

DECADE II SDC

Electrochemical Detector

User manual

174.0010, Edition 04, 2022



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**Declaration of conformity**

We Antec Scientific, Alphen a/d Rijn, Netherlands, declare that the product

DECADE II SDC TM Electrochemical Detector type 174

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

Safety (2006/42/EC)

Safety requirements for laboratory equipment **IEC61010-1:2001 2nd ed.**
(Class I, Installation cat. II, Pollution degree 2)

Particular requirements for laboratory **IEC61010-2-010:2003 2nd ed.**
equipment for the heating of materials

Immunity (2004/108/EC)

Electromagnetic immunity **EN61326-1:2006**
EN61000-4-2, EN61000-4-3, ENV50204,
EN61000-4-4, EN61000-4-5, EN61000-4-6,
EN61000-4-8, EN61000-4-11

Emissions (2004/108/EC)

Electromagnetic emission **EN61326-1:2006**
EN55011 (Class B), EN61000-3-2,
EN61000-3-3

Attention

Only use manufacturer-supplied cable(s) to connect with other devices. Part numbers 250.0122 (RS232 cable), 250.0130 (I/O cable) and 250.0128 (output cable). Thoroughly connect shielding to common. Manufacturer 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 6, 2019

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 Scientific 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 Scientific, 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. For more info: info@antescientific.com.

Shipping address for the end-of-life products:

Antec Scientific
Hoorn 131
2404 HH Alphen a/d Rijn
The Netherlands

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



ROHS directive

The DECADE II is ROHS compliant and in conformity with Directive 2002/95/EC Restricted use of Hazardous Substances in electrical and electronic Equipment (ROHS).

Antec Scientific is an ISO 9001:2008 certified company.



Symbols

The following symbols are used on the rear panel and oven compartment of the DECADE II:



Consult the manual for further safety instructions



Frame or chassis ground terminal

The following pictograms are used in the DECADE II manual:



Caution



Caution, risk of electric shock or other electrical hazard (high voltage)

Safety practices

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

Electrical hazards

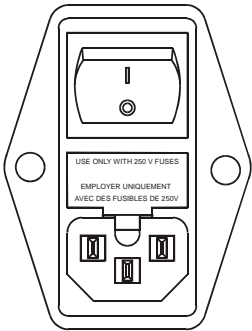


The removal of protective panels on the instrument can result in exposure to potentially dangerous voltages. Therefore, disconnect the instrument from all power sources before disassembly. Untrained personnel should not open the instrument.



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

Replace blown fuses with fuses of proper type and rating as stipulated on the rear panel and specified in the installation section of this manual. The fuse holder is integrated in the mains connector. Ensure that the instrument is never put in operation with fuses of a different type. This could cause fire.



V ~ 100-240 V	FUSE RATING	 <p>WARNING - RISK OF FIRE REPLACE FUSE AS MARKED AVERTISSEMENT - RISQUE DE FEU REMPLACEZ LE FUSIBLE COMME INDIQUÉ</p>
50 - 60 Hz		
260 VA	2.5AT / 250V	

Connect the detector to a grounded AC power source, line voltage 100 – 240 VAC. The instrument should be connected to a protective earth via a ground

socket. The power source should exhibit minimal power transients and fluctuations. Replace faulty or frayed power cords.

Place the detector on a flat and smooth surface. Do not block the fan located at the bottom of the detector. Blocking the fan will impair the cooling capability of the power supply.

General precautions



Perform periodic leak checks on LC tubing and connections.

Do not close or block the drain.

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.

This instrument has a lithium battery inside. Replacement of the battery should be performed by qualified service personnel. Dispose the battery according to chemical waste only.



LC equipments should be used by trained laboratory personnel only. Use proper eye and skin protection when working with solvents. Additional safety requirements or protection may be necessary depending on the chemicals used in combination with this equipment. Make sure that you understand the hazards associated with the chemicals used and take appropriate measures with regards to safety and protection.



Use of this product outside the scope of this guide may present a hazard and can lead to personal injury

Spare parts and service availability

Manufacturer 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 on an 'as available' basis.

Manufacturer provides a variety of services to support her customers after warranty expiration. Repair service can be provided on a time and material basis. Contact your local supplier for servicing. Technical support and training can be provided by qualified chemists on both contractual or as-needed basis.

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C H A P T E R 1

DECADE II SDC

Congratulations on your purchase of the DECADE II SDC. This detector enables you to perform all applications using electrochemical detection in the DC mode. The DECADE II SDC 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 DECADE II SDC is operated by PC control software using Antec Leyden data acquisition software or DECADE II Dialogue™.

CHAPTER 2

Installation guide

Unpacking

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. 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.

Installation

To unpack the DECADE II SDC, lift it from its box by both hands (Fig. 1).

Never lift the DECADE II SDC at its front door, but at its sides.

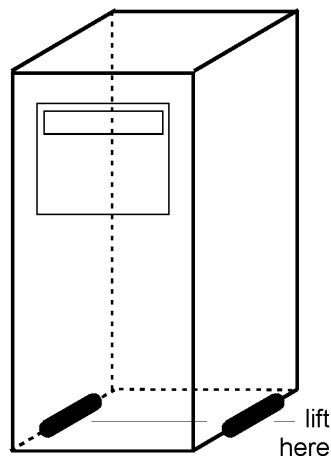


Fig. 1. Lift instructions DECADE II SDC.

Install the detector in an area which meets the environmental conditions listed below:

Table I. Environmental conditions

Parameter	Requirement
Storage temperature	-40 – 50 °C (-104 – 122 °F)
Storage humidity	0 – 90%, non-condensing
Operating temperature	4 – 40 °C (39 – 104 °F)
Operating humidity	20 – 80%, non-condensing

Place the detector on a flat and smooth surface. Do not block the fan located at the bottom of the detector (Fig. 2.). Blocking the fan will impair the cooling capability of the power supply.



Fig. 2. Location of power supply fan DECADE II.

Inspect the detector for possible damage and make sure that all marked (and ordered) items on the checklist are included. Switch ON the DECADE II by the mains switch on the rear panel. Ensure that the power (on/off) switch and power cord are always accessible.

Switch ON the DECADE II SDC by the mains switch on the rear panel. Ensure that the power (on/off) switch and power cord are always accessible.



Do not block the fan located on the rear panel. Blocking the fan will impair the cooling capability of the power supply.

HPLC connections



Use proper eye and skin protection when working with solvents.



The manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant EMC & safety standards. The pump connected to the system should be specifically designed for use in High Performance Liquid Chromatography and capable of delivering flow rates typically in the range between 1 µL/min

up to 10 mL/min. Please carefully follow the next steps for a successful installation and start-up.

