Standard Operating Procedure for Separation of Barite from Deep-Sea Sediments

Training Provided for Users:
Lab Safety Training (Environmental Health and Safety)
Paytan Laboratory Safety Training provided by lab manager (Ellen Gray)
Training on HF operations and HF First Aid Procedures by immediate supervisor for separations (typically Ellen Gray, Lab Manager, Adina Paytan, PI, or Liz Morris and Andrea Erhardt, grad students)

General Information:

Hydrofluoric acid is extremely dangerous. Spilling as little as 100 ml on your body can result in death. Read all safety information and complete lab training before using this highly dangerous chemical.

This operation is performed in Earth and Marine Sciences, Room C474.

The location of the eyewash/safety shower is next to the sink. Ensure that eyewash/safety shower is currently maintained on monthly basis (e.g., check the tag to confirm).

The location of the fume hood used for HF is next to the hall door. Ensure that the hoods are currently certified and sash below maximum height (check the sticker affixed to hoods - certified annually).

HF first aid instructions provided by EH&S are posted on the outside of the fume hood used for all HF procedures. The location of the calcium gluconate gel is in the refrigerator in the Main Lab in room C453 and is noted on the fume hood, above the sink, and on the refrigerator itself. Access to this refrigerator should be kept open and free of obstructions at all times. The calcium gluconate gel is to be retrieved and be present and accessible in room C474 when HF is in use. Return to the refrigerator when HF work is complete.

Frequency of this operation: HF is used on days 4, 5, and 6 of an 8-day procedure. This works out to 3 days every two weeks for most HF users. For batches of 8 to 16 sediment samples, this is a total of 80-160 ml HF on Day 4, 120-240 ml on Day 5, and 160-320 ml HF on Day 6.

Duration of this operation: It takes about one to two hours to carry out this procedure, depending on how many samples are being done at a time (about one hour for 8 samples).

Always check personal protective equipment (PPE) for functionality before using (e.g., check gloves for pinholes; goggles and face shield for cracks, coat for tears).

Chemicals Used
49% Hydrofluoric acid, in 1-lb plastic bottles with safety nozzles.
1 N nitric acid
6 N hydrochloric acid
5% sodium hypochlorite (bleach)
0.02N hydroxylamine in 25% acetic acid
saturated aluminum chloride (in 1N nitric acid)

**Personal Protective Equipment Required for HF Use:**
Safety Goggles (not glasses)
White lab coat
Thin nitrile double gloved or Neoprene gloves (*offer better protection than nitrile*)
Vinyl lab coat
Appropriate Street Clothing: long pants, closed toed shoes
Face Shield (optional)

**Personal Protective Equipment for the rest of the procedure that does not use HF:**
Safety glasses
Gloves (thin nitrile)
Closed-toed shoes
Long pants
Lab coat

**HF WORK IS NEVER TO BE CONDUCTED ALONE.**

**The procedure:**

Dry deep-sea sediment samples in 50°C oven until dry (or freeze-dry). Powder sediment samples as needed.

**Day 1**

1) Create labels for each 250 mL Nalgene bottle (one for each sediment sample). It is best to do sets comprising 8, 12, or 16 samples.
2) Tare the bottle and weigh 20-40 g of sediment sample into each bottle. Record weight of each sample in the data table.
3) Pour each sediment sample into 1 L plastic beaker. (It is convenient to label each beaker with the sample number, so as to not confuse them.
Under the hood, wearing nitrile gloves, lab coat, and safety glasses:
4) Pour ~25-50 mls of ~6N HCl into each beaker. (To make 6N HCl, use 50% acid and 50% DI water by volume since concentrated HCl is 12. In the hood, add the acid to the water [do NOT add water to acid]). It is important to add this slowly, as many samples that are carbonate-rich will react strongly with the acid. Swirl and stir the contents of each beaker (a plastic spatual works well); then wait a few moments until the reaction settles down and add a little more acid. Continue to do this until the sample stops bubbling (reacting).
5) Transfer the contents of each beaker back into the respective labeled bottles by rinsing the beakers with DIW from a squirt bottle. If not full, top up the bottles with 6N HCl. Put samples in secondary containment, label them with 6N
hydrochloric acid, and leave them in the hood. Leave standing to react overnight with bottle covers loosely on (at room temperature).

Day 2
Wearing nitrile gloves, a lab coat, and safety glasses:
1) Balance out pairs of bottles with DIW. (This is to ensure that they are all properly balanced in the centrifuge, which can often be highly sensitive and, if not balanced, can result in lost samples.).
2) Centrifuge at ~2500 rpm for ~10 minutes.
3) Hazardous waste containers are stored outside of the hood, by the standing freezer. Move the hydrochloric acid hazardous waste container to the hood. Decant supernatant into hazardous waste container (labeled with 6N hydrochloric acid). After securely capping, move the hazardous waste container from the hood back to the cart.
4) “Rinse” sediment residue (this is standard procedure after reacting sediment with various solvents):
   • fill each bottle with DIW and shake vigorously
   • centrifuge at 2500 rpm for 10 minutes
   • decant supernatant (water, if pH is neutral (5.5 or higher) can go down sink, if not goes in waste container)
5) Fill bottles with bleach (5%), shake, and put in 50°C oven with covers loosely on to react overnight.

