

APPENDIX A COMPLETE PROCEDURE DETAILS

This appendix contains the complete procedure details pertaining to this project. The procedures used were based primarily on those written by H-S. Mii (01APR94; Mii, 1996), but were adapted for use with a digital microscope camera and an image analysis system. Furthermore, certain procedures that were not specifically used in this project (i.e. partially embedding shells in Procedure B) as well as some extraneous information (e.g. general laboratory cleanup procedures) were deleted. Mii's original procedures can be obtained via http://geoweb1.tamu.edu/faculty/grossman/DiGPaST/BrachProcedure_Mii_01APR94.pdf. Finally, please note that I use the term "specimen" for shells and "sample" for an individual powder sample taken from a specimen.

Notes are given after certain procedures for further clarification.

Procedure A. Initial Preparation of Specimens

1. Make a list of specimens on an Excel spreadsheet, including the genus/species and all other relevant information, as well as a unique ID for each. **NOTE: See Appendix C1.**
2. Prepare and label suitable containers for storing specimens (e.g., plastic sample box of appropriate size).
3. Photograph the specimens and labels for future reference. Include a scale (e.g., scale card or ruler).
4. Determine the best line along which the specimens should be cut for thin-sectioning and sampling, and mark it with a pen/marker/etc. This cutting plane should encompass the thickest part of the shell.
5. Determine whether the specimens will be completely or partially embedded. **NOTE: I completely embedded all of my specimens.**

Procedure B. Embedding Specimens

NOTE: I fully embedded specimens, so I have removed the procedures for partially embedding specimens.

1. Select a suitable cardboard box for embedding specimens. Make sure that the specimens all fit in the box with at least 3 – 4 mm of space surrounding each specimen (including top and bottom). ***NOTE: I used between 3-6 specimens per box, which were on the order of 10 x 10 x 5 mm.***
2. Label the sides of the box with the specimen IDs to keep track of the specimens.
3. Mix enough epoxy to fill the box 3 - 4 mm from the bottom.
4. Place epoxy in vacuum chamber for 10 minutes at about 28-30 torr to de-gas the epoxy and prevent air pockets from forming around specimens as it cures.
5. Before putting specimens in box, pour in a 3-4 mm layer of epoxy and wait 48 hours for it to cure. This thin layer of epoxy prevents the specimens from being damaged when the bottom of the box is cut away.
6. Arrange the specimens in the box so that there is 3 - 4 mm of space around them. They should be aligned and propped up with supporting material such that the planned cutting plane of each specimen (from procedure A4) is perpendicular/parallel with the edges of the box. ***NOTE: I used yellow clay on a few specimens (which became messy) and then small pieces of Styrofoam on the rest.***
7. Mix, evacuate (see procedures B3 and B4), and pour enough epoxy to fill up the box in a layer no deeper than 2 cm and wait at least 48 hours for it to cure. Continue adding “layers” of epoxy until the specimens are completely embedded. Note that epoxy must be added in layers because it will not cure if too thick.

Procedure C. Cutting Specimens

1. After specimens are embedded and epoxy has cured, use the trim saw to separate specimens from each other and to cut away the sides of the cardboard box. Be sure to make the cuts as straight and perpendicular to each other as possible.
2. Label individual specimens with permanent marker now that they are separated.

3. Store the specimens separately in the labeled storage containers.
4. Using the Isomet saw and as thin a blade as is practical, cut the sample along the cutting line marked in procedure A4.

Procedure D. Preparing Glass Microscope Slides

NOTE: I used pre-frosted slides, so I have removed the procedures for preparing non-frosted slides.

1. Etch the sample ID on the non-frosted side of the slide(s).
2. Grind down the corners of each slide using 320-grit sandpaper and then 600-grit sandpaper. Then grind down the edges on the frosted side of each slide. This prevents you from cutting yourself while working with them and prevents the slide from “grabbing” the cloth of the polishing wheel and damaging it.

Procedure E. Making Thin-Sections

1. Determine which side of each cut specimen contains the thickest shell and proceed with that half. Store the other half in the labeled storage container.
2. Wash the specimen in distilled de-ionized water and ultrasonicate in distilled de-ionized water. Wash hands thoroughly.
3. Polish the surface of the specimen that is going to be glued to the glass slide using 320 grit polishing powder.
4. Wash the specimen in distilled de-ionized water and ultrasonicate in distilled de-ionized water. Wash hands thoroughly.
5. Polish specimen using 600 grit polishing powder.
6. Wash the specimen and glass slide in distilled de-ionized water and ultrasonicate in distilled de-ionized water. Wash hands thoroughly.
8. Glue the specimen onto the frosted side of the glass slide.