1. The installation of the flow cell and column is shown in Fig. 3.
2. If a manual injector is applied with position sensor, the sensor cable must be connected to 'C' on the rear panel to enable INJECT/LOAD functions.
3. If an electrically actuated valve is mounted, connect the 'digital input/output cable' to 'B' on the rear panel to enable INJECT/LOAD functions.



Fig. 3. Installation of the DECADE II SDC.



4. 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.
5. 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 an ISAAC™ reference electrode is used, make sure that the buffer contains 2 mmole/l chloride (KCl or NaCl) ions.

6. 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 fouling 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.
7. 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.
8. If the DECADE II SDC 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).
9. Consult your flow cell manual for installation of the flow cell. The cell connector inside the oven compartment is ESD sensitive. Make sure that the flow cell is OFF when removing or connecting the cell cable.



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.**

10. 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.
11. 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 electrical noise induced by 'dripping' of mobile phase.

12. Set the cell potential (see page 36 for optimisation of the potential), switch ON the flow cell (see page 17) and allow the system to stabilise for approximately 30 min. A 'good' stabilisation curve shows a mono-exponential decline without jumps and/or spikes.
13. Connect the data system to the output (see page 18).

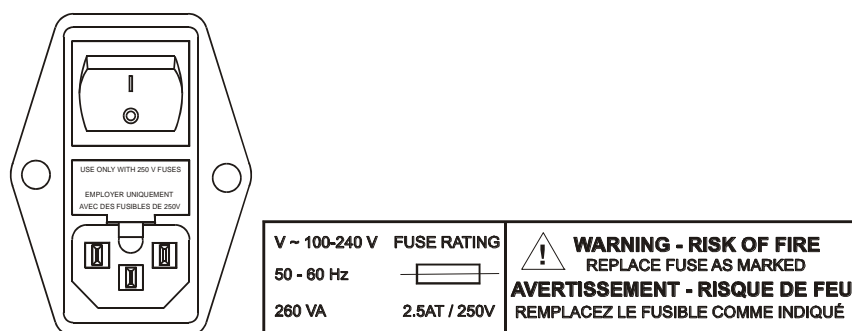
Your system is now ready for use. The DECADE II SDC has been developed for continuous operation. For maximum stability it is advised to leave the system ON continuously. If preferred, the flow cell may be switched OFF at night.

Maintenance

Perform periodic leak checks on LC tubing and connections and check if the drain on the bottom of the oven compartment is not blocked or closed. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Empty and clean waste container regularly. Never dispose of such products through the municipal sewage system.

This instrument has a lithium battery inside. Replacement of the battery should be performed by qualified service personnel. Dispose the battery according to chemical waste only.

Replace blown fuses with fuses of proper type and rating as stipulated on the rear panel and specified in the installation section of this manual. The fuse holder is integrated in the mains connector. Ensure that the instrument is never put in operation with fuses of a different type. This could cause fire.



Do not use any organic solvents to clean the exterior of the detector. Use a cloth wetted with water only to clean the detector.

Remove any dust on the protective screens that cover the fans in the oven compartment.

ALEXYS® DECADE II SDC driver

Full control and data acquisition is supported in the ALEXYS data system software. Important difference with stand-alone operation is that timed events are not executed from time files, but from the events page in the ALEXYS software.

DECADE II SDC time files are not supported in the ALEXYS data system. Software. Use events page instead.

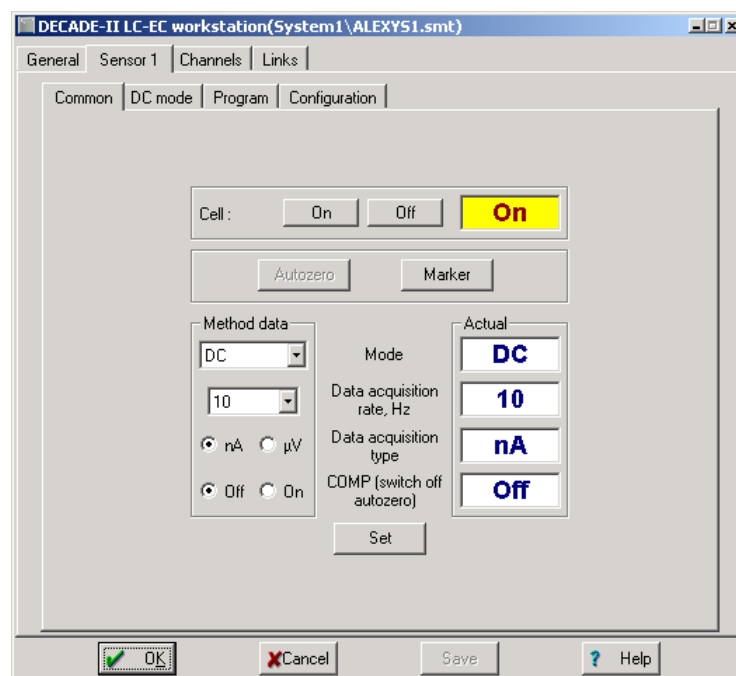


Fig. 4. DECADE II SDC driver in the ALEXYS data system software. Parameters in blue against white background are actual settings. New parameters on the left side (Method data) can be set by clicking the Set button.

For detailed information please consult the ALEXYS software user manual (p/n 185.0035).

DECADE II Dialogue for SDC

DECADE II Dialogue for Windows is a multi-functional program to control the DECADE II electrochemical detector. DECADE II Dialogue is a tool for:

- controlling all operational parameters of DECADE II
- creating and transmitting time files to DECADE II
- controlling DECADE II IO ports for external devices
- control of DECADE II SDC and (one) LC 100
- OQ and PQ measurements
- noise measurement

There are no tools for further data analysis, therefore DECADE II Dialogue can not be considered as fully featured chromatography software package.

DECADE II Dialogue is one software package containing functionality for all different versions. Depending on the license dongle PQ/OQ functionality will be unlocked. In the free version - without USB licence dongle - PQ, OQ, Start Run/Sequence are unavailable.



Fig. 5. Screenshot of the free version (no USB dongle) in combination with a DECADE II SDC.

