ABSTRACT

The following protocol describes how to prepare microbial fuel cells (MFCs) with Indium Tin Oxide (ITO) electrodes for use as a poised potential enrichment tool, specifically an oxic system to enrich for the cathode-oxidizing community. The protocol describes setting up four “online” MFCs that are connected to a potentiostat at a particular set voltage plus one “offline” unconnected control (to account for non-electrode related changes). One of the online MFCs is a “negative” killed substrate control (to account for abiotic medium-source current fluctuations) and the remaining three MFCs being replicate live samples. This protocol is based on advice primarily from Frauke Kracke and Annette Rowe.

ATTACHMENTS

Bioelectrochemistry protocol For CHI Potentiostat_v3.docx
Supplies:

- Four 2-chamber 100ml volume microbial fuel cells (Addams & Chittenden Scientific glass) (This system is 3-port. At least 1 port is necessary)
  - Note: handle with extreme care to avoid knocking the side-arms on the MFCs, as these are brittle and prone to breaking.
- For each 3-port 2-cell set (Also Addams & Chittenden Scientific glass): 25mm knuckle clamp and seal set, 2x GL45 open cap, 2x GL45 Tf/silicone septa, 6x GL14 open cap, 6x GL14 Tf/silicone septa. 2x 4mm glass tubing, 10 cm length (VWR), 25mm filter holders (Cole Palmer Advantec 43303010 Polypropylene), 25mm Whatman 0.2 um mesh filter membranes (VWR)
- One 100ml glass pyrex bottle with septum cap
- Indum Tin Oxide (ITO) electrodes (Delta Technologies Ltd. Catalog # CB-50IN-1111)
- Reference electrodes (Working reference: Gammry plastic skinny Catalog #932-00018 OR Analytical Instrument Systems (AIS) glass reference electrodes)
- Lab master reference electrode (Gamry Standard electrode Part No. 930-00015–DON'T USE FOR ANALYSIS. CALIBRATON ONLY)
- Ti wire (16’ gauge, surgical grade, 100% pure)
- Carbon cloth (Fuel Cell Store, Plain Carbon Cloth - 1071 HCB. Catalog #591342)
- Nafion 117 membrane (Fuel Cell Store. Catalog # 591539)
- Regular 3” x 1” glass microscope slides
- Wooden cocktail sticks
- Kimwipes
- Pencil (do not use pen!)
- Plastic bin for soap bath
- Ruler
- Wire cutters or scissors
- Paper towels
- 9V battery
- Crocodile clips
- Metal spatulas
- Metal tweezers
- Glass beakers
- Q-Tips
- Falcon tubes
- Glass cutter/diamond scribe
- Sharps waste container
- Reference electrode
- Scalpel or razor blade
- Beakers
- Vycor porus glass frit (Gamry Instruments)
- Plastic heat shrink tubing
- Hollow luer lock needles (20G x 1 ½ inch)
- 3mL Luer-lock syringes
- MB Biomedical DNA kit

Reagents:
- 100% ethanol
- 90% ethanol
- 80% ethanol
- 70% ethanol
- 60% ethanol
- 50% ethanol
- MilliQ water
- Conductive Silver Epoxy (MG Chemicals. Note: Needs lowest resistance rating possible)
- Epoxy (Loctite Quick-set)
- Double autoclaved media (composition dependent on experiment type)
- Simple Green detergent
- 150 mM NaCl
- 0.1M HCl
- 1M Sodium bicarbonate solution, autoclave sterile
- Butane gas
- 3M NaCl
- 3.5 M KCl saturated with excess AgCl (AIS) – Glass AIS
- 4 M KCl saturated with excess AgCl (Gamry – Catalog #955-00004) – Plastic Gamry skinny
- 3 M NaCl
- Hexamethyldisilane
- Test Solution (10 mM Potassium ferricyanide in 100 mM KNO₃)
SAFETY WARNINGS

- Safe laboratory procedures should be followed at all times.
- Users should familiarize themselves with MSDS forms for all chemicals to be used.
- Preparation of the electrodes requires use of epoxy and the generation of small amounts of chlorine gas – these steps should be done under a fume hood.
- Many things need to be autoclaved; autoclaving training is mandatory.
- Users should always wear gloves – in addition to chemical safety, gloves act as (limited) insulators in case of electrical charge buildup.
- Goggles must be worn when cutting ITO glass slides and handling cut slide pieces.
- Users should ensure no current is passing through the system before touching any leads, and should disconnect the counter lead (green) first.
- Users should NEVER allow leads and electrodes to touch that have current passing through – Caution is especially necessary while handling when connected to a battery.

