LacCore Grain Size Pretreatment SOP

Laura Triplett, Oct. 2002, March 2007
Jessica Heck, Oct 2013

Purpose: To prepare sediment samples for grain size analysis by removing unwanted components such as organic matter, carbonate minerals and cements, and diatoms.

Cautionary Notes: Care should be taken when deciding if and how to remove organic matter, carbonate minerals and cements, and/or diatoms from sediment samples prior to grain size analysis. Hydrogen peroxide is used to remove organic matter, nitric acid is used to remove organic matter (and will remove carbonates), sodium hydroxide is use to remove biogenic silica (and will affect non-biogenic silica), and hydrochloric acid is used to neutralize the sodium hydroxide solution (and will remove carbonates). The success of pretreatments varies widely with sample type, so each pretreatment should be tested and perfected before a new research project begins. Once a pretreatment regime has been selected, it should be carefully followed for each sample to ensure as little variation as possible. Researchers should have a plan for duplicate and replicate samples to be run throughout the course of the project. For example, one sample may be chosen to be a "control" that is run with every batch of pretreatments and analyses.

Each pretreatment has benefits and hazards, depending on the particular research question. For example, organic matter may bind sediment grains together creating artificially large particles when measured by the laser diffractometer, so it is important to remove organic matter. However, adding nitric acid (and possibly even hydrogen peroxide) to a sample is likely to at least partially dissolve any grains derived from carbonate bedrock. Therefore, if the sediment is derived in whole or in part from a carbonate bedrock, the data may be compromised by using nitric acid to help remove organic matter.

Safety: Most pretreatments should be done in a fume hood. Wear a lab coat, chemical splash goggles, and gloves when handling chemicals. The following are guidelines for safely managing the chemical reactions, but samples should always be closely monitored to prevent violent reactions.

Preparing samples

Supplies: Sediment samples, 50 mL centrifuge tubes with caps or tall 400-mL glass beakers, sub-sampling tool of choice.

Samples can be digested in tall 400 mL beakers or 50 mL centrifuge tubes. Centrifuge tubes are preferred because up to 48 samples can be processed at a time. Beakers should be used if violent chemical reactions cannot be contained in centrifuge tubes, but only 12 beakers fit in a hot water bath and care must be taken to prevent fine sediment particles from caking onto the sides of the beakers. The reactions are more likely to be violent if samples are organic-rich.

1. Label one centrifuge tube and cap for each sediment sample. Place 3 to 6 cubic centimeters (cc) of sediment in each centrifuge tube. The goal is to have ~2 cc of sediment remaining after the pretreatments. If the sediment is largely inorganic sediment, a smaller initial volume of sample can be used.
2. Observe proper sub-sampling technique. (See Jillavenkatesa, et al. (2001) for details.) CAUTION: Sub-sampling errors can skew the final results.
3. Cap and store in the cold room if there will be a delay before beginning pretreatment.

<u>Organic matter pretreatment</u>

The length of time required to completely digest organic matter may vary significantly for samples from different locations. Before beginning this pretreatment it is important for researchers to decide whether to: 1) continue the reaction until bubbling stops, 2) continue the reaction for a predetermined amount of time, or 3) use a predetermined volume of hydrogen peroxide.

Supplies: fume hood, hot water bath, 30% hydrogen peroxide (H_2O_2), squeeze bottle with DI water, empty squeeze bottle.

1. Place a hot water bath in the fume hood and fill it 2/3 full with water (D.I. water for most, tap water for the blue Isotemp hot water bath).
2. Preheat water bath to 85˚ C.
3. Place the centrifuge tubes with sediment in centrifuge tube racks.
4. In the fume hood, but out of the hot water bath, add ~1 mL H_2O_2 and monitor the reaction.

CAUTION: Some samples react violently to the H_2O_2 and will need to remain out of the hot water bath until the reaction slows down. Spread out the addition of H_2O_2 to the samples for ease in monitoring the reactions and make sure there is enough time respond to any violent reactions. Violent reactions can be sporadic and may not happen immediately. These reactions can be kept in check with a burst of air, DI water or ethanol from a squeeze bottle. The ethanol will reduce the surface tension of the bubbles but may speed up the reaction. Keep a beaker of cold water nearby for any samples that need to be cooled down to help slow the reaction. If the samples bubble over they need to be tossed and started over. The bubbles will preferentially remove the smaller grains from the sample and skew the final results.

5. For samples that react violently before being placed in the hot water bath, continue to add small amounts of H_2O_2 until the reaction can be managed in the hot water bath.
6. For samples that do not react violently, place them in the hot water bath. Gradually add another 5 mL, or less, H_2O_2 to the samples, and monitor the reaction.
   CAUTION: Samples that did not react violently in the fume hood may react violently as they get up to temperature in the hot water bath.
7. Add more H_2O_2, as needed, to keep the reaction active. Some samples can be filled with H_2O_2 (approximately 30 mL) and left for extended periods of time to slowly react. Others may need 2-3 smaller additions of H_2O_2 (5-10 mL at a time).
8. Once the centrifuge tube is full and, the reaction has stopped, the sample should be centrifuged, following the procedure below. The sample cannot be centrifuged until the reaction has stopped; gas bubbles forming in the sediment will make it impossible to form a pellet during centrifuging.
9. If it is suspected that more organic matter remains the researcher may choose to do one of the following:
   a. Repeat steps 4-8 (many samples can be restarted right in the hot water bath) to continue removing organic matter. Doing these steps more than three times is not necessary for samples less than 5 cc in volume.
   b. Add about 2 mL 11M nitric acid (HNO_3, 1:1 concentrated acid to DI water, always add acid to water) to each centrifuge tube. Let sit for ten minutes then centrifuge *and rinse three times* according to the procedure below and continue to next pretreatment or preparation for analysis.
      (nitric acid reacts violently with many organic matter, the hydroxyl group strips a hydrogen from the organic molecule to form water, and the remaining nitrogen group takes the hydrogen's place.)
      CAUTION: Nitric acid will aid in removing remaining organic material and speed up the process; however, it will also cause carbonates to dissolve.
10. When the reaction is complete, based on the method chosen (visible reaction, set time, or set volume) continue on to the next pretreatment.