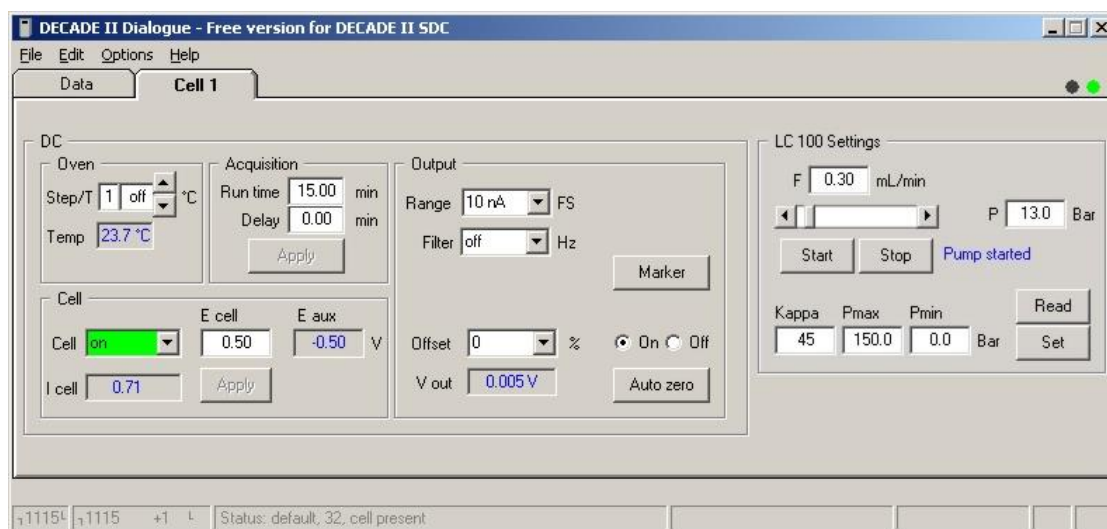


Fig. 6. DECADE II Dialogue for operational control of the DECADE II SDC electrochemical detector.

CHAPTER 4

Detection and parameters

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 DECADE II SDC covers such a wide range from 200 μA down to 10 pA full scale, without being limited by electronic noise. For this reason the DECADE II SDC is equipped with a 24 bit ADC and 20 bit DAC for analogue data output.

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 amplified by the current-potential (I/E) converter (Fig. 7).

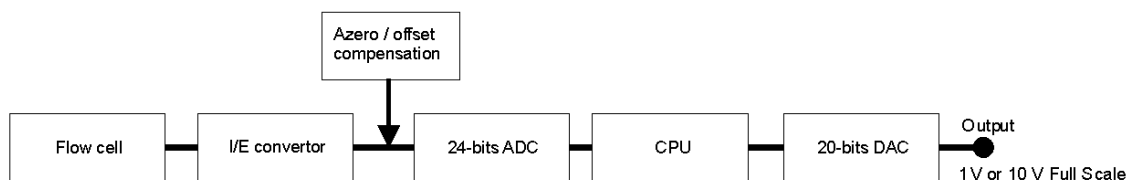


Fig. 7. DECADE II SDC signal processing from electrochemical flow cell to output.

The signal from the I/E converter can be compensated with auto zero or offset, and is digitised using a 24 bits ADC. In the CPU the signal is processed, for example noise filtering, or more complex data processing in PAD. Finally after the 20 bits DAC the signal is set to a 1 or 10 V full scale analogue output.

Parameters

Operational parameters are controlled using PC software.

Range

A number of ranges can be selected; the maximum current that can be compensated for using auto zero and offset differs. The high sensitivity ranges (10 pA - 5 nA) have the best noise specifications. In fact, there is a trade off between best noise specification at sensitive ranges, and maximum compensation at the less sensitive ranges. This is an inevitable consequence of the tremendous dynamic range that is covered by electrochemical detection.

Table II. DC ranges and maximum compensation.

Range FS	Max comp	Range FS	Max comp
200 μ A	2.5 mA	20 nA	2.5 μ A
100 μ A	2.5 mA	10 nA	2.5 μ A
50 μ A	2.5 mA	5 nA	250 nA
20 μ A	2.5 mA	2 nA	250 nA
10 μ A	2.5 mA	1 nA	250/25 nA*
5 μ A	2.5 mA	500 pA	250/25 nA*
2 μ A	25 μ A	200 pA	250/25 nA*
1 μ A	25 μ A	100 pA	25 nA
500 nA	25 μ A	50 pA	25 nA
200 nA	25 μ A	20 pA	25 nA
100 nA	25 μ A	10 pA	25 nA
50 nA	2.5 μ A		

* From firmware > 3.00 the noise level in ranges 200, 500 pA and 1 nA has been improved considerably by selecting a different amplifier setting. As a consequence max. compensation is changed to 25 nA for these ranges.

Offset

A maximum offset of +50% and - 50% in 5% steps can be set. For example, 20% is a 200 mV offset when the maximum output is 1.0 Volt (2 V at max. 10.0 V).

Polarity

The polarity of the output can be inverted. Oxidative and reductive analyses generate opposite currents. For data acquisition, traditionally chromatographic peaks have a positive amplitude. Therefore selection of polarity is useful.

Filter

High frequency noise is efficiently removed and chromatographic peaks can be detected with better signal to noise ratio.

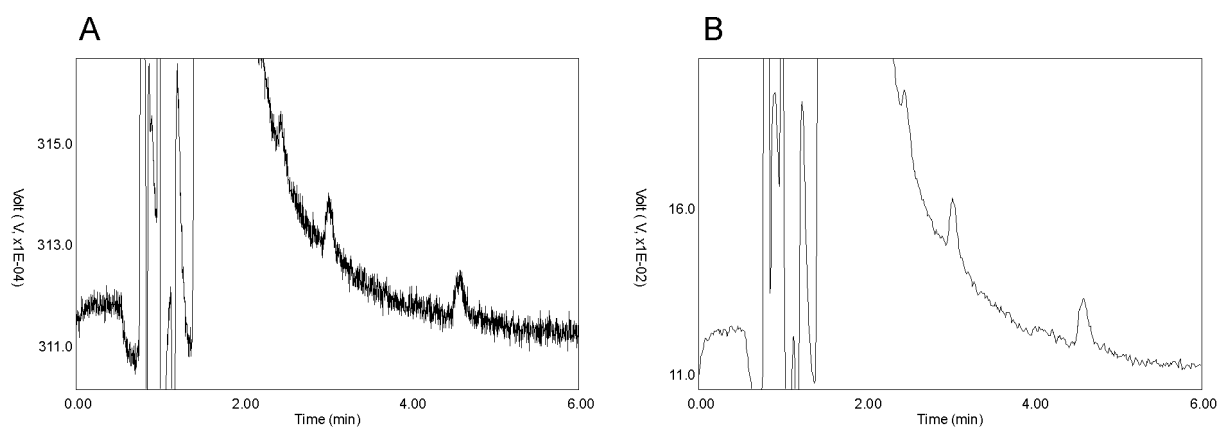


Fig. 8. Signal to noise ration is improved using a filter (A vs. B).

CHAPTER 5

Noise suppression: ADF™**Introduction**

Besides for its tremendous linear dynamic range and selectivity, electrochemical detection is well-known for its very low limits of detection. To further improve these detection limits the Antec Leyden engineers have developed ADF (Advanced Digital Filter) and the DECADE II has been equipped with it as a standard. The improvement factor in signal-to-noise (S/N) ratio depends on the frequency relation of signal and baseline noise. S/N improvements from a factor 5 up to more than 100 have been obtained. To understand how a digital filter works, first the importance of frequencies in chromatographic analysis will be explained. Then we will look at peak width, filter settings, cut off frequency, amplitude response plots and finally at a few chromatograms before and after applying ADF.

