ANTEC
LEYDEN

DECADE
Twin option

user manual

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C H A P T E R 1

DECADE Twin option

Introduction

The DECADE can be equipped with an additional flow cell. To control the additional flow cell[1] a hardware and a software upgrade of the DECADE is required, the so-called Twin option. The potential of the Twin cell can be set and the cell current is displayed in the DECADE screen. The Twin cell can be used in different ways to achieve better selectivity or sensitivity, however, it cannot be used as a second detection cell.

It is not possible to direct the signal of the Twin cell to a DECADE output.

The Twin cell can be applied for ***cleaning*** of the mobile phase. The Twin cell is placed before the injector and after the pumping system and pulse dampener. A high potential is chosen to convert any electroactive contamination so that it does not interfere with the detection. The resulting decrease in background current improves the detection limit. A typical application in the reductive amperometric detection is removal of oxygen from the mobile phase.
The Twin cell can also be applied to improve ***selectivity***. In certain analyses interfering peaks occur in a chromatogram which cannot be removed by optimisation of the chromatography, or by sample pre-treatment. If the interference has a considerably lower oxidation (or higher reduction) potential, it is possible to remove the interference by electrochemical conversion with a Twin cell. The Twin cell is positioned between the chromatographic column and the detection flow cell. The potential of the Twin cell is carefully optimised to convert electroactive interferences so that they are not detected in the detection flow cell. Obviously, the analytes of interest should *not* be converted by the Twin cell.
Another application of the Twin option is to connect a ***reactor*** flow cell before the detection flow cell. The Twin cell is positioned between the chromatographic column and the detection flow cell. In the Twin cell a potential is applied that converts the analyte of interest into an electroactive species that can be detected by the detection flow cell. This approach is

[1] To avoid confusion: the flow cell controlled by the Twin option is referred to as “Twin cell” or “reactor flow cell”. The flow cell used for detection is referred to as “detection flow cell”.

usually applied to obtain a better selectivity or sensitivity, and is only applicable for analytes with reversible electrochemical reaction properties. An example is the pre-reduction followed by oxidative detection of vitamin K.

Flow cell

In principle any electrochemical flow cell can be used as Twin cell with the DECADE. However, depending on the application, certain requirements as to cell volume and to conversion rate have to be met. For example the VT-03 flow cell (Antec Leyden) has a large dead volume *after* detection, which makes this flow cell unsuitable as Twin cell.

The Twin cell required depends on the application.

1. If the Twin cell is used for **cleaning** up the mobile phase, a high pressure resistant flow cell with a high conversion rate (large working electrode area) is required. At this moment the only cell suitable is available from ESA (type no. 5020 'Guard Cell').
2. Application of a Twin cell to improve the **selectivity**, requires a high conversion rate and an acceptable cell volume (< 20 µl for standard LC). A suitable flow cell for standard LC is the ESA 5010 'Analytical Cell'.
3. The same ESA 5010 cell can be used as **reactor** flow cell in standard HPLC applications. The high conversion rate yields a high recovery.

For micro LC the ESA 5010 flow cell is not suitable due to the large cell volume (ca. 20 µl).

Antec Leyden does not supply, support or warrant flow cells of other manufacturers.

Optimisation of a cleaning flow cell

Before considering the use of a Twin cell for cleaning the mobile phase, it is advisable to check the source of contamination. Passivation and cleaning of the LC system may already improve (decrease) the background current. Optimisation of the mobile phase by using high quality chemicals (including water), or by using in-line degassing and gas-impermeable tubing, may also decrease the background current in LCEC.
Optimisation of a Twin cell used for cleaning of the mobile phase is done by monitoring the background current in the detection flow cell. In oxidative amperometric detection the Twin cell is switched on and the potential is increased. The signal on the detection cell (I_{cell}) should decrease with increasing E_{twin}. In reductive amperometric detection, the potential of the Twin

cell is decreased (more negative E_{twin}) which should result in a smaller background current (I_{cell} closer to zero).