BEFORE START INSTRUCTIONS

Useful Resources:


Appendix 5. Basic principles

1 Preparing ITO “working” electrodes and carbon cloth “counter” electrodes

- Measure and cut 5 lengths of Ti wire 8cm long. Rub well with 100% ethanol and kimwipe to remove impurities (These will show up as black marks on the kimwipe.
- Measure and cut four 1.5” x 1.5” pieces of carbon cloth for the counter electrodes, and lay out on a piece of paper towel. If placed on a plastic tote lid, this makes for convenient carrying.
  - *Note*: It is important for the counter electrodes to be larger than the working electrodes, to avoid overflow errors
  - *Note*: ONLY USE PENCIL TO MARK
Take 5 ITO electrodes and lay them on a piece of paper towel. Look carefully for scratches and brown patches in the violet film; dispose of any with these imperfections.

Find out which side has the ITO coating by placing nibs of multimeter on the glass and measuring resistance (Set dial to Ohms – Ω) and seeing when you get resistance. Place the electrical resistant sides up.

*Under the hood,* thoroughly mix a lentil-size amount of silver epoxy on a plain glass slide with a cocktail stick.

Transfer ~3 mm³ on the middle edge of each ITO working electrode and carbon cloth counter electrode (Fig. 1).

Immediately press up to 0.5 cm of one end of the Ti wire into each epoxy drop, carefully but firmly.

Leave under hood for ~24 h to cure.

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**Two days before starting experiment**

2  **Soaking membranes**

- Cut Nafion 117 membrane into four disks of ~25 mm diameter.
  
  **Note:** Drawing around the seal of the MFC WITH A PENCIL is an acceptable way to do this.

- Put membranes in ~ 200 mL MQ water in a glass or plastic beaker with a cover to exclude dust.
- Soak overnight to pre-swell material.

**Preparing electrodes, continued**

- *Under the hood,* thoroughly mix a pea-size amount of plain epoxy pastes together on the slide with cocktail stick, and then place a small blob on top of the cured silver epoxy junction on each ITO and carbon cloth electrode (Fig. 1).

- Leave for at least 24 h to cure. This seals the silver from oxidizing (and increasing resistance) and strengthens the junction.

- Check calibration of at least four reference electrodes (more is good, just in case) and regenerate if necessary (see Appendix 1)
Figure 1a) Fully built ITO electrode

Figure 1b) Fully built carbon cloth electrode
If regeneration of reference electrodes was necessary, confirm regeneration was successful and repeat, if necessary.

Pre-clean ITO electrodes by sonication for 15 min in detergent (1 cap of Simple green in 500 mL MilliQ water, then rinsing under a constant stream of D.I. water for 30 min.

- Note: An efficient way to do this is to have a plastic beaker constantly overfilling, with the electrodes in the beaker.

- Sterilize the ITO electrodes by either:
  - irradiating slides under UV for 15 min per side.
  - Adding electrodes to MFC build before autoclaving (20 min at 121°C), so they are included in the sterilization process in MFC.

- Note: Make sure there is no chance the glass will knock against the MFCs during autoclaving – this will cause the junction to weaken and the glass slides to part from the wire. Have spare electrodes built, if this is your preferred option.

- Note: DO NOT autoclave wrapped in foil, contact with metal and coating will cause damage to the coating, causing it to become black and peel off.

Pre-clean carbon cloth counter electrodes (note: once prepared, these can be autoclaved repeatedly, and re-used many times):

- Under a hood, Attach an electrode to each pole of a 9V battery with crocodile clips.
- Immerse built electrodes in 150 mM NaCl solution for 30 s.
- Switch poles and repeat.
- Carefully dip in 0.1 M HCl for 1 s ten times, then 80% ethanol for 1 s ten times, then MQ water for 1 s ten times.

Construct MFC as shown in Figure 2 and fill each cell with 100ml of DI water. Also fill the 100ml glass bottle with the same.

- Note: knuckle clamp should be finger tight only.