Frequency

A scientific definition of frequency is “the number of completed alterations per unit time”. It has two dimensions: count and time. Frequency is usually expressed in Hz, which is counts per second.

The counts themselves can run in a regular, evenly spaced manner, as with sine waves whose curve shapes do not change. Alternatively, the counts can run in an irregular manner within the specified unit of time. If the latter happens, frequencies would vary if broken down into smaller units of time. In the example of Fig. 9 a signal is shown with a frequency of 12 alterations in 5 minutes. To express its frequency in a more scientific way a full period is precisely determined and expressed in Hertz (or s^{-1}). It is a sine wave with a frequency of 0.04 Hz (Fig. 10).

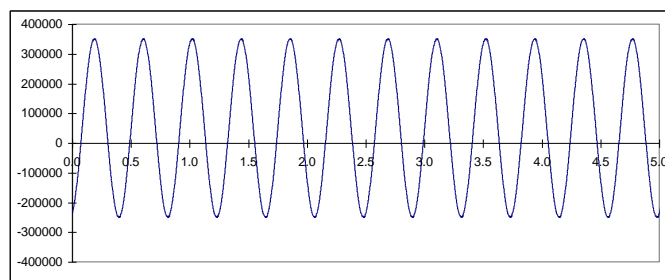


Fig. 9. Example of a signal with regular evenly spaced alterations: a sine.

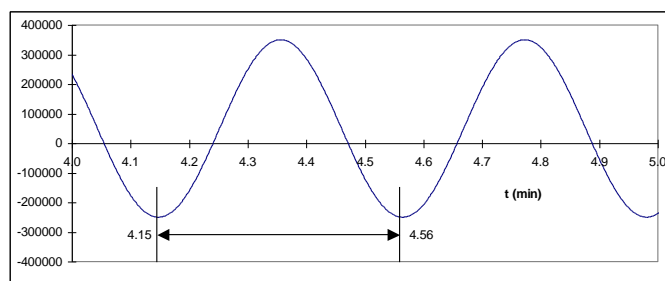


Fig. 10. Sine of Fig. 9. A full period is 0.41 min (25 s) which corresponds to a frequency of $1/25 = 0.04$ Hz.

Frequency of signal and noise

Also a chromatographic peak can be expressed in terms of frequencies. The way to determine this frequency is the same. The duration of the full peak is measured and expressed in Hz.

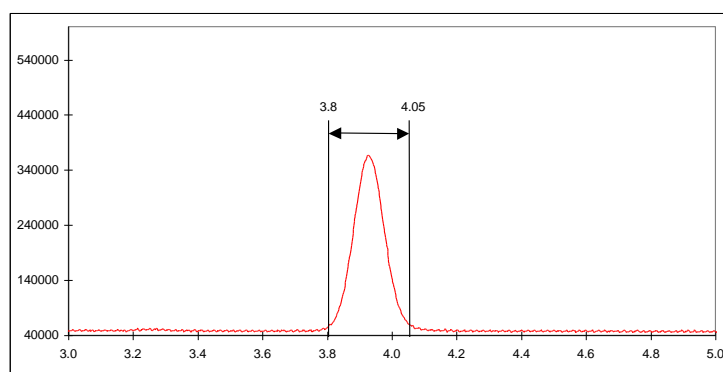


Fig. 11. Frequency tells how often something happens: 1 peak in about 0.25 min (15 s), $f = 1/15 = 0.07$ Hz.

This is further illustrated by an overlay of the same chromatographic peak with a sine of 0.07 Hz (Fig. 12).

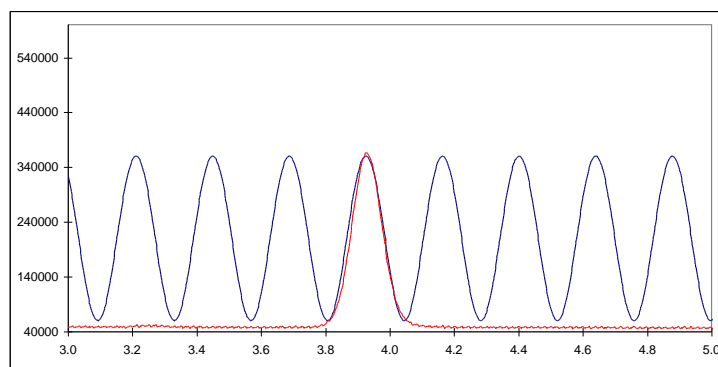


Fig. 12. Overlay of a chromatographic peak with 0.07 Hz sine.

Typically in chromatography narrow peaks are in front of a chromatogram while peaks with longer retention times get wider. As a consequence frequencies are not constant but vary between 0.1 – 0.01 Hz, which corresponds to 10 – 100 s peak width.

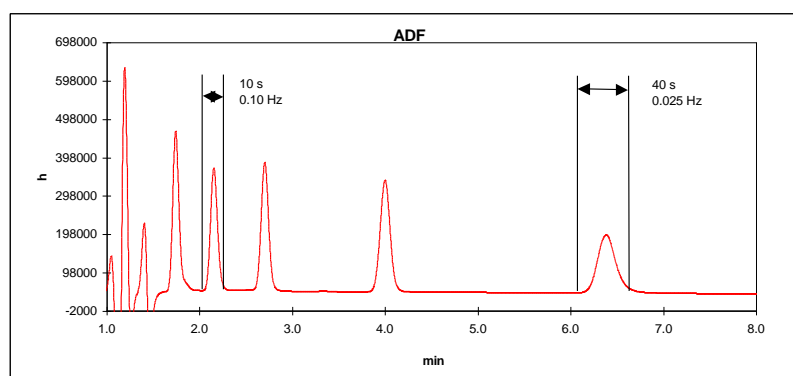


Fig. 13. Typical chromatogram with peak widths between 10 – 100 s..

Noise in chromatography can come from different sources. Pump pulsations are typically shown as a very regular noise pattern, while electronic noise has a more random character. This is illustrated in Fig. 14 where a noise trace is shown with an overlay of a 10 and 0.4 Hz sine.