Day 3
1) Remove samples from oven, let cool, and gently decant some of the bleach (can go in the sink but rinse down with water).
2) Rinse with DIW as in Day 2, Step 4 three times.
3) In the hood, add 0.02N hydroxylamine in acetic acid (1.4 g in 750 mL of DI water and 250 mL of acetic acid, make this in the hood). Shake vigorously. Put in 80°C oven overnight with covers loosely on.

Day 4
Wearing safety goggles, (face shield), vinyl lab coat, long pants, closed toed shoes, and either 2 layers of disposable nitrile gloves or 22mil neoprene gloves:
1) Remove samples from oven and let cool completely. Decant hydroxylamine carefully into waste container moved from the cart to in the hood.
2) Rinse with DIW as in Day 2, Step 4 three times. During the rinse process, transfer sediment samples from 250 ml Nalgene bottles to 50 ml centrifuge tubes (Fisherbrand blue tops or Corning orange tops, caps must have recessed threads or they will leak).
3) Alert others present in the lab that you will be using HF. If no one is present IN THE LAB (not just down the hall!) you can’t use HF. Turn the sign above the hood to read “HF in use.” Ensure that the calcium gluconate gel is in the lab with you. Ensure that the calcium carbonate container is full and present in the hood.
with you. Calcium carbonate will neutralize the hydrofluoric acid if small spills or leaks occur.

4) Move 500ml plastic HF container with safety cap on from storage below the hood to in the hood. If you need a new container of HF, follow directions in package for replacing packaging cap with safety cap.

5) In hood with the sash as low as you can comfortably work, place the measuring centrifuge tube and the sample centrifuge tube into the Styrofoam centrifuge tube holder (do not hold it with your hand). Add 30 mL of 1:2 HF:HNO₃ (1N) (10 mls HF and 20 ml HNO₃) to the measuring tube in the hood. Add the HNO₃ first, then the HF. Add this mixture from the measuring tube into the sample tube. Swirl and cap securely.

6) After adding HF to each sample, if you need to shake the samples by hand to loosen the sediment, make sure they are securely capped and only shake under the hood. Leakage is possible. Put a kimwipe around the edge of the cap so you can tell if it leaks. Inspect your gloves for any trace of HF and replace them with fresh gloves if found. Contaminated gloves and kimwipes should go in a ziplock bag before disposal.

7) Let react with covers loosely on overnight (at room temperature) in the hood. Write on tape on secondary containment: Samples in hydrofluoric and nitric acid, your name, the date, and time).

Day 5
Wearing safety goggles, (face shield), vinyl lab coat, long pants, closed toed shoes, and either 2 layers of disposable nitrile gloves or 22mil neoprene gloves:

1) In hood with the sash as low as you can comfortably work, fill bottles to 40mls with DIW, securely fasten caps, put in centrifuge adapters, put in secondary containment. Alert people working in the lab that you are moving HF from the hood to the centrifuge. Centrifuge. Transport samples in centrifuge adapters in secondary containment to hood. Inspect centrifuge adaptors for any spills and if found neutralize with calcium carbonate, rinse and let dry.

2) Alert others present in the lab that you will be using HF. If no one is present IN THE LAB (not just down the hall!) you can’t use HF. Turn the sign above the hood to read “HF in use.” Ensure that the calcium gluconate gel is in the lab with you. Ensure that the calcium carbonate container is full and present in the hood with you. Calcium carbonate will neutralize the hydrofluoric acid if small spills or leaks occur.

3) Move HF waste container to into the hood. Decant supernate to waste container under the hood. Securely replace cap before moving container out of the hood.

4) Move 500ml plastic HF container with safety cap on from storage below the hood to in the hood. If you need a new container of HF, follow directions in package for replacing packaging cap with safety cap.

5) In hood with the sash as low as you can comfortably work, place the measuring centrifuge tube and the sample centrifuge tube into the Styrofoam centrifuge tube holder (don’t hold it with your hand). Add 30 mL of 1:1 HF:HNO₃ (1N) (15 mls HF and 15 ml HNO₃) to the measuring tube in the hood. Add the HNO₃ first,
then the HF. Add this mixture from the measuring tube into the sample tube. Swirl.

