

## PROTOCOL FOR SAMPLE TREATMENT

**Laboratory Safety:** The isotope lab is used by many people, and it is the responsibility of each user to work in a way that is safe for themselves, safe for others, and does not compromise the integrity of anybody's work. Flammable liquids are used in the lab, along with a number of acids, exposure to some of which can be lethal. To protect yourself, it is mandatory that you wear

1. eye protection
2. gloves
3. lab coat
4. shoe covers

when you are working in the lab. Additional personal protection equipment may be required for specific tasks (e.g., transfers of > 1 L of acid solutions). All procedures involving acid solutions in open containers should be performed in chemical fume hoods. In all circumstances, if you are unsure of the correct procedure, ASK!

Please familiarize yourself with the location of fire extinguishers, safety shower, eyewash facilities, spill control and cleanup kits, and HF treatment gel.

**Overview:** The samples are predominantly organic. The target is to extract Pb (and, for some samples, Sr) for isotopic analysis.

We assume Pb is bound to the sample in several different ways; some Pb is surface-bound (hydrogen bonds or electrostatic bonds), some is incorporated structurally in the organics, and some is bound into the inorganic fraction of the samples. We would like to determine the isotopic composition and relative abundance of Pb in each of these different “reservoirs.”

We do selective extractions that help us separate the different sources of Pb, with a relatively mild extraction as the initial step, and increasingly aggressive dissolutions of the residues from prior steps. At each step, we will generate subsamples for isotopic composition (IC) determination and concentration measurement (isotope dilution or ID), as well as a residue for further treatment. The initial extraction step is with ethanol; the residue from ethanol treatment is extracted with hot 1 M hydrochloric acid; the residue from hydrochloric acid treatment is extracted with aqua regia (a mixture of hydrochloric and nitric acids), and the residue of aqua regia treatment is fully dissolved in a mixed hydrofluoric acid – nitric acid solution. Thus, for each sample, we will have up to 8 subsamples to analyze. Because we will be working with low levels of Pb in some of these samples, working cleanly is a must.

## **Protocol:**

### **A. Sample Weighing**

1. For each sample and the blank, prepare two 15 mL teflon beakers. Empty 6M HCl from clean beaker to waste jar. Label beakers (cap and body) with sample number; one of the two beakers for each sample should be labeled with “R” (residue), the other with “S” (solution). Set beakers on hot plate to dry. When beakers are dry, remove from hot plate, cap, and allow to cool. When cool, weigh each beaker with cap.
2. Remove 10 15-mL centrifuge tubes from the acid bath; rinse them twice with MQ H<sub>2</sub>O, and shake them “dry.” Label tubes with sample numbers; label last tube as “blank.”
3. Weigh up to 1 g of sample (0.3 – 1 g) and transfer to centrifuge tube.

### **B. Ethanol Extraction**

4. To each sample, add 3 mL of ethanol. Mix the sample on the vortex mixer, then place into the hot water bath for ~1 hour. During the hour, mix sample on vortex mixer every 15 minutes.
5. After ~ 1 hour, centrifuge the samples for 5 minutes. Pipette the ethanol solution into the “S” beaker prepared in step 1. Cap the beaker and set it aside. To each sample, add 5 mL hot MQ H<sub>2</sub>O. Mix on vortex mixer, and place into the hot water bath for ~ 15 min.
6. After ~ 15 min., centrifuge the samples for 5 min. Pipette the water solution into the beakers with the ethanol. When the ethanol+water solutions have cooled, weigh the beakers with the ethanol+water sample.
7. Using a minimum of MQ H<sub>2</sub>O, transfer the solid residue from the ethanol extraction into the second Teflon beaker prepared in step 1. Place these samples on the hot plate to dry. (Go to step 11 for further treatment of residues.) Reserve the 15 mL centrifuge tubes for later use.
8. For each sample, prepare a 15 mL Teflon beaker for IC and ID. Empty 6M HCl from clean beaker to waste jar. Label beakers with sample number followed by “EtOH Pb IC” or “EtOH Pb ID.”
9. To the IC beaker, pipette 1 mL of the ethanol sample solution. Add 1 mL of concentrated HNO<sub>3</sub> (2-9-11 batch!) and place the beaker, uncapped, on the hot plate. Note: concentrated nitric acid can react violently with organics like ethanol. Watch the beakers closely and remove from the hot plate if the reaction is too vigorous. *The purpose of this step* is to destroy the organic compounds that are in solution; these interfere with ion exchange and analysis.

**9a.** Dry down the ethanol – nitric acid solution. When dry, add 1 mL of concentrated nitric acid and dry down again.

**9b.** To each sample, add 0.2 mL conc HNO<sub>3</sub>. Then add 0.1 mL 30% H<sub>2</sub>O<sub>2</sub> (Seastar, not Suprapur), place on hot plate, and allow to react. This reaction produces a lot of spatter, so samples should be well spaced on the hot plate. When the reaction has died down, add another 0.1 mL aliquot of H<sub>2</sub>O<sub>2</sub>, and allow to react. Then add another 0.1 mL aliquot of H<sub>2</sub>O<sub>2</sub> and allow the sample to dry. *The H<sub>2</sub>O<sub>2</sub> treatment is a more aggressive procedure for destroying organics.* At the end of this step, the samples should be free of organics.

**9c.** When the sample is dry, add 2 mL 6M HCl. Cap the beaker and allow to reflux on hot plate for at least 2 hours. After ~ 2 hrs, dry the samples down. They are now ready for processing through Pb ion exchange columns. *The purpose of this step is to collect the spatter from the walls of the beaker, and to convert the sample from nitrate salts to chloride form, which is necessary for the subsequent ion exchange procedure.*

**10.** Take the ID beakers into the weighing room. Tare each beaker, open, on the balance. Add 2 drops of the <sup>208</sup>Pb spike (kept in hood in zircon lab). Record spike weight. Seal spike bottle and return to zircon lab.