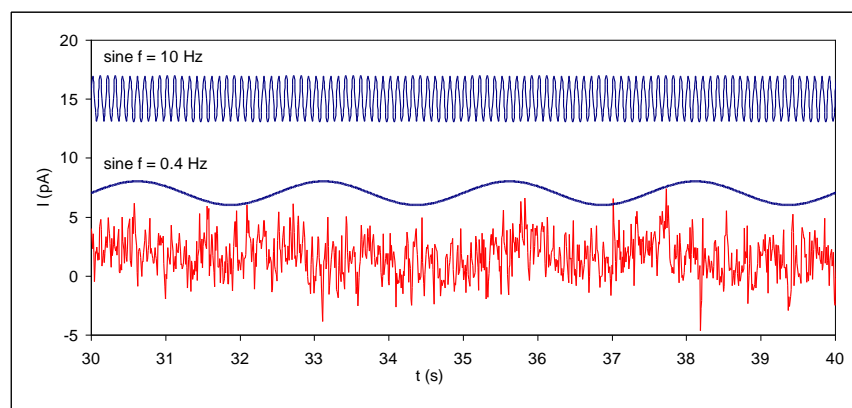


Fig. 14. Typical random noise in chromatography (lower trace). Both frequencies (0.4 and 10 Hz) can be recognised amongst others.

Looking closely to the lower noise trace both frequencies (and others) can be recognised. This is typical for noise in chromatography: a collection of more or less random frequencies.

Low pass noise filters

The way noise filters work is by suppressing certain frequencies in the acquired signal. Typically low pass filters allow chromatographic peaks (low frequency) to pass, while high(er) frequency noise is attenuated. No matter how advanced, it is impossible to use a low pass filter successfully if there is no difference in frequency of signal and noise.

Analogue filters are made of hardware, from capacitors, resistors and amplifiers (opamps). Digital filters are mathematical routines to process an acquired signal.

Traditionally, in many detectors for chromatography an analogue low-pass filter is applied (rise time filter). A 'passive' RC filter consists of resistors and capacitors. An active higher order filter can be considered as a number of these RC filters in series. In a 4th order filter the signal coming from the first filter is filtered again in a second, third and fourth filter. During these steps, loss of signal occurs simply because of all the resistors that are applied.

Operational amplifiers, which are 'active' components, are applied in each stage to restore the signal to its original value.

With the availability of powerful processors, digital signal processing has become an excellent alternative for hardware filters. In its most simple form a running average filter takes the average of n data points to create a new data point. For example in a 5-points running average filter output data point $y[80]$ is calculated from measured data points $x[80] - x[84]$ as:

$$y[80] = \frac{x[80] + x[81] + x[82] + x[83] + x[84]}{5}$$

Each input data point has the same weighting factor of 1/5. In more advanced digital signal processing a more complicated equation is used to calculate the output data point $y[n]$:

$$y[n] = a_0 x[n] + a_1 x[n-1] + a_2 x[n-2] + a_3 x[n-3] + \dots$$

In contrast to the previous equation, each data point has a different weighting factor a . Sum of these weighting factors $a_{0...n}$ will always be 1.

Characteristic of noise filters is that processing the signal will result in a delay. This is inevitable, as the mathematics of digital signal processing requires a number of previous data points to process a new data point.

The filter characteristic in DSP is often named after the scientist who 'invented' the mathematics behind the signal processing routine. Well-known names in this field are Bessel, Chebychev, Savitsky, Golay, Hamming and many others.

Amplitude response plot

There are several ways to describe the filter characteristics. An amplitude response plot gives important information on filter behaviour. Suppose our signal of interest has a frequency between 0 - 1 Hz, and all higher frequencies are noise. An ideal filter is shown in Fig. 15 where signal frequencies between 0 – 1 Hz completely pass while frequencies of higher than 1 Hz are completely blocked.

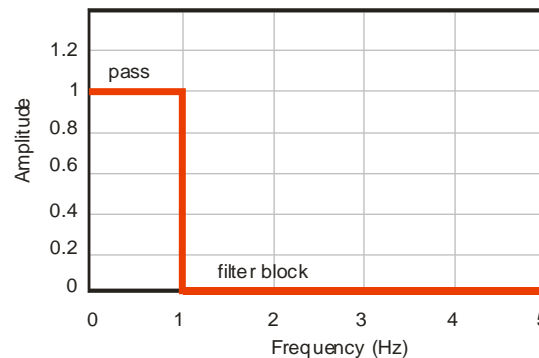


Fig. 15. Amplitude response plot of an ideal low pass filter with a cut-off frequency of 1 Hz.

In practise filters behave a bit different from the ideal situation. Amplitude response plot shows a more gradual attenuation profile at higher frequency.

This cut off frequency is where the output signal amplitude is 70% of the input signal, also known as 3 dB point.

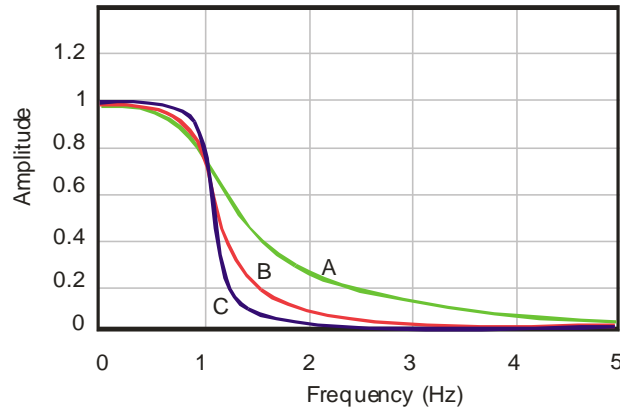


Fig. 16. An amplitude response plot of a low pass filter with a cut-off frequency of 1 Hz. It is a 2 (A), 4 (B) and 8 (C) pole Bessel filter.

In Fig. 16 it is shown that the number of poles is important, a filter behaves more ideal with increasing number of poles. In a hardware filter the number of poles is the number of filter circuits that are placed in series.

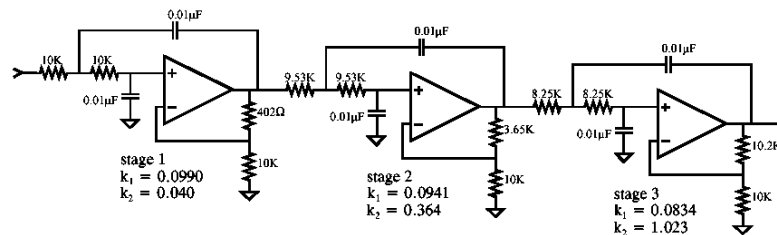


Fig. 17. Analogue 6 pole Bessel filter.

A digital filter does not have poles, but it is characterised by the number of input data points used to calculate a new output data point. For example a 9-point digital filter (Savitzky-Golay) is given as:

$$\begin{aligned}
 Y[1] = & -0.090909091 X[1] + 0.060606061 X[2] + \\
 & 0.168831169 X[3] + 0.233766234 X[4] + \\
 & 0.255411255 X[5] + 0.233766234 X[6] + \\
 & 0.168831169 X[7] + 0.060606061 X[8] + \\
 & -0.090909091 X[9]
 \end{aligned}$$

Note that the sum of coefficients is exactly 1. $Y[n]$ is the output data point, $X[n]$ are input data points. Generally spoken, the performance of a digital filter

improves with more input data points, but also more processor capacity is required for the large number of calculations.