6) After adding HF to each sample, if you need to shake the samples by hand to loosen the sediment, make sure they are securely capped and only shake under the hood. Leakage is possible. Put a kimwipe around the edge of the cap so you can tell if it leaks. Inspect your gloves for any trace of HF and replace them with fresh gloves if found. Contaminated gloves and kimwipes should go in a ziplock bag before disposal.

7) Let react with covers loosely on overnight (at room temperature) in the hood. Write on tape on secondary containment: Samples in hydrofluoric and nitric acid, your name, the date, and time).

Day 6

Wearing safety goggles, (face shield), vinyl lab coat, long pants, closed toed shoes, and either 2 layers of disposable nitrile gloves or 22mil neoprene gloves:

Note: If samples clean easily you may skip this step and go directly to Day 7. I recommend testing one set of samples from a site with the shorter procedure first, if it works, there is no need to risk using more HF.

1) In hood with the sash as low as you can comfortably work, fill bottles to 40mls with DIW, securely fasten caps, put in centrifuge adapters, put in secondary containment. Alert people working in the lab that you are moving HF from the hood to the centrifuge. Centrifuge. Transport samples in centrifuge adapters in secondary containment to hood. Inspect centrifuge adaptors for any spills and if found neutralize with calcium carbonate, rinse and let dry.

2) Alert others present in the lab that you will be using HF. If no one is present IN THE LAB (not just down the hall!) you can’t use HF. Turn the sign above the hood to read “HF in use.” Ensure that the calcium gluconate gel is in the lab with you. Ensure that the calcium carbonate container is full and present in the hood with you. Calcium carbonate will neutralize the hydrofluoric acid if small spills or leaks occur.

3) Move HF waste container to into the hood. Decant supernate to waste container under the hood. Securely replace cap before moving container out of the hood.

4) Move 500ml plastic HF container with safety cap on from storage below the hood to in the hood. If you need a new container of HF, follow directions in package for replacing packaging cap with safety cap.

5) In hood with the sash as low as you can comfortably work, place the measuring centrifuge tube and the sample centrifuge tube into the Styrofoam centrifuge tube holder (don’t hold it with your hand). Add 30 mL of 2:1 HF:HNO₃ (1N) (20 mls HF and 10 ml HNO₃) to the measuring tube in the hood. Add the HNO₃ first, then the HF. Add this mixture from the measuring tube into the sample tube. Swirl.

6) After adding HF to each sample, if you need to shake the samples by hand to loosen the sediment, make sure they are securely capped and only shake under the hood. Leakage is possible. Put a kimwipe around the edge of the cap so you can
tell if it leaks. Inspect your gloves for any trace of HF and replace them with fresh gloves if found. Contaminated gloves and kimwipes should go in a ziplock bag before disposal.

7) Let react with covers loosely on overnight (at room temperature) in the hood. Write on tape on secondary containment: Samples in hydrofluoric and nitric acid, your name, the date, and time).

Day 7
Wearing safety goggles, (face shield), vinyl lab coat, long pants, closed toe shoes, and either 2 layers of disposable nitrile gloves or 22mil neoprene gloves:

1) In hood with the sash as low as you can comfortably work, fill bottles to 40mls with DIW, securely fasten caps, put in centrifuge adapters, put in secondary containment. Alert people working in the lab that you are moving HF from the hood to the centrifuge. Centrifuge. Transport samples in centrifuge adapters in secondary containment to hood. Inspect centrifuge adaptors for any spills and if found neutralize with calcium carbonate, rinse and let dry.

2) Ensure that the calcium gluconate gel is in the lab with you. Ensure that the calcium carbonate container is full and present in the hood with you. Calcium carbonate will neutralize the hydrofluoric acid if small spills or leaks occur.

3) Move HF waste container to into the hood. Decant supernate to waste container under the hood. Securely replace cap before moving container out of the hood.

1) Rinse with DIW as in Day 2, Step 4 three times. Supernatant for all but the last rinse should go in the waste container.

2) Add ~15 mL saturated aluminum chloride (in 1N HNO₃), shake, and let react for ONLY one hour in 80°C oven with covers loosely on. Remove, add DIW, and let cool.

3) Balance and centrifuge.

4) Rinse with DIW as in Day 2, Step 4 three times.

5) Filter solid residue onto 0.4 micron vacuum filters and place filters in labeled plastic petri dishes. Let dry with petri dish covers loosely on overnight.

Day 8

1) Weigh and record masses of each sample in data table (subtract mass of filter paper).

2) At this point, you may wish to examine the samples under the SEM to assess purity/cleanliness.

3) Carefully remove sample from filter with a brush into labeled crucible.

4) Ash samples in furnace at 700°C for one hour, let cool, and weigh into labeled aluminum foil envelopes.

You can stop and take off if needed after any one of the water rinsing steps. Just keep track of where you are in the procedure.

You are done with the separation. Samples should have barite and some other insoluble contaminants. You are ready to make the SEM stubs.