ANTEC
ANALYTICAL TECHNOLOGY

THE ANTEC EC-DETECTOR

October 16, 1992

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I INTRODUCTION

An electrochemical detector consists of an electrochemical flow-through cell, preferably mounted in a shielded cabinet and an electronic controller. Included in an optimized HPLC-system, it allows the trace analysis (pico- to femtomol range) of a number of -electrochemically active- substances.

Although different cell designs are marketed by several producers, all with specific advantages and disadvantages, we feel that the VT-03 detector design, after extensive tests in practice, is ahead of competiting designs with respect to S/N ratio and userfriendlyness.

The VT-03 has a wall-jet configuration, which implies that a high signal to noise ratio may be accomplished in a very small effective cell volume (< 0.35 µl), making the detector pre-eminently suitable for microbore and capillary LC.

The control unit (CU-04-AZ) contains four stages. The first fixes a certain potential (the voltage clamp), versus the reference electrode, between the so-called working and auxiliary electrode. The voltage clamp is critical in the sense that any change in this voltage is strongly amplified in the next stage. The current resulting from oxidation or reduction of a solute reaching the working electrode is amplified and converted to a voltage (I/E converter) in the second stage. Usually the signal is filtered slightly (time constant about 0.1 s) in the I/E converter to suppress high-frequency (50/60 Hz) noise. Then the signal is adapted in discrete steps to cover the ranges of interest. The signal can be further filtered to smoothen the output. Now the detector signal is suitable to provide a flatbed recorder or an integrator. The -non-filtered- output signal is monitored by an auto-zero circuit which, if activated, zeroes the output signal. This circuit can be controlled by the front panel or by external instruments.

The present configuration only functions in the oxidation mode. The reduction mode is possible after changing the internal wiring of the controller.

II SYSTEM DESCRIPTION

II.1 Controller

The CU-04-AZ controller is supplied for either 220-240V or 110 - 120 V and it is grounded via the mains cord. The controller comprises the following subunits:

- The voltage clamp or potentiostat, which delivers the potential to the detector cell. The potential is adjusted with a 2mm screwdriver within the "cell on/off" frame and is set via the display.

- The I/E converter or current amplifier, which measures the current in the detector cell and converts it to voltage. In this stage the signal is slightly filtered.

- The amplifier. Ranges from 0.1 to 100 nA/V are available as standard, for the control of μ-cells the range covers 0.01 - 10 nA/V (in 1, 2, 5 steps). Other ranges can be supplied on request.

- The auto-zero circuit, able to zero the output signal. It compensates a cell current of maximally 120 nA in the standard and 12 nA in the μ-controller.

- A low-pass filter with time constants of 0.1 - 5 s (1, 2, 5 steps).

- An LCD display, showing the (ref)potential with mV, the output with 0.01 V and the cell current with 10 pA (standard) or 1 pA (μ-version) resolution. The output of the controller nominally amounts to 1 and maximally to about 10 V. A voltage divider (second output) decreases this value by a factor 10. The maximal current through the I/E converter amounts to 1 μA (standard) or 0.1 μA (μ-version).

Convince yourself that the concentration of the solutes causes no higher output level, since the concentration then has no linear relation with the output any more. The maximal potential on the detector cell is about 1.5 V. This value is far too high for normal purposes. It is strongly advised not to work with potentials higher than 1.3 V. Higher values may foul or even damage the working electrode. Such treatment by the customer is not covered by the warranty of ANTEC.
The controller is constructed for continuous use. To obtain optimum stability it is advised to keep it in the on position (the mains switch is therefore mounted on the back of the instrument). This does not hold true for the detector cell itself.

II.2 Flowcell

The detector cell is the result of a development period of a number of years. It may deliver very sensitive analyses provided that it is properly used.
The cell is build up of two blocks, which, during use are clamped together by 4 bolts (M4) with a spacer of 25 (μ-version), 50 (standard) or 100 μm in between. The bolts should not be overtightened (max force 30 N.cm), since this can damage the cell fittings. As a rule check the pressure of the HPLC system during (crosswise!) tightening of the bolts but prevent a notable increase in system pressure. Overtightening sometimes evokes pulsations which are identical to the pulsations caused by your HPLC pump.

