Fabrication of Carbon Fiber Electrodes for Single Cell Amperometry

Originally designed by Chad Grabner in A. Fox's lab, modified by Billy Roden in A. Harkins lab

Reagents:

Epoxy Resin: Miller-Stephenson, Epon Resin 828

Aliquot epoxy by adding 18.5 g of Epon Resin 828 to 20 ml glass scintillation vials. Use gloves when pouring epoxy. Store aliquots under a fume hood.

Epoxy Hardener: Sigma Aldrich, 1,3-phenylenediamine flakes (MPDA)

Catalog No. P23954-100G04716AJ

Aliquot hardener by weighing 0.7 g into 1.5 ml eppendorf tubes. Place eppendorf tubes in a beaker with foil wrapped around the outside because hardener is light sensitive. Store beaker with eppendorf tubes containing hardener in a dessicator. Caution! MPDA is a messy compound. It should be weighed and poured with care to prevent dispersion, because MPDA turns things brown. Lay out paper towels and use gloves when weighing and pouring.

Materials:

Carbon fibers with sizing removed
Epoxy Resin: Miller-Stephenson, Epon Resin 828
Epoxy Hardener: Sigma Aldrich, 1,3-phenylenediamine flakes (MPDA)
Glass capillary tubes: Fisherbrand, Micro-Hematocrit Capillary Tubes (fire-polished)
Dissecting scope
Electrode puller – Narishige Japan Model PP-830 (1^{st} pull = 24.8°C, 2^{nd} pull = 62.7°C)
Oven set to 100°F
Hot plate

Carbon Fiber Preparation

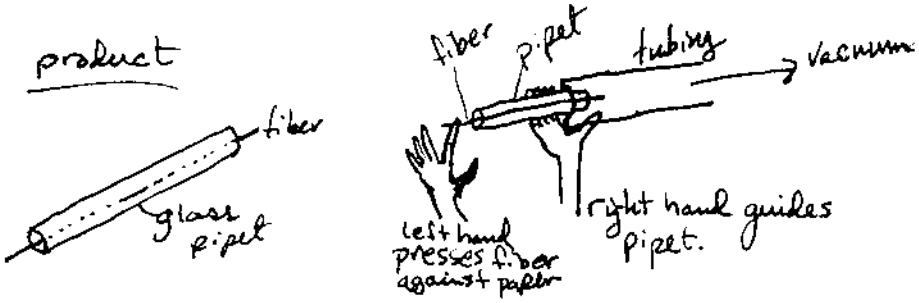
1) Cut a bundle of carbon fiber at a length that is ~1.25 X the length of the capillary pipette. Note: Once the bundle is cut, you want to limit the amount of handling, because handling eventually leads to tangling of the fibers. Tangled fibers are hard to separate in later steps.
2) This step is the removal of "sizing." Most carbon fibers come with "sizing," which is a thin coat of epoxy on the surface of the fiber. The sizing is helpful in the manufacturing of boats and golf clubs. The sizing needs to be removed when making electrodes. This will assure that electrical contact between the fiber and KCL/amplifier is not impeded by a thin layer (insulating) of epoxy sizing.
3) When stripping the sizing remember to avoid tangling the fibers in the bundle. Add a bundle of fibers to a wide-mouth tissue culture bottle (250 mL bottle), or a beaker.
4) Add ~100 mL of acetone to the container and warm the solution just below the boiling point of acetone. (Once the solution starts to boil, lower the temp until the solution stops boiling).
5) Incubate the fibers for ~30 min or longer.
6) Placing a lid on the bottle (not sealed) will reduce acetone evaporation.
7) Rinse fibers. Remove the fibers from the warm acetone and transfer them to a clean beaker with 50-100 mL acetone.
8) Dry fibers. Place fiber bundle onto aluminum foil and allow the acetone time to evaporate.

Fire-polishing Glass Capillary Tubes

1) The glass capillary tubes should be fire-polished on each end over an open flame to avoid ripping of teflon coated Ag/AgCl pellet on electrode holder. Fire-polish each tube by holding the tube in the center with forceps and exposing the end to the flame. Rotate the tube about 3 times while in the flame in order to make the entire circumference smooth.
2) Invert the tube and place the new end into the flame, again rotating 3 times.
3) Check each end for smoothness by running your finger horizontally across the end. If it still feels rough, put the end back into the flame. When checking for smoothness, be sure to let the end cool down before placing your finger on it. Also, be careful not to leave the end in the flame too long because this will cause the end to close off either completely or too much where it cannot be used later. Repeat steps 1-3 until an entire vial of glass capillary tubes have been fire-polished.
4) Place the fire-polished tubes into the oven until they are ready to be filled with carbon fibers.