The E_{twin} setting giving the highest S/N ratio must be selected.

Optimisation of selectivity

Optimisation of the Twin cell potential used to improve selectivity is done by construction of an I/E curve. The potential of the Twin cell is varied and the peak heights of the analytes and interference are measured.

The E_{twin} setting giving the highest S/N ratio must be selected.

In this case N is “chemical noise”, which is the peak height of the interfering peak.

Optimisation of a reactor flow cell

Optimisation of a detection system using a reactor flow cell in line with a detection flow cell is done by construction of a hydrodynamic voltammogram for both flow cells. First, a voltammogram for the reactor flow cell is acquired, while the potential of the detection cell is kept high and constant (i.e. resulting in a maximum signal). Next, at the optimum potential of the reactor flow cell a hydrodynamic voltammogram for the detection flow cell is made.

An example of such an optimisation is given for the reductive conversion followed by oxidative detection of vitamin K. The hydrodynamic voltammogram for the reactor flow cell is obtained at an oxidation potential of 1.0 V vs. Hy-REF (Fig. 1).
It is important to realise that an optimum exists in the voltammogram of vitamin K at the reactor flow cell. This optimum is compound dependent, and in practice it appears that this optimum may also vary for different reactor flow cells. It is therefore recommended to make such a voltammogram each time a new reactor flow cell is installed.
The next step in the optimisation of the detection of vitamin K is the construction of a hydrodynamic voltammogram at the detection flow cell using a constant potential of -400 mV at the reactor flow cell. An optimum working potential of 500 mV is found, however to be able to detect the other vitamins as well a potential of 1050 mV (vs. Hy-REF) is used in Fig. 2. The construction of a hydrodynamic voltammogram is described in the DECADE manual.

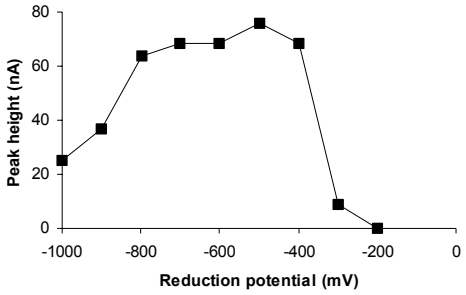


Fig. 1. Hydrodynamic voltammogram of vitamin K at the reactor flow cell. An optimum appears at -400 mV.

In Fig. 2 a multi-vitamin mixture is analysed. After switching on the reactor flow cell (Fig. 2B) the vitamin K1 peak appears in the chromatogram.

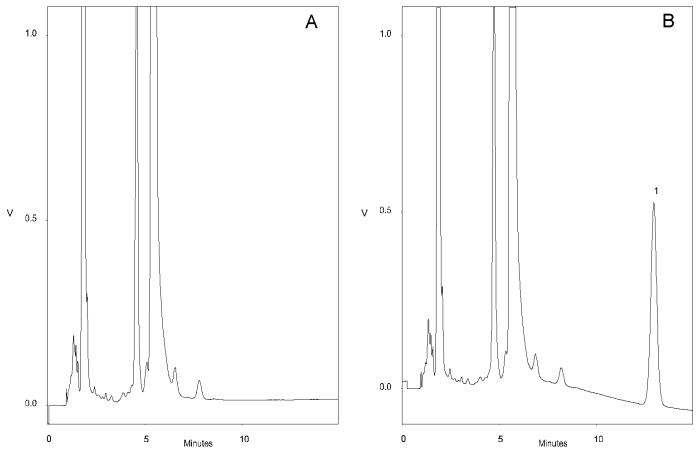


Fig. 2. Analysis of a vitamin sample at 1050 mV vs. *Hy-REF, and a reaction potential of 0 mV (A) or -400 mV (B). Peak 1 is vitamin K1.*

If the DECADE is operated using a reactor flow cell, the potential of this flow cell should be optimised. A maximum (negative or positive) potential is not always the best, an optimum may exist.