The solution to be analyzed is directed perpendicular to the working electrode to obtain a maximum conversion of compounds with a minimum of noise. The diameter of the working electrode is 1.9 or 3 mm, resulting in an effective cell volume of 140 and 330 nl resp. (50 μm spacer). The auxiliary electrode is positioned concentrically with respect to the working electrode and is built up of 316 stainless steel.

The reference electrode is of the Ag/AgCl type and consists of a silver wire coated with a melt of AgCl. Electrical contact with the detector cell is accomplished by a salt bridge of saturated KCl.

Filling the detector with the HPLC eluent should be done with great care, since air bubbles in the cell increase the cell noise greatly. Special attention should be paid to filling the reference chamber in the injector block, since, if the electrical contact with the eluent is blocked by an air bubble, the residual current increases strongly, leading to strong fouling of the working electrode. It is suggested to fill the cell via the column outflow, while the outlet of the cell is blocked. By keeping the cell in an angle of about 45° with the reference chamber on top of the cell, the chamber is easily filled without air bubbles.

The controller may be switched on provided that the cell is in the "off" position. Check the cell potential via the display and, if necessary, adjust it.

II.3 Tuning detector parameters

In cases, where reprodicibility is of paramount importance, it is preferred to work in the so-called limiting current of a solute. Therefore it is necessary, before performing routine analyses, to check the I/E relationship of the solutes of interest, in the present setting of your phase system! This is important since your phase system parameters also determine the I/E relationship of a solute. However, avoid too high potentials to reach this purpose: The working electrode can be fouled (see above) increasing the residual current and thus the noise level may reach inacceptable levels.

If sensitivity is the main goal, try to compromise between sensitivity and reproducibility. With the above in mind, the detector cell can be connected. Be sure (!!!!) that the black female plug is connected to the reference electrode (is it bubble-free?), the red one to the glassy carbon (or Au, Pt, Ag, Cu) working and the blue one to the stainless steel auxiliary electrode.

II.4 Important points

Never make electrical contact between the cell and the controller if you are not sure of:
a) the adequate potential and
b) that the flowcell is connected. In the ANTEC cells malconnection is impossible if the connectors supplied are used.

To avoid hum it is usually necessary to make ground on the injector by the crocodile clamp on the cell cable. Further, if the isolated cell/column cabinet is used, the cabinet is preferably grounded by the banana jacket on the backside. In addition it may be necessary to check whether the front-plate of this cabinet makes electrical contact with its housing. This contact is guaranteed if the front is fixed with the screws supllied. If PEEK tubing is used between pump and injector it might be required to make an electrical connection between the steel of the pump and a metal point close to the detector cell (e.g. the injector loop).

If the buffer in use is circulated, be sure that the returning buffer is not dripping: the minute changes in pressure that occur during dripping are observed by the flowcell. Set the recorder on 1 V full scale and the sensitivity of the potentiostat at 100 nA/V. Set the switch in the "cell on/off" frame to "on". You should observe a large signal which is

called the "charging current". This signal will gradually decay over a period of minutes to hours, depending on
1) the required sensitivity setting of the instrument, and
2) the potential setting.
If a more or less horizontal baseline is attained, it may be shifted by the background current compensation. Now it should be possible to start analyses.

The background current delivers important information as to the operational status of the detector cell and the degree of fouling of the buffer. As a rule of thumb it should not be higher than about 50 nA, but is usually much lower than 10 nA (standard cell). An unusually low background current is indicative for a fouled working electrode, a very high background points at a malfunctioning reference electrode or a contaminated buffer. The latter is traced by stopping the flow through the detector cell: If without flow the current decreases quickly the contaminated buffer caused the high background current; if the current remains unchanged or slowly increases, the reference or working electrode are to be suspected.

At high sensitivity settings slight changes in temperature may cause drift. Even movements in the room leading to such slight changes may result in an slowly up and down going background current ("wander"). To prevent such problems a separate cabinet can be supplied as detector cell housing (optional). It is strongly suggested to mount the column, the injector and, for top performance, also a -high efficiency- pulse dampener in this cabinet. It has been demonstrated that it improves detection limits to a factor 5 or even 10. It is important to make the connection between the column and the cell as short as possible, to exclude temperature effects. The housing also eliminates electromagnetic influences.