Applying ADF in chromatography

If noise frequencies in LC-EC differ from the frequency of the signal, noise can be suppressed. Using the right filter setting (cut-off frequency) will specifically attenuate noise and improve the signal-to-noise (S/N) ratio. No matter how 'advanced' a filter is, it is only possible to apply low pass filtering if noise frequencies are higher than the frequency of the signal.

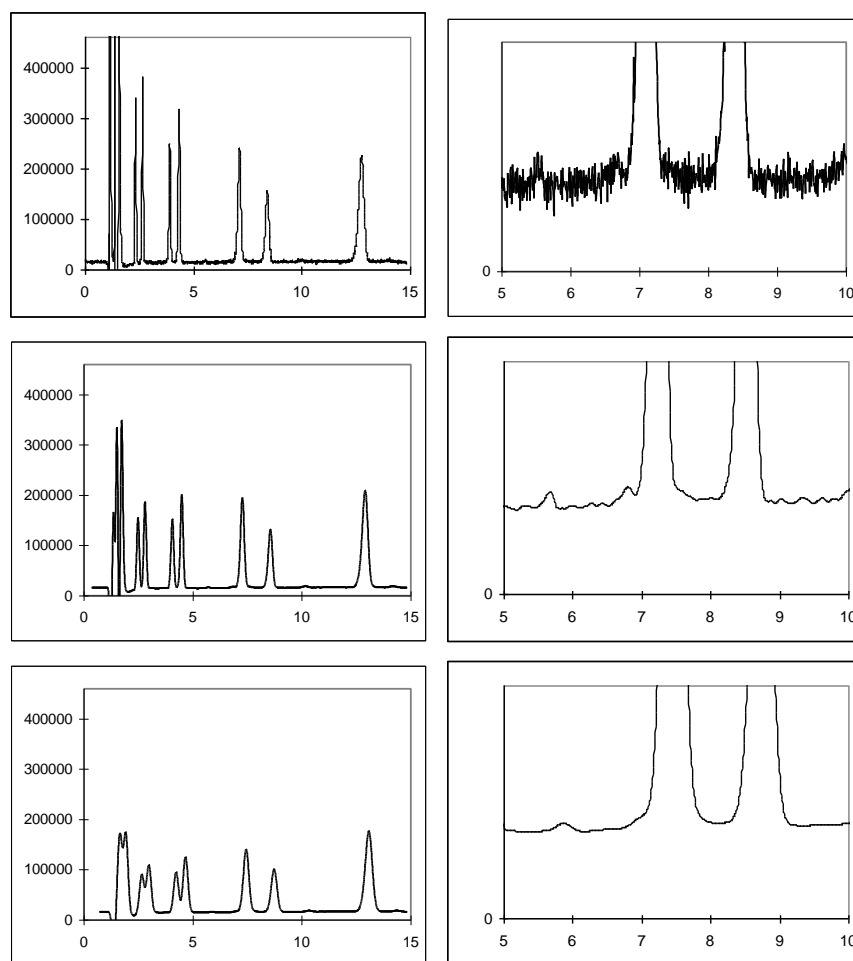


Fig. 18. From top to bottom filter setting of 0.5, 0.02 and 0.002 Hz. Narrow peaks in front of the chromatogram are deformed at 0.005 Hz, whereas wider peaks show hardly any deformation (see peak at $t \sim 13$ min). Attenuation of noise is shown in the close up on the right.

Prerequisite for a 'good' noise filter for data acquisition in liquid chromatography is that it improves the S/N ratio without significant distortion of the signal of interest. This is particularly difficult if the frequency of the signal is close to the frequency of the noise.

The DECADE II has a number of filter settings to optimise for best possible signal-to-noise ratio. The width of the peaks of interest is important because wider peaks allow stronger filter settings simply because of the lower frequency of such peaks. Advised filter setting to start further optimisation is given as:

$$\text{Filter setting} = 1 / [2 * (\text{peak width})]$$

So at a 10 s peak width a 0.05 Hz filter setting is advised. If peaks are 50 s a 0.01 Hz filter is advised to start with. Note that if a chromatogram has interesting peaks of 10 s as well as 50 s, it may not be possible to work with one filter setting. In that case it is advisable to switch to a stronger filter setting for the second half of the chromatogram using a timed event. To optimise for the best S/N ratio, use the lowest acceptable cut-off frequency. After optimisation, do not change the cut-off frequency setting during analysis of a calibration sequence. Use the same settings for analysis of samples and calibration standards.

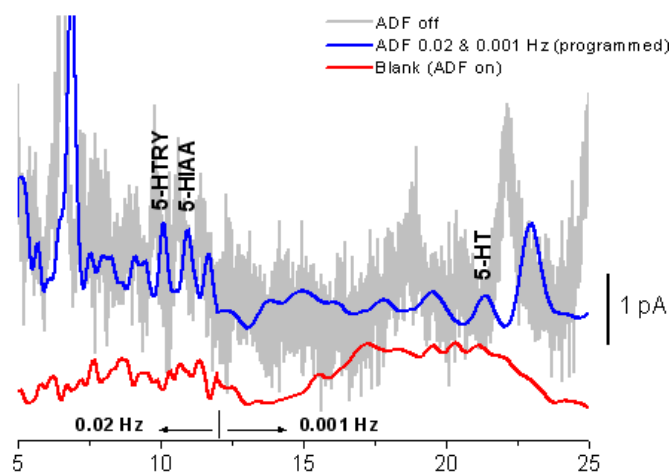


Fig. 19. Analysis of 20 pmole/L 5 hydroxytryptophan, 5-HIAA and 5HT using ADF for improving detection limits.

The S/N improvement depends on the composition of the frequency spectrum. Improvement up to a factor 100 may be obtained compared to an unfiltered signal. As high frequency noise is suppressed, remaining noise components will be in the same frequency range as chromatographic peaks.

As suppressing noise will always result in (some) suppression of signal it is advised to switch the DECADE II to the highest acceptable sensitivity.

C H A P T E R 5

Events and time files

Introduction

Running an Events table 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, auto zero or control of external equipment (i.e. trigger to start integration software etc.).

Output events

Connector A and B on the rear panel enable control of (or by) external equipment. Together with time files this supplies a powerful tool for development of automated methods.

Inject marker

A manual valve with position sensor can be connected to 'C' on the rear panel of the DECADE II SDC which enables the inject marker on connector B. The contact is high when the valve is in 'load' position, and low in the 'inject' position. It can be used to start the integration software when injection is done.

