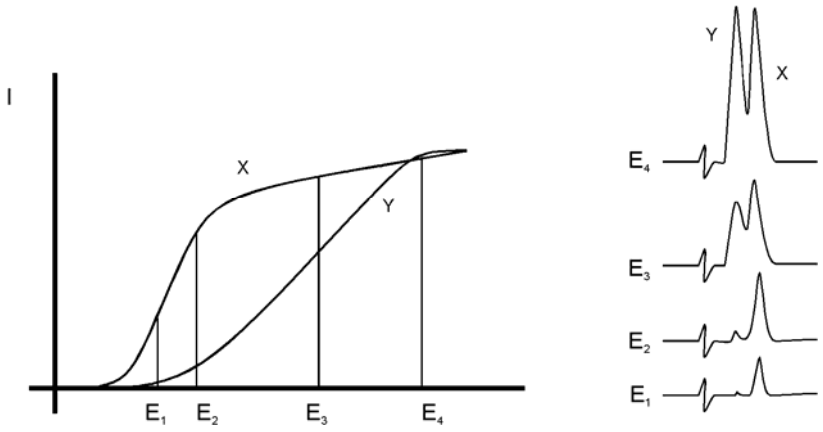


Fig. 10. Selectivity in LC-EC of compound X and Y is optimised by choosing the working potential with the largest difference in peak height.

Electrochemical reactions are pH sensitive (Fig. 11). 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.

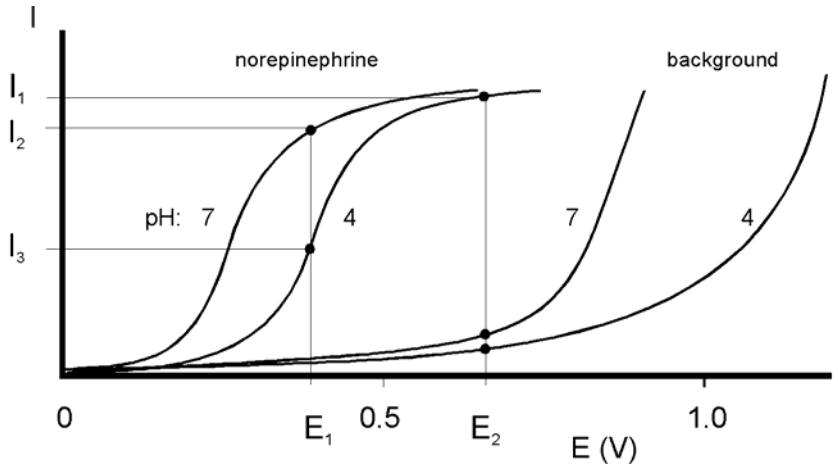


Fig. 11. At a higher pH the I/E curve of norepinephrine is shifted to the left.

Reaction kinetics predict that electrochemical detection is mass-flow dependent. When the LC flow is stopped in LC-EC, the analyte will be oxidised 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 flow rate. Therefore, it is important to use a pulse-free solvent delivery system.

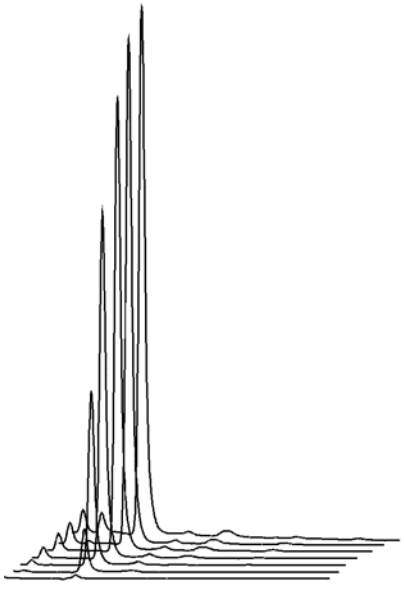


Fig. 12. Construction of a hydrodynamic voltammogram for norepinephrine. Chromatograms are obtained at cell potentials ranging from 1.0 V (back) to 0.4 V (front), with 100 mV steps.