If pump pulses are observed, these may be caused by
1) a malfunctioning pump,
2) an overtightened detector cell,
3) the presence of Fe^{++}, released by the stainless steel parts of the HPLC system. The detector oxidizes Fe^{++} to Fe^{+++} leading to these pulses. They can be minimized by the addition of a minute amount of EDTA to the buffer. To prevent the release of Fe^{++}, it is advised to periodically (e.g. once in a month) flush the system (disconnect the column and detector!!) during 30 min with 10 - 15% nitric acid.

NB: 1) It has been found that at potentials above 1 V EDTA contributes to the cell signal.

2) Buffers made up from citric acid give similar problems.

If Teflon tubing is used, overtightening of the fingertight fittings on the column outlet and the cell in- and outlet will generate noise, caused by increased pressure and/or turbulences.

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III MAINTENANCE

III.1 Detector cell

Resurfacing the working electrode is necessary when the response of the cell is relatively low. Indicative, as mentioned above, is a low background current. Visual inspection of the working electrode will reveal a spot if the electrode is fouled. This mostly is caused by fouled or concentrated samples depositing reaction products of the oxidation process on the working electrode. Since the detector has a very high signal to noise ratio it is worth considering to dilute the samples to postpone resurfacing.
A high background current and/or increased noise points at an unsaturated reference electrode or a fouled mobile phase.

Resurfacing should be performed if cleaning of the electrode surface with a tissue soaked in methanol or ultrasonic cleaning of the whole working electrode block during about 30 min in acetone is without result. Resurfacing is done by polishing the electrode block with diamond slurry (0.25µm) and **distilled** water. A silk disk is supplied with your detector. In switching the cell on then, again a large charging current is observed.

An alternative and in our hands very effective solution is possible with a short treatment of the working electrode with so-called chromic acid (concentrated sulfuric acid with potassiumdichromate). To do so, put a small drop of chromic acid solely on the surface of the working electrode (max. 30 sec). Rinse the electrode then for at least 2 min with tap water and distilled water respectively. To prevent fouling, do not touch or wipe the electrode any more. After connecting the cell to the controller a much slower decay of the charging current is observed which is characteristic for this treatment.

The reference electrode should be filled with saturated KCl (or, if necessary in perchlorate containing buffers, with saturated NaCl). It is advised to check whether KCl cristals are present in the electrode chamber, to ensure saturation of the electrode.

Air bubbles, especially in the reference chamber or its connection to the cell, may be a constant annoyance or an occasional problem. To prevent such problems the following suggestions are of interest:
- expel dissolved gases by continuous bubbling of the mobile phase bottle with helium, or by vacuum treatment.
- expel bubbles from the detector by shortly blocking the outflow of the cell during flow, and abruptly release the blockade.
- refill the reference electrode.

IV LISTING OF KNOBS, SWITCHES AND CONNECTORS.

- Power (on/off): Is situated on the back of the instrument and applies mains electrical power to the unit. Whenever the power is switched (ON or OFF) the cell should be in the OFF position.

- Cell (on/off): Applies the necessary potential to the working electrode referred to the Ag/AgCl electrode.

- Potential adjustment: Establishes the value of the working electrode potential which can be read on the display.

- Offset: This is accomplished by the switch in the auto-zero frame, or it can be externally controlled (TTL-compatible). The offset level (see potmeter in blue frame on back panel) can be set to max. ± 0.13 V. This level can be set/monitored by pressing the autozero knob and simultaneously checking the V_{out} in the display.
This function is very helpful if drifting baselines are observed, since after activation the autozero function will jump to this preset level.
The offset can be switched off in the reset position.

- Sensitivity: The current sensitivity range in nA/V is selected, compatible with the recording device used. Therefore it is most easily to use the 1 V setting on the recorder or integrator.

- Filter: Smoothens the output signal, but also affects the peak shape of sharp peaks.