Overload

Activated when a overload occurs, see also page 44 for details.

Auto zero

Enables external activation of the auto zero command. This function is active only when the 'I-cell' is displayed.

Cell on, off

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

Table III. I/O contacts connector A.

No.	Name	I/O	Function
1,2,3	Relay 1	Out	Contact between 1 (common) and 2 (default) or 3. Activated by time file Outp 0100
4,5,6	Relay 2	Out	Contact between 4 (common) and 5 (default) or 6. Activated by time file Outp 1000
7	Cell on	In	Trigger to switch on cell
8	Reset	In	Resets a running time file
9	Overload	Out	Active when overload occurs ('out of range')
10	AUX1	Out	Free programmable TTL output Activated by time file Outp 0001
11	AUX2	Out	Free programmable TTL output Activated by time file Outp 0010
12	Cell off	In	Trigger to switch off cell
13	Start	In	Starts a time file
14	Auto zero	In	Auto zero command, always accessible when 'I-cell' is in display
15	Common		Ground

Outputs 7, 8, 12, 13 and 14 are level triggered. When active, output status 9, 10 and 11 is low (default is high).

Table IV. I/O contacts connector B.

No.	Name	I/O	Function
1 - 3	Common		Ground
5	Mark	In	Baseline spike of 10% FS, duration: 0.1 s
8 - 11	Common		Ground
13	Inject marker	Out	In combination with manual valve connected to connector C, high: 'load', low: 'inject'.

Outputs 4 – 7, 12, 14 and 15 are level triggered.

Level triggered TTL input: contacts require a minimum TTL-low pulse duration of 100 ms. If multiple activations are required the next pulse should be given after 100 ms TTL high. When the input is kept low, only one activation will occur.

TTL output: default = high (5 Volt)



The manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant EMC & safety standards

CHAPTER 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.

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 DECADE II SDC: the voltage runs between two pre-set values and the current is measured.

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.

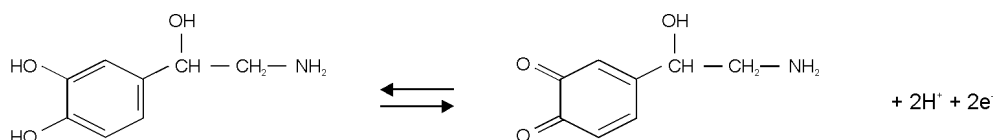


Fig. 20. Oxidation/reduction reaction of norepinephrine.

An example of an electrochemical reaction is shown in Fig. 20, 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

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 difficult.

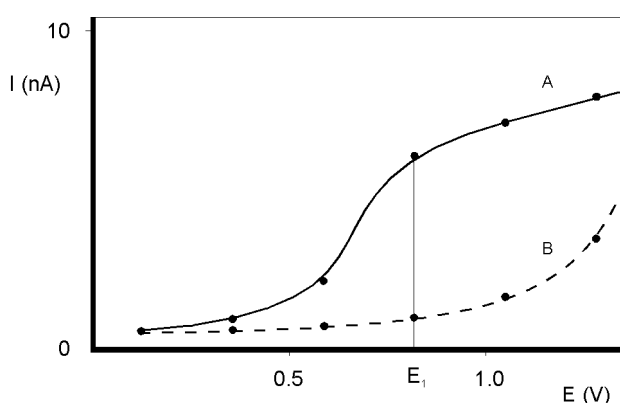


Fig. 21. Hydrodynamic voltammogram of norepinephrine (A) at a glassy carbon working electrode, and the current of the baseline (B). At E_1 the electrochemical signal becomes diffusion limited.

As can be seen in Fig. 21, 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 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. 21 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. 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 Fig. 22 the selectivity for compound X is improved considerably by decreasing the potential to E_2 or E_1 . Obviously, if 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.

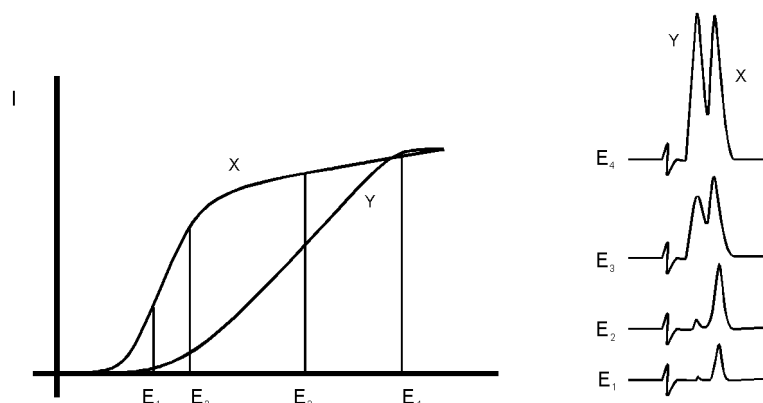


Fig. 22. Selectivity in LC-EC of compound X and Y is optimised by choosing the working potential with the largest difference in peak height.

Electrochemical reactions are pH sensitive (Fig. 23). 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.

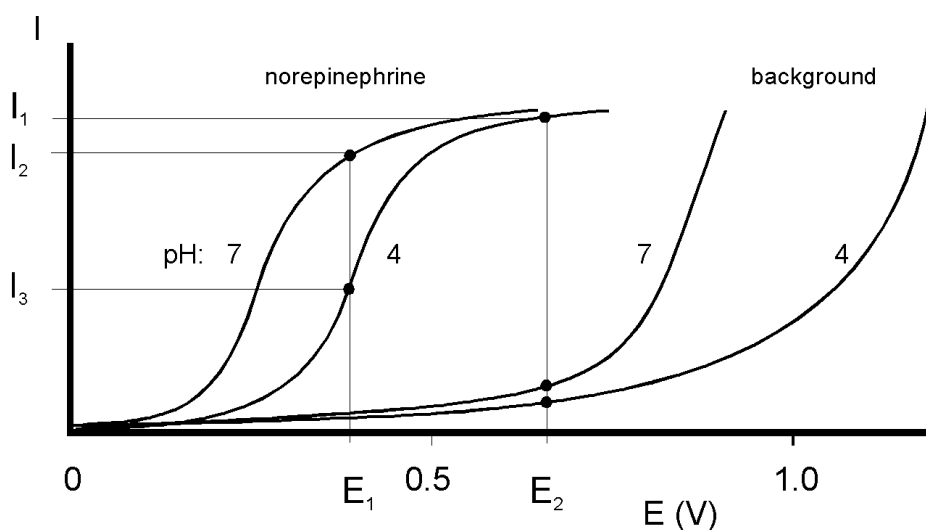


Fig. 23. At a higher pH the I/E curve of norepinephrine is shifted to the left.

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 the flow rate. Therefore, it is important to use a pulse-free solvent delivery system.