<u>Biogenic silica preatreatment</u>

Supplies: hot water bath, samples in centrifuge tubes with caps, 1M sodium hydroxide (40g/L), 0.5N hydrochloric acid (42 mL con. HCl +958 mL water),

If the sediment has a large amount of diatoms (as may be observed in a smear slide), researchers may choose to destroy biogenic silica particles. Diatoms, sponge spicules and siliceous plant phytoliths will be counted as sediment particles, and they are most often in the silt size range. **CAUTION: NaOH dissolves biogenic and non-biogenic silica.** Biogenic silica particles are dissolved faster than mineral silicates, but a reduction in mineral silicate grain size

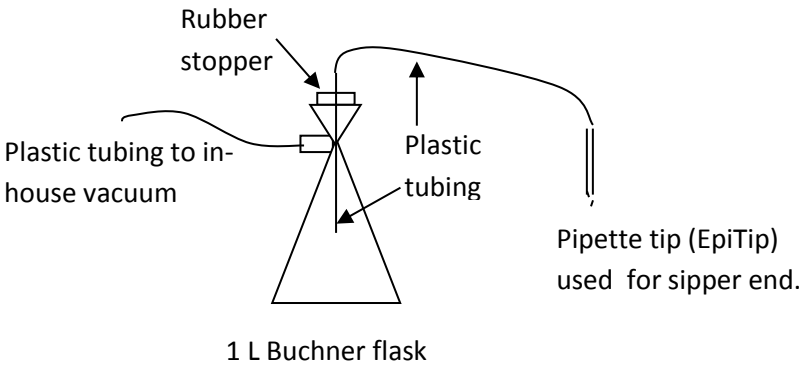
or the complete dissolution of clay sized silicate particles could result from NaOH use. **HCl is used to neutralize the NaOH and could result in carbonate dissolution.**

1. Add 40 mL of 1M sodium hydroxide (NaOH) solution to each centrifuge tube.
2. Cap and shake.
3. Loosen caps (but leave them on the tubes) and place the samples in the centrifuge tube rack in the hot water bath.
4. After 10 minutes, tighten the caps and shake well. Loosen caps and place back in the hot water bath for 10 additional minutes.
5. Centrifuge, following steps 2-3 in the centrifuge procedure below. Centrifuge time may need to be increased to 40 minutes because NaOH acts to disperse particles. Remember to make sure the supernatant is clear, not cloudy, before sipping.
6. Add 8-9 mL of 0.5 N HCl, cap, shake, uncap, fill to 35 mL with DI water. This will neutralize the solution and flocculate the sediment to aid in the centrifuging process. **CAUTION: HCl may lead to carbonate dissolution.**
7. Follow the centrifuge and rinse procedure.

<u>Centrifuging and rinsing procedures</u>

Supplies: samples in centrifuge tubes with caps, centrifuge, sipper assembly, soda ash (only if acids are used),

1. Remove the centrifuge tubes from the hot water bath.
   If beakers were used, remove them from the hot water bath and rinse samples into labeled centrifuge tubes with DI and methanol. Methanol may be more effective at rinsing the small particles off the beaker walls. A stir rod with rubber policeman may be used to scrape off the caked-on particles. Caked on particles may be clumped together and lead to larger grain size results.
2. Centrifuge at 3500 rpm (~2400 x g) for 15-30 minutes. Centrifuging is complete and successful when the supernatant is transparent. After the organic matter pretreatment, the supernatant may be yellow but it should also be transparent. If the supernatant is cloudy, return the tubes to the centrifuge for another 15-30 minutes.
3. Do not pour the supernatant out of the centrifuge tubes. Instead, use a sipper mechanism hooked up to the in-house vacuum (see schematic below). Draw down most of the liquid in each centrifuge tube taking care not to disturb the sediment. The in-house vacuum should only be on enough to remove the liquid but not so much as to disturb the sediment. Waste can be poured down the drain if neither nitric nor hydrochloric acid were used. If acids were used, neutralize the contents of the Buchner flask by adding soda ash to the flask before pouring the waste down the drain.



Schematic 1: Sipper assembly

4. Once the supernatant has been sipped off, continue on with the procedure or rinse the sample following the directions below if nitric acid was used.
    1. Add a few mL of DI water to each centrifuge tube. Shake (and stir, if necessary) to get all the sediment back into suspension.
    2. Fill the tube to about 40 mL with DI water, shake or vortex well, and repeat the centrifuge/sip/rinse procedure twice more.
    3. Move on to the next pretreatment, refrigerate the sample for future analysis, or prepare the sample for grain size analysis
5. Be sure to clean the centrifuge when done. Follow the directions posted above the centrifuge.

Preparation for analysis

1. At this point, each sample should be in a centrifuge tube: still wet, but with minimal supernatant remaining. Fill each centrifuge tube with sodium hexametaphosphate solution (5g Na-HMP in 1L low purity deionized water).
2. Label all caps, and use electrical tape to secure the tubes onto the wrist action shaker. (Electrical tape may remove any labels stuck to the tubes and those that are written with Sharpies onto the tubes themselves. Shake the samples overnight.)

References

Jiilavenkatesa, A., Dapkunas, S. J., and Lum, L. H. 2001.

Particle Size Characterization. U.S. Department of Commerce. *Chapter 2: Powder sampling.*