- Autoclave assembled MFCs and 100ml glass bottle at 121°C for 20 minutes. Allow to cool overnight on the bench.

- Autoclave media and negative substrate control for a second time for 1 hour at 121°C, then cool to incubation temperature for experiment. For 4C incubations, this means leaving out for a couple hours, then in the fridge overnight.
Figure 2a. Schematic of cells complete and ready to go. Note, gas exchange ports are not included in working cell for this configuration. RE = reference electrode, WE=Working electrode (ITO glass) and CE= counter electrode (Carbon cloth). Filters are 25 mm 0.2 um mesh Whatman filters in autoclavable filter holders. PEM = pre-soaked Nafion 117 proton exchange membrane and gasket, secured with knuckle clamp. Right cell is counter, left cell is working. See “day of” instructions for detailed information about placement.

Figure 2b. Schematic of cells. RE = reference electrode, WE=Working electrode (ITO glass) and CE= counter electrode (Carbon cloth). Filters are 25 mm 0.2 um mesh Whatman filters in autoclavable filter holders. PEM = pre-soaked Nafion 117 proton exchange membrane and gasket, secured with knuckle clamp. Right cell is counter, left cell is working. See “day of” instructions for detailed information about placement.
4 **T₀ - Day to Start Experiment**

- Set up the laptop controller and potentiostat near the location of incubation (i.e. near a fridge if experiment is being done cold)
- Make sure computer is connected to Potentiostat and is on.
- Turn on Potentiostat (little round button – will light green).
- Turn on software on computer.
- Run a hardware check (Figure 3). If this comes back as anything other than ‘OK’ there is a fault. If so, contact manufacturer.

![Figure 3](https://dx.doi.org/10.17504/protocols.io.xihfkb6)

**Figure 3. Screenshots of hardware check**

- Label MFCs – live samples, negative controls, offline positive control, killed control, etc.
- *Under the HOOD*, sterilize each reference electrode by rinsing well with 100% ethanol, then
waiting for a minute or two.

- Add reference electrodes and caps with o-rings
- Empty working cells of MFCs of autoclaved water and fill with 100 mL of sterile media (or raw sample fluid, if appropriate). Also fill the 100ml glass bottle with the same.
  o Note: Pipetting works best here, particularly with precious media
  o Note: It is most important that the reference electrode is submerged. When choosing a higher port to operate it from, it may be necessary to add an additional 5-10 mL of fluid. This should be consistent across the cells.
- Empty counter cells of MFCs of autoclaved water and fill with 100ml of sterile media.
- Add 1ml of 1 M bicarbonate solution to all cells and offline control to act as a pH buffer (10 mM final concentration).
  o Note: A buffer is necessary to account for proton movement caused by electrodes. The exact buffer and concentration should be tailored to the media but should be biologically safe.
- To the working cells of the MFCs for live samples (and the offline positive control), add a portion of sample using sterile spatulas
  o Before transferring, tare the balance, then weigh the sample vial with sample.
  o Transfer sample to replicate MFC and POSITIVE CONTROL using sterile spatula. Remember that ‘online negative’ control should have none.
  o Weight the sample vial again, and subtract the weight from the initial measurement to get the weight of sample added
  o Aim for ~2 cm3 of volume or 3g of sample per MFC
  o Transfer the same volume of sample to a sterile cryotube and freeze for a T₀ microbial community assessment.
  - To the negative substrate control, add previously autoclaved substrate where necessary.
  - Insert the reference electrodes into the MFCs in whichever port makes the most sense.
  o Note: I use top center, but a lower port would be more efficient. BE CONSISTENT IN PORT CHOICE.
  o Make sure the conductive side of the ITO working electrodes are facing the reference electrodes in the working cell, and that they are approximately 4 mm apart
  o Note: they should not be touching and they should not be more than 4mm apart.
  o Use tape to secure their position
- Connect each MFC to the potentiostat cables, in the following order: Reference (white clip) to reference electrode, Counter (red clip) to carbon cloth electrode then working (green clip) to ITO electrode. Secure the latter with tape to keep it from moving.
- If experiment is being run at cold temperature, carefully transfer MFCs and closed positive control to fridge and tape door closed to incubate in the dark (Figure 4). If the experiment is being done at room temperature in the dark, a cupboard, foil wrap or upturned box are options.

protocols.io | https://dx.doi.org/10.17504/protocols.io.xihfkb6
Set up software settings for a cyclic voltammetry (CV) sweep as for Figure 5. A cyclic voltammetry sweep at the beginning generates a “before” profile of the electrochemically active substances in the experiment, for comparison to after.