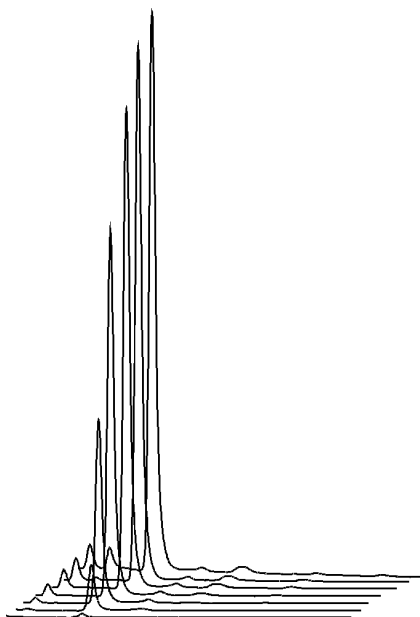


Fig. 24. Construction of a hydrodynamic voltammogram for norepinephrine. Chromatograms are obtained at cell potentials ranging from 1.0 V (back) to 0.4 V (front), with 100 mV steps.

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 $\mu\text{mol/l}$, is prepared in mobile phase.
2. The DECADE II SDC is stabilised in the DC mode 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 is repeated until the lowest potential setting (Fig. 24).
5. The peak heights and the background currents are plotted against the working potential (Fig. 21).

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

Specifications DECADE II SDC**General specifications**

Power	100-240 VAC, 50/60 Hz, 260 VA max., auto-sensing
Operating mode	DC
Potential range	between +2.00 and - 2.00 V in 10 mV increments
Output	between +1 and - 1 V or between +10 and -10 V (20 bit D/A converter)
Offset	between +50% and - 50% of max. output voltage, 5% steps
Event marker	pulse of 10% of max. output
Auto zero	triggered by rear panel TTL or RS232C control
RS232C	full parametric instrument control, data acquisition at 1, 2, 5 and 10 Hz
Oven	height 37 cm, from 7°C above ambient to 45°C, accuracy 0.5°C, stability 0.1°C; accommodates column and flow cell(s)
Environmental	operating temperature: 4 – 40 °C, RH 20 to 80%, non-condensing
DC mode ranges	10 pA – 200 µA in 1, 2, 5 steps
Filter (ADF)	Advanced Digital Filter, 0.5 - 0.001 Hz, 1, 2, 5 steps
Noise	better than 2 pA with dummy cell in 1 nA/V range, filter off, E_c +800mV and temperature of 30 °C.
Auto mode	DC mode (5 files), cycle time, number of cycles and oven temperature. Time-based control of 50 time points as to range, filter, output contacts (2 TTL, 2 relays), auto zero, board id, offset, valve position (if present) and cell potential.

Physical specifications

Dimensions	Dimensions 44 (D) x 22 (W) x 44 (H) cm = 17.3" (D) x 8.7" (W) x 17.3" (H)
Weight	14 kg (32 lbs) without flow cell and column

CHAPTER 9

Error messages

Table V. Error messages.

Error	Message
01	Incompatible boot version
02	Control board error
03	Sensor board x error (x = board number)
04	Firmware program error
05	Record error
06	Incompatible FW version
07	Incompatible FW
08	Control board FW erase failed
09	Sensor board x
10	Upload checksum error.
11	Checksum error.
12	Temperature sensor 1 error.
13	Disconnect flow cell x
14	Control board SRAM error.
15	Sensor board x SRAM error .

Please contact your local supplier if one of the above errors occur.
Furthermore the following messages can occur during a measurement:

Table VI. Messages.

Message	Advice
01 Out of range	Output is either above +1.0V or below –1.0V. Pressing AZERO may give an adequate read-out again. If the message remains after pressing AZERO, the autozero function is unable to compensate the background cell current. Advice: use a less sensitive range in the SETUP menu.
02 PAD overload	Charging current in pulse mode out of range. Pressing AZERO may give an adequate read-out again. If not, it is advisable to change the pulse settings (increase t1) or use a less sensitive range.

CHAPTER 10

Rear panel

Connectors A, B and C

For detailed information on the I/O contacts see page 35.



Fig. 25. DECADE II SDC rear panel.

RS232C

The RS232 interface provides full parametric control from a PC. Programmable parameters comprise cell potential, range, auto zero, offset, filter and control of DECADE II SDC output contacts for control of external equipment.



The manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant EMC & safety standards.

C H A P T E R 1 1

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 13
Cell disconnected, or switched off	Check connection
Output disconnected	Check connection
Fouled 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 ²⁺ 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 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
Leaking REF or cell	Tighten connections with care

Decreased sensitivity (low S/N ratio)

Possible cause	Remedy
Fouled 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

Base line 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
Fouled WE	Clean WE
Fe^{++} in buffer	Add EDTA, passivate metal parts with HNO_3

Saturation of output

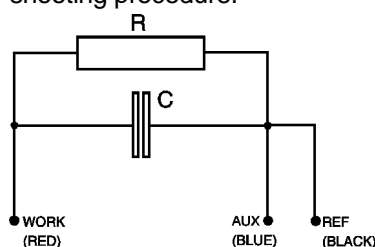
Possible cause	Remedy
Damaged REF	Check with spare REF, replace if necessary
Damaged WE	Replace cell block
Cell incorrectly connected	Check connections (REF: black, WE: red, AUX: blue)
Cell potential too high	Optimise cell potential

C H A P T E R 1 2

Dummy cell

External dummy cell

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



The dummy consists of a resistor (R) of 300 M Ω and a capacitor (C) of 0.47 μ F in parallel. The current is measured over the resistor according to Ohm's law ($V = I \times R$), 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 ($\pm 1\%$). 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 2 pA if the filter of the DECADE II SDC is set to off, provided that the dummy is within the fully closed Faraday shield at the same position as the flow cell (see Table VII for settings). With a 1 second risetime the noise should be better than 1 pA.

Table VII. Dummy cell test settings.

Parameter	Setting
Cell potential	800 mV
Cell current	2.67 nA (read-out)
Oven	35 °C, stable
Filter	off (or as specified)
Range	1 nA/V



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

CHAPTER 14

Detector accessories

The electrochemical detector is shipped together with a number of parts. The listing in Table below may not be complete, see check list of delivery for complete listing.

Table VIII. Accessories electrochemical detector.

Part number	Component
250.0040	External dummy flow cell
250.0107	Column clamp 12 mm
250.0113	Fuse 2.5 AT 250 V
250.0122	RS232 cable
250.0130	External I/O cable
250.0128	Output cable
250.0116	Mains cable (Europe)
250.0118	Mains cable (USA)
250.0126	Cell cable D connector

For these and other DECADE II SDC parts or flow cells contact your local supplier.

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