Threading Carbon Fiber into Capillary Tubes

1) Spread fibers (sizing already removed) out onto a white sheet of copy paper. Hold in place with tape. Orient individual, long (1.25 X) fibers into rows across the paper. Use tape to help isolate single threads.
2) Connect plastic tubing to house vacuum with a filter and make sure vacuum is turned on. Insert a glass tube into the free end (blue marker facing out from vacuum tubing). The glass capillary tubes should be taken from a stock of tubes stored in the oven over night for at least one night in order to ensure that the tubes are dry and not affected by humidity. These tubes should also be fire-polished on both ends with an open flame before they are stored in the oven and filled with fibers.
3) Suck the fiber into the glass tube (the tape will keep the fiber in place while you hold the glass tube in the tubing with your hand). Leave ¼ inch of thread out of tube.
4) Cut the thread using a scalpel. Transport the glass tube to a petri dish with tape in the center to hold it in place.

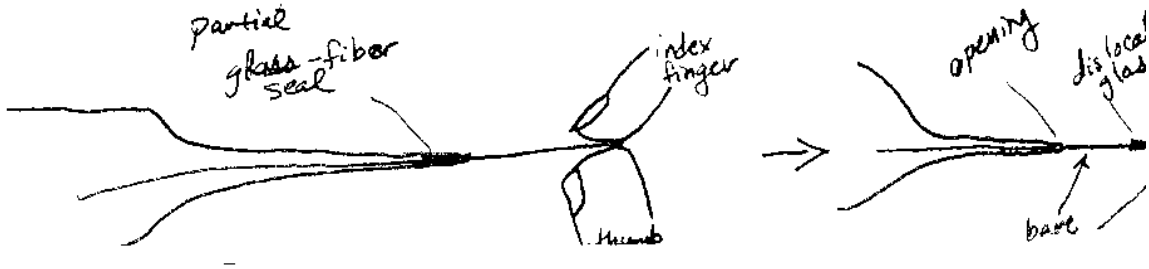


(Nothing is drawn in proportion or to scale)

Pulling Pipette-Fiber in an Electrode Puller

1) Place capillary tube in the top slot. Orient the tube with the blue marker on top and leave about 1/8 inch of free space above the bottom slot. Tighten the tube using the top silver notch.

2) Move black top notch, move the bottom silver notch up. Flip switch on the side. Press start button.
3) After the drop, wait for the coil to cool down. Lower black notch. Move side switch. Press start button.
4) Once the pipette is pulled in half after the drop, the two halves need to be separated enough (~0.7 cm) so that only a segment of bare fiber, no glass connects the two halves. Cut bare fiber at the center by cutting it with scissors. The result is two halves that should be close to identical (assuming the puller settings are optimal).
5) Take out the top electrode. Pull on the protruding fiber by grabbing the tip of the fiber, which enables it to move slightly in relation to the tube. The fiber should move ~1 mm. This breaks the glass seal that has formed where the glass melts onto the fiber. Trim the fiber with scissors so that only 1/8 inch is left outside of the tube. Place in clay to keep it stable and be careful not to smash the tip.
6) Repeat step 5 with the bottom electrode.
7) Repeat steps 1-6 until about 16 fiber-electrodes are pulled.



Making Epoxy-Glass Seal

1) Take a pre-made aliquot of Epoxy. Loosen cap, place vial on hot plate and heat at about 55°C for 6 minutes in a fume hood.
2) While Epoxy is heating, wrap the pulled electrodes by using tape. Obtain a long piece of scotch tape and place sticky side up on the bench. Secure this piece with two small pieces of tape placed on each end. Place a large, full length capillary tube in the center. Line up the electrodes on one side of the large tube, with the electrodes' tips protruding away from the full-length capillary tube. Align the tips with one another.
3) Fold tape over the tubes.
4) Roll up tubes into a bundle. The bundle should not be too large and should only hold about a tray of the pulled electrodes (~16 electrodes).

5) Once the electrodes are wrapped and the Epon Resin is heated for 6 minutes, add 0.7 grams of MPDA to the hot Epon Resin (14%, w/v). Add in quickly and use gloves. Mix by swirling the vial. The MPDA will dissolve rapidly.
6) Dip the bundle of electrodes into the Epoxy fluid about 2 mm above the bottom of the vial. Be careful not to smash the tips. Rotate the bundle, and then lift up slowly. Dip a second time. Rotate the bundle, and then left up slowly again. After about 20 sec, a sufficient amount of epoxy will enter the glass tips via capillary action. Once the epoxy cures (discussed next), this portion of epoxy will seal and separate the inside of the electrode from the outside.
7) Undo the bundle and remove each electrode, one by one. Inspect each electrode. Dispose of electrodes without carbon fiber. Place the electrodes that have fibers on a piece of cardboard with slots to hold the electrodes.
8) Take the cardboard with electrodes and place in an oven set at 100°F.
9) After at least 48 hours, take the cardboard with electrodes out of the oven. Examine each electrode under the dissecting microscope. The baked electrode should have an amber colored epoxy that is hard, and a thin epoxy coat will cover/insulate the fiber tip. Discard electrodes that did not fill with epoxy, have a smashed tip, or have more than one fiber, etc. After inspecting each electrode, place back on the cardboard and back into the oven. Electrodes are usually good for about 1 month after making.