- Do 3x CV sweeps. Press play to initiate analysis.
  - Note: I label my analysis thusly: (Project code)(Technique code)(Run sequence)(time)(date)
  - (run subset sequence).
  - Note: The machine is set to autosave, but it is better to save your data each time with a useful filename.
Note: See Appendix 5 for troubleshooting

Check for OVERFLOW error (A chime will happen, and the word OVERFLOW will appear in the lower left corner of the screen). If this happens, STOP IMMEDIATELY.

- Change software setting to chronoamperometry (CA) (Figure 6).

Figure 6a. Software settings for chronoamperometry run. These set the machine going for the longest it can (~3 days) but it is HIGHLY RECOMMENDED you stop, save and re-start once a day (except weekends, obviously). Just in case

Figure 6b. View of a live CA scan

- Note: All settings should be exactly as they are in figure 6 a. Sensitivity may be changed,
depending on the amount of current being generated (see Overload above for more on this).

- The other key value is the voltage at which the set is poised. This is the energy level at which the electrons are transferred. The value depends on the question posed by the experiment—it is recommended that the literature be consulted prior to voltage choice. Additionally, values approaching +/- 0.8 mV and higher risk generating H₂.

- Note: All measurements are recorded relative to the reference electrode—Ag/AgCl here. The axis for the on-screen graphs are auto-generated, and the figure updated in real time. When running, time left for analysis appears in the upper right hand corner of the screen, also updated in real time (Figure 6 b).

- Check for OVERFLOW error (A chime will happen, and the word OVERFLOW will appear in the lower left corner of the screen). If this happens, STOP IMMEDIATELY.

- See Appendix 5 for troubleshooting
  Check for overflow error after an hour or so (See above and Appendix 5).

### Three days before the end of the experiment (Tend). Making ...

5
- Begin making new ITO electrodes for T_{end} cyclic voltammetry analyses as above (to determine pattern of minerals that may have formed during the experiment).
  - Note: These can be used as the electrodes for the next run as well, with cleaning.

### Tend - Breaking up the experiment

6
- Perform 3 x CV runs (Software settings in Figure 5). Save these after each run.
- Turn the power off, disconnect MFCs from Potentiostat and move cells to hood.
- One at a time from each MFC, remove an ITO electrode slide, handling it by the wire with flame-sterile tweezers, and place it sterile aluminium foil.
  - Note: DO THIS ONE AT A TIME FOR EACH SLIDE – letting them dry out will destroy any biofilm and ruin microscopy data.
- Take a sterile cotton swab and dip it in autoclave sterile MQ water for a couple of seconds to thoroughly wet it.
- Rub this firmly and vigorously across ½ - 3/4 of the slide (on active side) to remove biomass. Cut off the end of the swab with flame-sterilized scissors, and drop into MP biomedical kit FastDNA bead tube (Figure 7.)
Store these in the fridge until time for DNA extraction (Appendix 3).

- OPTIONAL: Add a coverslip and view other half of the slide under light microscopy (I use x100 oil immersion lens). Take micrographs. Don’t linger over this.
- Score the bottom fifth of the slide with a glass cutter and snap off with sterile tweezers (Lay tweezers pointing in same direction as wire for best results). Then, preserve slides for SEM (Appendix 4). *Be careful of cut glass* (Figure 7)
- Add fresh, precleaned ITO electrodes to the cells. Run another CV sweep, with the same settings as before.
- OPTIONAL: Take out fluid for pH and preserve some for other analysis
- OPTIONAL: Fish out rock fragments with a sterile scoop and preserve for other analysis.
- To clean up the equipment, remove, rinse and store reference electrodes. Nafion membranes cannot be re-used. Rinse glass well, scrub with angled brush and put into a deturgeant bath and then an acid bath to clean. For plastic fittings and counter electrodes, skip acid bath. Ti wire can be cut from cut electrodes and re-used if the piece is long enough. Cut ITO electrodes go into sharps waste. Every now and again, it’s worth giving the fridge a quick go-over as well.