It is recommended to repeat this optimisation each time a new reactor flow cell is installed.

C H A P T E R 2

Installation

Installation of hard- and firmware

1. Disassemble the DECADE according to DECADE Service Manual.
2. Release the strip which locks all DECADE boards.
3. Gently pull the CPU Board (second from left) from its slot until the EPROM is easily accessible.
4. Remove old EPROM from the CPU board and place the Twin-EPROM in the same position.
5. Pull the old Oven Control Board (third from left) from its slot and loosely slide the new Oven/Twin Board into the same position.
6. Remove the black sleeve from the loose cable assembly hanging between the CPU and the Oven/Twin Board.
7. Connect the female plug to JP2 such that the red wire is connected to 'work'.
8. Firmly push the Oven/Twin Board into its slot and fix the locking strip.
9. Reassemble the DECADE according to the DECADE Service Manual.

Antec Leyden makes no representations or warranties either express of implied that the information contained in this manual is complete or accurate. It is understood that the purchaser must assume all risk in the use of this manual.

Installation Twin cell (ESA 5010 'Analytical Cell')

The ESA type 5010 'Analytical Cell' is placed inside the DECADE (Fig. 3A). In order to prepare the Twin cell for use, the following steps have to be done:

1. Follow the manufacturer's (ESA) recommendations regarding the installation of the flow cell.
2. As the next steps in this installation will void all ESA warranty, it is advised first to test the flow cell performance.

The next steps in this installation procedure will void all warranty on the ESA flow cell.

Opening and modification of a flow cell is entirely at the users own risk. Antec Leyden and its affiliated companies shall not be liable for errors or incidental or consequential damages.

3. Label the in- and outlet tubing with the ‘in’ and ‘out’ stickers.
4. Open the housing by unscrewing the four bolts on the bottom of the cell.
5. Without breaking the wires, take out the reactor flow cell which is packed in insulating material.
6. Remove this insulating material (foam + tape).
7. From the flow cell several wires are connected to the 15 pins D connector. These connections should be left as they are.
8. From the flow cell a black grounding wire is connected to the housing, and leads to the 15 pins D connector.
9. Cut the metal clip to which the grounding wire is attached, so that the grounding wire is free from the housing, but still connected to the 15 pins D connector.
10. Remove the D connector from the housing by unscrewing the two bolts.
11. To prevent clogging of the porous carbon working electrode, connect an inlet filter to the inlet side of the cell.
12. Position the cell between column and Antec flow cell and install the flow cell by connecting the LC tubing.
13. Connect the flow cell with the flow cell cable to the DECADE.
14. Start the LC system and check that all LC connections are free of leakage.
15. Continue to install the detection flow cell as described in the DECADE manual.

The Twin option is now ready for use.

Installation Twin cell (ESA 5020 ‘Guard Cell’)

The ESA type 5020 ‘Guard Cell’ is connected without modifications. Follow the manufacturer’s (ESA) recommendations regarding the installation of the flow cell.

Make sure that the flow cell housing and body is not connected to ground. Otherwise it is not possible to measure a cell current, although it is functioning.

This means that the flow cell must be 'isolated' from the LC pump and DECADE by means of PEEK tubing (Fig. 3B).