- A. Using a glass stir rod spread a few drops of loctite or epoxy (no need to evacuate it) around to completely cover the surface of the specimen that will be glued. Attach specimen to slide.
 - B. If using loctite, put the sample(s) in the "heating box" overnight.
 - C. If using epoxy, allow it to cure for at least 48 hours (at room temperature).
 - D. Use kimwipes and absolute alcohol or acetone to wipe off the excess loctite/epoxy around the edges of the specimen after it has dried/cured.
9. Cut the specimen from the slide with the Isomet saw, leaving a thin-section roughly 500 microns thick.
10. Use the grinding wheel on the right side of the Hillquist petrographic saw to grind the thin-section to proper thickness. ***NOTE: Some of my specimens started to come off the slide during this step as a result of poor loctite/epoxy quality, so some of the thin-sections are several hundred microns thick. This problem might be avoided by heating billet in a warm oven for 24 hours and storing the billet in a desiccator for at least two hours before attaching it to the slide.***
11. Polish the thin-section(s).
- A. Polish the thin-section(s) using 320 grit polishing powder.
 - B. Clean the thin-section(s) with the ultrasonic cleaner. Wash hands thoroughly.
 - D. Polish the thin-section(s) using 600 grit polishing powder.
 - E. Clean the thin-section(s) with the ultrasonic cleaner. Wash hands thoroughly.
 - F. Polish the thin-section(s) using 9.5 μ m polishing powder.
 - G. Clean the thin-section(s) with the ultrasonic cleaner. Wash hands thoroughly.
12. To see the specimen IDs better on the glass slides, mark over the specimen ID etchings with a permanent marker/pen.

Procedure F. PL/CL Photography and Specimen Preservation Analysis

NOTE: The images I acquired during these procedures can be found in Appendix B and full resolution versions can be found at <http://jordan.noret.info/research/UGR>.

1. Load the thin sections into the specimen compartment of the Technosyn Model 8200 MKII cathodoluminescence (CL) stage (cathodoluminoscope) and seal the compartment.
2. Turn on the cathodoluminoscope power and start the vacuum pump. Put pressure on the CL stage gun plate and door to make sure O-rings seal. Leave the vacuum pump running throughout the rest of this procedure. After the specimen chamber is sufficiently evacuated (this usually takes a minimum of 30 minutes), the electron beam will function properly.
3. While waiting for the chamber to fully evacuate, turn on the computer and digital camera attached to the microscope. Open the imaging software on the computer and center/focus the specimen on the screen using plane light in the microscope.
4. **Plane light image.** Using an exposure time that does not result in too dark or too light of an image (the brightness can be previewed on the computer monitor), capture the plane light image and save to computer. If the thin-section is of proper thickness, this exposure time should be less than 250 milliseconds (usually around 50-100 ms). Thicker sections will require longer exposures. ***NOTE: For the thicker thin-sections described in procedure E10, I had to use exposures of up to 1 second.***
5. **Cathodoluminescence image.** Turn off the microscope light, any other lights in the room, and cover the microscope with a dark cloth to prevent light from the computer monitor from leaking into the microscope stage.
 - A. Turn on the electron beam (kV switch). The kV meter should read above 10 kV.
 - B. Using an exposure time of 5 seconds, capture the image and save to computer.
 - C. If image in previous step is too bright, try shorter exposure times. If image in previous step is too dark or completely black, try longer exposure times until a strong orange luminescence appears on the specimen (usually the calcite cements exhibit CL). Exposure times should not exceed 60 seconds.
 - D. If image is still completely black, either the kV knob needs to be turned up or there is no luminescence in the specimen. Use a previously photographed luminescent specimen to determine which is the case. ***NOTE: For most thin-sections I used exposure times of***

between 10 and 20 seconds, but for the thicker thin-sections described in procedure E10, I had to use exposures of approximately 1 minute.

Step G. Powder Preparation for Stable Isotopic Analysis

1. Obtain powder samples from specimens.
 - A. Select areas of shell to drill based on the PL/CL photographs, avoiding luminescent areas. Sampling locations should be distributed across whole shell. If possible, serial samples should be taken between the inner and outer shell margins.
 - B. Working under a binocular microscope, use a dental drill with a stainless steel dental bur to drill holes into the shell and collect the carbonate powder residue (about 300-500 μg from each hole).
 - C. Give each drill hole an ID and label a corresponding microcentrifuge tube. ***NOTE: For example, the tube corresponding to the first sample hole in specimen BT1-1 was labeled as BT1-1-1 or BT1-1(1).***
 - D. Carefully sample the shell and transport the powder from each drill hole into its labeled microcentrifuge tubes.
2. Using a microbalance, take about 150 μg of each powder sample and place in a test tube to be analyzed by a stable isotope ratio mass spectrometer.
3. Follow the appropriate procedures in the stable isotope lab to analyze samples for collecting $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ data.