Figure 7. Schematic of ITO slide sample collection

You need to save the files as csv. Voltage output needs to be normalized by electrode area at the least. Appendix 4 gives direction on common CV scan issues. CV files can then be plotted as they are. CA files will need combining into one time series. You will probably need to convert A to uA and seconds to something more meaningful as well. You are looking for a difference in current between negative control (which should stay stable) and working cells that increases over time (See Appendix 4).

A note on reporting: All data will be vs. Ag/AgCl (or whatever your reference electrode may be). When reporting, it is good practice to convert to vs. SHE (Use this to help: http://www.consultrs.net/resources/ref/refpotls3.htm).

To help with keeping track of the different physics, chemistry and biology terms, table 1 may be helpful.
Cathode | Anode
---|---
Electrons are … | Donated | Accepted
Electrons move … | Away from | Towards
Electrical Charge (see principles of electrical circuits) | + | -
Redox terms | Oxidized/Reducing agent | Reduced/Oxidizing agent
Standard Redox Potential | - | +
Microbe uses it as an… | Electron donor | Electron acceptor
It is … for the microbe | Food | Respiration
Environmental conditions are .. | ??? | Anaerobic

Table 1. Summary of all the terms, charge symbols and redox values associated with poles of a bioelectrochemical circuit. The TOTAL or NET energy of a redox reaction (its Gibbs free energy) is a separate thing but is a Redox reaction, so the terms and symbols are derived from Redox.

**Appendix 1. Reference electrode calibration and regeneration**

Reference electrodes are the biggest source of error in this system after humans. They rely on equilibrium principles, and therefore somewhat temperature dependent. The Vycor frits (porous glass tips) need to be kept immersed where possible. Reference electrodes are also UV reactive, so need to be kept in the dark. Treat them like pH probes (which they are, in a way), and you can’t go too far wrong.

**Calibration**

Compare reference electrode with Lab master by placing both in 3 M NaCl solution, connecting Lab master to negative and reference electrode to positive of multimeter. It should read 0 mV (+/- 20 mV). If it does, no regeneration needed. If it does not, following regeneration steps below.
Regeneration

- Slice through heat shrink tubing with scalpel blade and remove Vycor frit.
  - Note: Used frits may be regenerated by soaking in 80% ethanol, then sonicating for 15 min and soaking in MQ water.
- Check for cracks and damage to glass and wire.
- For serious cases (i.e. +/- 50 mV out), attach ‘–‘ pole of 9V battery to electrode and Pt wire to ‘+‘, then touch wire to fluid in electrode for 1 min. Switch poles on battery and repeat. Then, switch poles back and repeat again.
  - Note: This ‘blows off’ any impurities and crud from the electrode wire surface that is interfering with its efficacy – when working you'll see bubbles of chlorine gas come off the electrode wire)
- Remove old fluid from glass tube with hollow needles and syringe and flicking.
- Soak overnight in 80% Ethanol, then rinse.
- Fill with 0.1 M HCl, and leave soak for at least 30 min, preferably a couple hours.
- Thoroughly wash with D.I. water x3
- Fill with filling solution – Gamry for Gamry electrodes, AIS for AIS electrodes.
- Attach ‘+‘ pole of 9V battery to electrode and ‘–‘ to Pt wire. Touch wire to fluid for 1 min exactly.
  - Note: This causes Cl⁻ to deposit on the Ag wire as a purplish-brown discoloration. This causes an ion gradient, which is how the electrode works. Get your battery poles mixed up, and you have to start the process all over.
- Cut approximately 2 cm of heat shrink tubing and fit to the throat of the electrode
- Add frit
- Use heat gun on low to carefully shrink heat wrap to fit frit and electrode end *Be careful of heat gun*
  - Note: Be wary of heating end to much. It will cause fluid to boil, expelling the vycor tip at great velocity and causing bubbles. It may also crack the frit or soften the heat-shrink tubing so it goes gooey.
- Trim tubing and frit so that the end is flush with a sharp razor blade.
- Check for bubbles. Flick to remove to far end of electrode, or try again.
  - Note: Bubbles at the frit interfere with efficacy of electrode
- Store in filling solution in the dark. Foil wrapped 15 mL falcon tubes with a hole in the top and parafilm are your friends here.
- Leave for a day before using, to allow electrode solution to reach equilibrium.