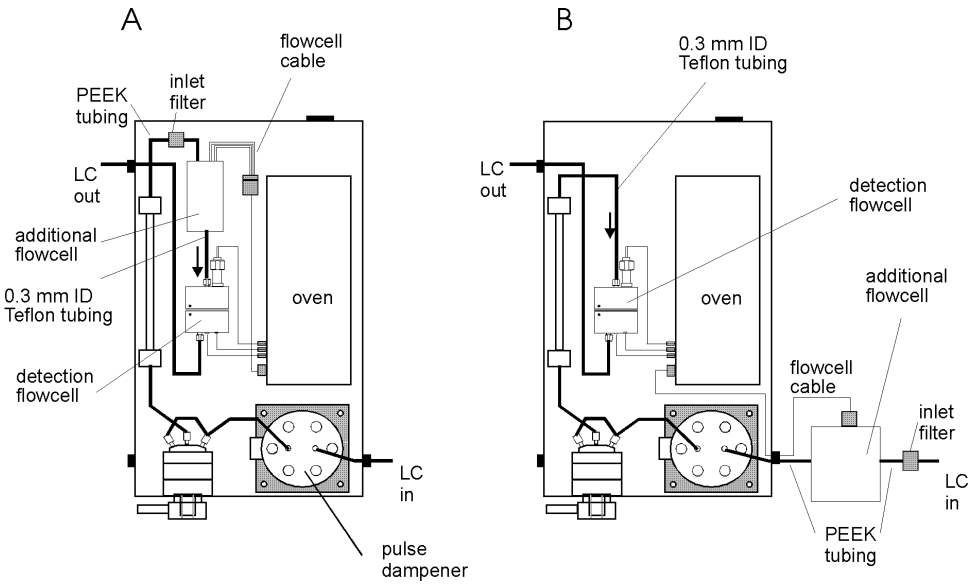


Fig. 3. Installation of a reactor flow cell (A) or a cleaning flow cell (B) with the DECADE.

DECADE Twin screen

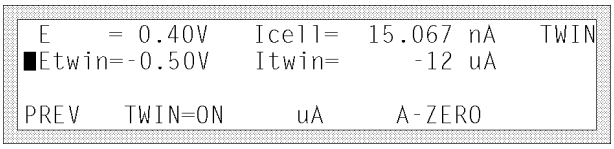
If the DECADE is equipped with the T-version software (version x.xxT) the main screen reads:

```
                DECADE                MAIN
              ANTEC Leyden
            (version 3.01T)
           DC     PULSE    SCAN
```

For control of the Twin cell TWIN is selected in the DC SET screen

```
■Eox. =+0.80V#                          DC
□Range= 50 nA  □Offs.=  0 %            SET
□Filt.= .1 s   □Toven= 30 °C
 PREV   CELL=ON   TWIN   AUTO    STATUS
```

In the TWIN screen the potential of the Twin cell can be set and the cell current can be read.



On the first line the flow cell potential (E), and the cell current (Icell) of the detection flow cell are displayed. Changing this cell potential is done in the previous, i.e. DC SET screen.

Twin parameters

µA/nA

TWIN	function

Toggles between the µA and the nA range

A-ZERO

TWIN	function

Sets the recorder and integrator output to 0 V, or to the offset voltage. This function only affects the measuring cell.

Etwin

TWIN	control

Controls the potential of the flow cell. Can only be set or changed after confirmation with the '#'-button. Selectable values are between -2.40 and +2.40 V.

Itwin

TWIN	status

Displays the true, non compensated cell current, i.e. unaffected by zero and offset. The maximum current in the µA range is 1.5 mA (max. readout 256 µA). In the nA range the maximum current is 15 µA (max. readout 2560 nA). If 'OVLD' is displayed, an overload occurs, switching to µA range may help.

PREV

TWIN	function

Returns to the DC SET screen.

Twin=ON

TWIN	function/status

Displays or switches the flow cell status.

Twin connector

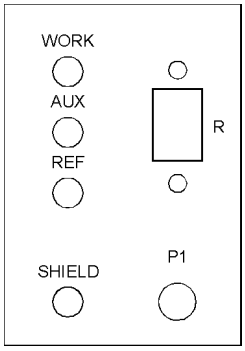


Fig. 4. The flow cell connector panel (R: D9 female connector) in the oven compartment of the DECADE for connection of the Twin cell.

Table I. D9 female connector

Pin no.:	Name:
1	Reference electrode 1
1	Reference electrode 1
2	Auxiliary electrode 1
3	Working electrode 1
4	
5	Ground
6	
7	Reference electrode 2
8	Auxiliary electrode 2
9	Working electrode 2