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Loss of Response

INTRODUCTION

Loss of response in an HPLC-electrochemical detector (ECD) system will usually be perceived as a dysfunction with the detector. This is where the operator will see the problem. Consequently, the ECD is usually the first component to be suspect. In actuality, problems with the ECD itself are rare – the cause is more likely to be issues with other components in the HPLC system, the analytical chemistry being used, or analyte integrity. Assuming that the problem is with the detector and/or cell will be misleading. Methodical troubleshooting will pinpoint the trouble. It is always a good idea to keep a daily log of system performance and conditions to help diagnose the situation. The following parameters should be recorded:

- Flow rate and backpressure
- Response for external standard/test mixture
- Column age and approximate number of samples run to date
- Age of EC cell
- Applied potential and typical background current (for each electrode; use autozero off for CoulArray® cells)
- Composition and age of mobile phase—length of time it was recycled
- Prior use of column or EC cell for different chemistries or biological matrices
- Major event (e.g., leak, system shut down, brown out)

When an EC cell is first installed and has been given adequate time to stabilize, it is imperative to construct an hydrodynamic voltammogram (HDV). (See Technical Note 101.) Evidence of change in a cell's current-voltage behavior may help to guide the process of troubleshooting the system.

WHERE TO START?

First, it is important to understand what the problem is. For example, are the peak responses a fraction of their normal height or has the response gone completely? Are all analytes affected to the same extent? Has the retention time changed significantly? Is there an issue with peak detection or integration? Also, troubleshooting will proceed very differently if the loss of response corresponds to an event such as a change of column or mobile phase, compared to a gradual decrease in response over time.

TROUBLESHOOTING GUIDE

To start, make sure that all components are powered up and functioning correctly. If in doubt, do a full-system reboot. Are the values for backpressure, background current, etc., within normal ranges (refer to your log book)?

1. Check the Fluidics

- Are there any leaks? If yes, tighten or replace the fittings.
- Is the backpressure normal? If not, check for leaks or blockages.
- Is the backpressure fluctuating more than a few psi? If yes, refer to the pump's operating manual for troubleshooting.
- If you are using a bubble-trap, make sure that it is full of liquid.
- Does the measured flow from the outlet of the cell correspond to the flow rate set on the pump (use a graduated cylinder to check)? Refer to the manual or technical note, as above.

2. Check the Autosampler (refer to the operation manual for details)

- Is the response for all compounds in the external standard/test mixture reduced by the same percentage? If so, then the problem is most likely due to the amount injected.
 - Was the standard made properly?
 - Is the injection volume correct?
 - Is there sufficient sample in the vials?
 - Is there a bubble in the syringe—if yes, use the syringe wash function, or other physical means to remove it.
- Is the autosampler injecting? Replace the injector rotor seal if necessary
- Is the needle straight? If not, replace the needle.
- Is there fluid in the wash bottle? Is it compatible (soluble) with your mobile phase?
- If using Microliter Pick-up mode (Dionex autosampler only), is there a filled transport vial with fresh mobile phase on the autosampler tray?
- Is the autosampler injection from the correct vial?
- Is the tray temperature too cold (i.e., sample freezing) or too warm (sample degradation)?
- Is the autosampler draining correctly – make sure the waste bottle is empty and the hose is draining correctly.

3. Check the Column

- As there any leaks?
- Are peaks broadened or tailing?
- Does the guard column need replacing?
- Does the column need replacing/regenerating?
- Is it at the correct temperature (check column oven temperature)?
- Is the correct flow rate being used?

4. Check the Electrochemical Detector

- Are the applied potentials and background currents normal (refer to log book)?
- Are the cell(s) on?
- For the Coulochem[®]—do you get an event mark when this button is pushed?
- Check if the potentiostat is working normally by performing the Detector Test Protocols (see operator's manual)
- Are the correct gain ranges being used? (Coulochem only)

5. Check the Cell

- Are there any leaks?
- With the 5040 and 5041 cell—is the cell assembled correctly? Is the working electrode wire attached correctly?
- With the 5041 and 5014B cell—was the cell reference port purged correctly?

6. Check the Data Station

- Is the data station set up correctly?
- Is the display axis correct?

7. Check the Analyte (external standards)

- Is the analyte stable? If not, prepare fresh and reanalyze.

8. Check the mobile phase:

- Was the mobile phase prepared in the same way?
 - This is important when mixing aqueous and organic solvents.
 - Was the pH adjustment done before or after addition of organic solvent?
- Was a new bottle of reagent used?

If all of the above items check out normally, then set the system to run a sample.

- Do you get a 5% deflection when the event mark is pushed (Coulochem only)? If not, recheck Cell Simulator Test.
- Do you get a void (solvent front) response when a blank (diluent) or standard is injected? Make sure that the gain range is at an appropriate setting. Is the solvent front at its normal time?
- Inject a high concentration standard—do you see a peak? Is it the correct size? Does it have the correct retention time?
- Construct an HDV. How does this compare to the original (see your log book)? If the response is decreased for a given amount of analyte, then try cell cleaning (below) or replace with a new cell.

Some comments on Peak size and Retention time

- Peak is present but much later than normal—check flow rate, check pump, purge to remove bubbles, check column temperature, age of column, mobile phase preparation, and composition.
- Peak is present at the correct retention time but is much smaller than normal—its size is decreased relative to the void response. Check standard (make up fresh) and check dilution. Possibly a problem with auto-oxidation.
- Peak is present at the correct retention time but is much smaller than normal—Both it and the void response have decreased equally.
 - Check injection volume, check for bubbles in the autosampler syringe, check applied potential and gain range setting.
 - Do a clean cell (e.g., +1000 mV, 5 mins) to refresh the electrode.

Sometimes cells are damaged (response usually decreases rapidly and catastrophically—e.g., brown out, running cell with no electrolyte in the mobile phase, running the cell with zero flow), or age (usually a slow decrease in response over time—e.g., use of high potentials, build up of adsorbed materials due to low organic mobile phase). Aged cells can sometimes be revived by using electrochemical treatment or by cleaning with organic solvents (see Technical Note 93). However, the effectiveness of such treatments vary, and sometimes the defective cell has to be replaced.

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