Appendix 2. DNA extraction from ITO working electrodes

- Add T⁰ to a bead tube.
- OPTIONAL: Do a heat freeze-thaw lysis step in a thermocycler (15 min at 95C).
  - Note: If you do this, put Q-tips in a 0.2 mL Eppendorf tube with 20 uL Molecular grade MQ, then transfer to a bead tube, rinsing out the Eppi with ~2 uL of Molecular grade MQ.
- Add MP Biomedical kit step 1 reagents
- Take all tubes down to an -80C freezer, place in for 5 minutes to freeze then leg it up the
stairs so the samples thaw in the fridge.

- Follow MP biomedical kit instructions, with one exception. Instead of initial agitation with a vortex, use to Pete’s Retsch MM 400 shaking machine (Program: Frequency: 20 1/S for 5 min).
- Measure DNA concentrations
- Store at -20C.
- If DNA concentration is low, you will need to do a PCR to ascertain if amplifiable DNA is present before sending for sequencing.

**Appendix 3. SEM prep.**

10 IN BIOHOOD

1. Prepare 10 mL solutions of 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100 % sterile MQ water: Ethanol (filter again for sensitive applications) and 10 mL Hexamethyldisilane (AMOS) (find this in the flammables cabinet in the basement) in petri dishes. Label well.
2. With sterile tweezers, take each piece of freshly cut slide (active side up) and immerse in each solution for 10 seconds sequentially, finishing with the AMOS.
3. Air dry in hood for 48h.
4. Gold-coat as per instructions (Machine is with SEM in basement of C wing).

**Appendix 4. Data output guide**

11 *Chronoamperometry (Figure 8)*

The WE becomes a source/sink of electrons. A constant voltage is applied and variation in current (within a set range) is measured. In this way, the electrode can function essentially as a mineral.

![Figure 8. Example chronoamperometry readout (Rowe et al., 2015)](https://dx.doi.org/10.17504/protocols.io.xihfkb6)
Chronopotentiometry
Using the electrical potential generated by electrodes in an electrochemical cell to hold the contents of that cell to a narrow redox potential range, forcing a certain set of reactions to occur. In multiple cell systems an abiotic reaction can be generated at the WE, which generates a product that can pass through the membrane to a second cell containing the culture. The CE is located in the final cell in the chain.

**Cyclic Voltammetry (Figure 9)**
Voltage is varied across a range to scan for peaks that correspond to a certain redox reaction happening at an electrode, to identify if particular redox reactions are occurring. This is useful to determine if a particular redox reaction is happening in the system, and its magnitude. This can be calculated from the volume of space beneath the curve.

![Figure 9. Example Cyclic Voltammetry readout (Rowe et al., 2015)](image)

**No current**
- Is everything turned on?
- Is everything connected?
- Is membrane facing the correct way?
- Is there any damage to membrane?

**Low current**
- Dropping the pH in the CE chamber will increase current (change in redox potential). However, if the difference in potentials becomes great, this causes an electrical current to which stresses...
the microbes.
- Replace CE (fouling/depletion)

**Excessive noise in readings**
- Check for gas bubbles interfering with RE frit. Careful positioning of gas inflow relative to RE should assist in this issue.
- Replace RE

**Overflow error**
This error indicates that resistance is causing an overload in the system, and is damaging the reference electrode calibration. If caught quickly, you can drop the sensitivity of the analysis. If the error continues, make the counter electrode bigger to ease electron flow pressure. If the error was not noticed for a while, then regeneration of the reference electrode may be necessary.

**CV**

*The experiment doesn’t register the run command*
If the run command does not respond, update then run the experiment from the EXPERIMENT menu. The Run command does both these steps in one go, but can get confused easily.

*Current warning light is on (AIS only – analog of Overflow in CHI system)*
Current is too high, and must be reduced.

*Voltage warning light is on*
Voltage is wrong. The most likely cause is a reference electrode malfunction. Swap for a new, good reference electrode.

*Dummy cell result is poor (Fig. 10)*

![Figure 10. A cyclic voltammetry sweep with correct settings but wrong cable configuration](https://dx.doi.org/10.17504/protocols.io.xihfkb6)
A dummy cell connects the cables to a resistor in the order Working, Reference, resistor and counter. This checks the cables are working. First, check that the cables are connected correctly, then run a CV sweep. Finally, replace the cables as they may be damaged.