- Display: Three functions are monitored, the cell potential, the cell current and the output potential.

- Output: Two output ranges are covered, the 0.1 V range limits to 1V, and the 1 V range to 10 V.

- Cell cable: the plug should be inserted with the red dot on top. It can be released by pulling on the shielding of the connector.

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V SPECIFICATIONS

CONTROLLER

Potential range: 0 - 1.500 V, oxidative.

Sensitivity range:
Standard version: 0.1 - 100 nA/V (max. 1000 nA), in 1, 2, 5 sequence).
µ-version: 0.01 - 10 nA/V (max 100 nA), in 1, 2, 5 sequence).

Current offset: 120 nA (standard), 12 nA (µ-version).

Power: 110 - 120 V or 220-240 V AC, 50/60 Hz at less than 100 mA.

Time base recorder: 1 V or 0.1 V full scale (maximum linear output of the potentiostat is 10 or 1 V resp.).

Time constant: 0.1 - 5 sec in 1, 2, 5 sequence.

Offset level continuously adjustable from -0.13 V to +0.13 V

FLOW-THROUGH CELL

Conversion efficiency: 5-10% (spacer 50 µm).

Noise: about 1 pA (mainly determined by flow fluctuations).

Cell volume: WE diam. 3 mm, 0.33 µl (spacer 50 µm).

Detection limit: better than 0.5 pg for dopamine (applying a 100 x 4.6 mm analytical column with spherical 3 µm particles, capacity factor for dopamine about 3, cell potential 800 mV, time constant of 2 sec).

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VI TROUBLESHOOTING GUIDE

PROBLEM	PROBABLE CAUSE	REMEDY
Absence of detector response	No power	Plug powercord in
	Powerswitch off	Turn this switch on (at the backside)
	Faulty fuse	Replace it (reserve is in mains switch housing, 100 mA)
	Divergent mains voltage	Connect to 220-240V or to 110 - 120V
	Cell switched "off"	Switch it "on"
	Cell potential "0"	Check on display
	Working electrode(WE) disconnected	Check WE connection
	Cell cable disconnected	Check connection
	Recorder/integrator disconnected	Check connection, also: check on display (output)
	Fouled WE	Clean WE (e.g. with chromic acid)
Noisy baseline	Salt bridge in reference electrode(RE) not saturated	Refill with saturated KCl (or NaCl)
	Air bubble in RE or in cell	Remove it
	Slow temperature fluctuations in laboratory ("Wander")	Isolate detector cell
	Fouled WE (low cell current ---> display!)	Clean WE
	Leaking RE or cell	Tighten connections
	Contaminated buffer	Replace buffer
	Low time constant	Increase time constant
	High WE potential	Optimise potential

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PROBLEM	PROBABLE CAUSE	REMEDY
High cell current	Contaminated buffer	Replace buffer
	Fouled WE	Clean WE
	Salt bridge in RE not saturated	Refill with KCl cristals
	Cell potential too high	Check it (display)
	Strongly retained peaks from previous runs are passing the WE	Wait for elution of these (very) broad peaks
	Column is too "old"	Replace column
	High amount of Fe^{++} in buffer	Add EDTA to buffer and/or rinse metal parts with 10-15% HNO3
Saturation of output	Damaged RE	Check RE against saturated calomel electrode. If difference is greater than about 70 mV, replace it
	Damaged WE	Replace this cell block
	Cell incorrectly connected (esp. RE!)	Check connections (RE: white, WE: red, AE: blue)
	Cell potential too high	Check it (display)
Baseline oscillations	Malfunctioning pump	Check pump (seals, valves)
	Overtightened or wrongly tightened cell bolts	Adjust cell bolts, check pump pressure!
	Air bubbles in cell and/or RE compartment	Remove them
	Temperature oscillations	Prevent oscillations
	Contaminated buffer	Replace buffer
	Fouled WE	Clean WE
	Fe^{++} in buffer	Add some EDTA, or passivate metal parts with HNO3
Decrease in sensitivity	Fouled WE by determination of "dirty" samples	Clean WE or dilute samples
	Cell potential too low	Check it (display)

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