Construction of a hydrodynamic voltammogram

Before a hydrodynamic voltammogram can be obtained, the chromatographic conditions should be optimised. 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 stabilised at a high potential. After stabilisation 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 derivatisation 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 are repeated until the lowest potential setting (Fig. 12).
5. The peak heights and background currents are plotted against the potential (Fig. 9).
6. The working potential which gives the best sensitivity is obtained by plotting the signal-to-noise ratio against the working potential.

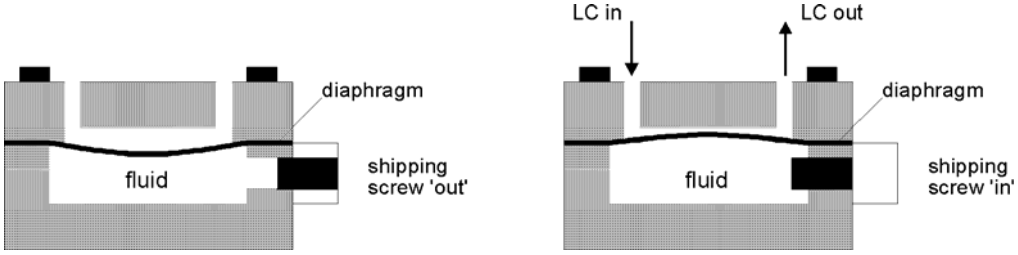
C H A P T E R 8

Pulse dampener

SSI pulse dampener part no. 12-**06**25 is ready for use, as supplied. The older version of the SSI dampener (part no. 12-**01**25) is supplied with shipping screw in the full out (counter-clockwise) position to provide additional space for the fluid to expand during the temperature extremes of transit. Failure to tighten the shipping screw before operating can result in the loss of compressible fluid and subsequent damage to the diaphragm.

Installation

1. **SSI 12-01<u>25</u> dampener only**: Turn the shipping screw fully clockwise and tighten to 120 inch-pounds or 'as tight as possible by hand' using the factory supplied 1/4" hex key.
2. The dampener should be placed between HPLC pump and injector. Connect the stainless steel pulse dampener using the factory supplied standard connection set (ferrules and nuts). The flow geometry through the pulse dampener is symmetrical, i.e. each one of the two connections can be used as inlet or outlet.
3. Flush the dampener with 20 ml HPLC grade water before use.



The shipping screw on the SSI pulse dampener part no. 12-**<u>01</u>**25 must be firmly tightened (turn clockwise) before operating, or damage to the unit will result. The SSI pulse dampener part no. 12-**<u>06</u>**25 is ready for use as supplied.

Operation

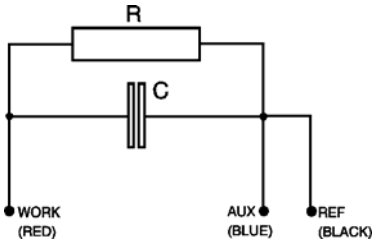
Operation of the SSI pulse dampener is automatic. The pulse dampener works best if the pressure is at least 500 psi (3.5 MPa) and preferably 1000 psi (7.0 MPa) or more. In case of low pressure it may be desirable to install a restrictor between dampener and injector to the pulse dampener outlet to enhance unit performance. Advised operating temperature is between 15 – 35 ^{o}C.

Always release pressure from the system slowly. A rapid pressure release could cause the pulse dampener diaphragm to rupture.

C H A P T E R 9

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 MOhm 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 VT-03 flow cell in an ideal HPLC set-up.
The noise generated via the dummy should be less than 3 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 VIII for settings). With a 1 second risetime the noise should be better than 1 pA.

Table VIII. Dummy cell test settings.

Parameter	Setting
Cell potential	800 mV
Cell current	2.67 ± 0.05 nA (read-out)
Oven	30 °C, stable: HEATING-LED flashes
Zero	ON/SET
Risetime filter	0.1 s
Range	0.01 nA/V
Output	Recorder 10.0 V (dip 1: off), data acquisition at 10 V Recorder 1.0 V (dip 1: on), 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.

C H A P T E R 1 0

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