*Test Cyclic voltammogram looks wrong*

If the peaks in the cyclic voltammogram have shifted (e.g. Fig. 11) then the reference electrodes require regenerating.

![Figure 11. Cyclic voltammograms (a CV program with Test Solution) showing shifted curve due to malfunctioning reference electrode.](https://dx.doi.org/10.17504/protocols.io.xihfkb6)

If the curves are jagged and not smooth using the a CV program and TEST SOLUTION then the instrument is picking up electrochemical ‘noise’. It is also worth checking the reference solution was made correctly. Sources of noise can include other instruments, desktop computers, fluorescent lighting, aircon systems and strong radiowaves (a faraday cage is recommended for this issue, such as a static protection bag). The AC power of a building can also be a cause. Powering the instrument off the battery can also assist in diagnosing this.

*Help! My curve looks wrong*

Option 1: There is an overlapping tail (Fig. 12).
Solution: Add a preconditioning step. The overlap is because there is not a chemical equilibrium at the beginning of the experiment, so the end point of the experiment has shifted.

Option 2: There is a long tail on the voltammogram (Fig. 13)

Answer: Drop the voltage range. The experiment has taken place well within the voltage range specified. This isn’t a large problem, but decreasing the voltage range speeds up the reaction.

Option 3: Lots of ‘noise’, despite the test CV showing none (Fig. 14)
Answer: Current range is too high, so decrease it. The instrument is having difficulty distinguishing the data when the range is too great.

Option 4. The curve is clipped (Fig. 15)

Answer: Current range is too low, meaning that the rest of the information is not recorded. Increase the current range.

Option 5. The curve is wide, and the delta (gap between tails) is large (Fig. 16)
Answer: Scan rate is too high, reduce the scan rate. An artificially elevated current reading is generated because there is insufficient time between scan pulses for the electrical signal to drop back down, so the digital and electrical pulses are no longer quite in sync.

Appendix 6. Basic principles and Glossary

13 (Note: A helpful resource for very basic electricity concepts https://learn.sparkfun.com/tutorials/tags/concepts)

**Current** (Amps; I). The rate of electrons passing through a particular point in a circuit. 1 amp = 6.241x18 electrons/s. It may be useful to think of it as analogous to flow rate.

**Electrical Potential** (Joules per coulomb; J). The amount of energy available to move electrons.

**Energy** (Joules; J). The capacity for a system to perform an action.

**Linear current** (DC). Electrons flow one way through a system.

**Voltage** (Volts; V). The difference in charge between two points with different energy potentials. It may be useful to think of it as an energy gradient.

**Power** (Watts; W). The amount of energy available to do actions (Energy/Time)

**Resistance** (Ohms; Ω). This describes the amount of obstruction to electrons passing through a system. Like having a thick crowd of people in your way vs. no-one.
A potentiostat is an instrument that measures and controls **voltage (electrical potential)** and **current** through a circuit formed of electrodes and a conductive substance (solid or liquid) in between these in order to perform certain techniques. For example, given a particular set of circumstances current (electron flow) can act as an electron donor/acceptor in a REDOX reaction. Ohms law illustrates the relationship between these (Fig. 17)

![Ohm's Law Diagram](image)

**Figure 17.** Illustration of the relationship between voltage (V; Volts), current (I; amps) and resistance (R; ohms), according to Ohm’s Law.

Electrochemical systems work by DC or **linear current** (as do batteries). Modern potentiostats use digital signals, meaning that the information is released and read in discrete packages. So, the signal the potentiostat reads is stepwise rather than linear. The electrical pulse signal itself is a wave. Data points are taken along the intercept of these two signal types, according to how a potentiostat is configured. Data points are then joined together to produce the smooth curve (Fig. 18).

![Potentiostat Data Curve](image)

**Figure 18.** How a potentiostat generates data curves from signals. Left: Signal intercept Right: curve generation. X axis is time, Y axis is current.

The more data points the potentiostat takes, the more accurate the data is (what happens between data points is not recorded) but the more inherently ‘noisy’ it is. More pulses (scan rate) increases sensitivity but reduces resolution in the same way.