**10a.** Tare each beaker, open, with spike. Pipette 0.1 mL of the ethanol sample solution into the beaker. Record sample weight.

**10b.** In the Sr lab, add 0.5 mL conc. HNO<sub>3</sub> to each beaker. Place the beakers, open, on the hot plate to dry down.

**10c.** When dry, process with 30% H<sub>2</sub>O<sub>2</sub> and the HCl conversion steps, as the IC samples were processed in steps 9b and 9c above.

**11.** When the residues are dry, cap the beakers, take them off the hot plate and allow them to cool to room temperature. When they are cool, weigh the beakers.

### **C. HCl Extraction**

**12.** For each sample, prepare two Teflon beakers, labeled with the sample number and “HCl-S” and “HCl-R.” Place the beakers on the hot plate, open, to dry. After ~ 1 hr., remove the beakers, cap them and allow them to cool. When cool, weigh the beakers with caps.

**13.** To start the HCl extraction step, pipette 1 mL of hot 1 M HCl into the beakers and transfer the samples from the Teflon beakers back to the 15 mL centrifuge tubes. Then pipette 1 mL of hot 1 M HCl into the beaker, rinse it, and add the acid to the centrifuge tube. Bring the total volume of HCl to 3 mL by pipetting another mL of hot 1M HCl into the centrifuge tube. Mix the sample and acid on the vortex mixer, then place the centrifuge tube into the hot water bath for ~ 10 min.

**14.** After ~ 10 min, centrifuge the samples for 5 min. Pipette the HCl solution into the “HCl-S” beakers. Cap the beakers and set them aside. Pipette 10 mL of hot MQ H<sub>2</sub>O into the centrifuge tubes. Mix the samples on the vortex mixer, and place the centrifuge tubes into the hot water bath for ~ 10 min.

**15.** After ~ 10 min, centrifuge the samples for 5 min. Pipette the water solution into the Teflon beakers. Cap the beakers and allow the samples to cool. When cool, weigh the beakers with the HCl solution.

**16.** Using a minimum of MQ H<sub>2</sub>O, transfer the solid residue from the HCl extraction into the second Teflon beaker prepared in step **12**. Place these samples on the hot plate to dry. (Go to step 20 for further treatment of residues.) The 15 mL centrifuge tubes should be reserved for step **24** below.

**17.** For each sample, prepare a 15 mL Teflon beaker for IC and ID. Empty 6M HCl from clean beaker to waste jar. Label beakers with sample number followed by “HCl Pb IC” or “HCl Pb ID.”

**18.** To the IC beaker, pipette 0.5 mL of the HCl sample solution. Add 1 mL of concentrated HNO<sub>3</sub> (2-9-11 batch!) and place the beaker, uncapped, on the hot plate. *The purpose of this step* is to destroy the organic compounds that are in solution.

**18a.** Dry down the HCl – nitric acid solution. When dry, add 1 mL of concentrated nitric acid and dry down again.

**18b.** To each sample, add 0.2 mL conc HNO<sub>3</sub>. Then add 0.1 mL 30% H<sub>2</sub>O<sub>2</sub> (Seastar, not Suprapur), place on hot plate, and allow to react. This reaction produces a lot of spatter, so samples should be well spaced on the hot plate. When the reaction has died down, add another 0.1 mL aliquot of H<sub>2</sub>O<sub>2</sub>, and allow to react. Then add another 0.1 mL aliquot of H<sub>2</sub>O<sub>2</sub> and allow the sample to dry. *The H<sub>2</sub>O<sub>2</sub> treatment is a more aggressive procedure for destroying organics.* At the end of this step, the samples should be free of organics.

**18c.** When the sample is dry, add 2 mL 6M HCl. Cap the beaker and allow to reflux on hot plate for at least 2 hours. After ~ 2 hrs, dry the samples down. They are now ready for processing through Pb ion exchange columns. *The purpose of this step* is to collect the spatter from the walls of the beaker, and to convert the sample from nitrate salts to chloride form, which is necessary for the subsequent ion exchange procedure.

**19.** Take the ID beakers into the weighing room. Tare each beaker, open, on the balance. Add 2 drops of the <sup>208</sup>Pb spike (kept in hood in zircon lab). Record spike weight. Seal spike bottle and return to zircon lab.

**19a.** Tare each beaker, open, with spike. Pipette 0.1 mL of the HCl sample solution into the beaker. Record sample weight.

**19b.** In the Sr lab, add 0.5 mL conc.  $\text{HNO}_3$  to each beaker. Place the beakers, open, on the hot plate to dry down.

**19c.** When dry, process with 30%  $\text{H}_2\text{O}_2$  and the HCl conversion steps, as the IC samples were processed in steps 18b and 18c above.

**20.** When the residues are dry, cap the beakers, take them off the hot plate and allow them to cool to room temperature. When they are cool, weigh the beakers.

#### **D. Aqua Regia Extraction**

**21.** All subsequent procedures will use the HCl-R beakers, because the reactions take longer and because the reagents are too strong for the plastic centrifuge tubes. Prepare 40 mL of aqua regia. To a 60 mL Teflon beaker, add 10 mL concentrated  $\text{HNO}_3$  (2-9-11). Then add 30 mL of concentrated HCl. Mix.

**22.** Add 3 mL of aqua regia solution to each HCl residue sample (and blank). Swirl the beaker to suspend the sample. Place on the hot plate, uncovered. There should be an active reaction as the aqua regia begins to oxidize the organics in the sample. Monitor the reaction; if it is too vigorous, remove the sample from the hot plate, and let the reaction settle down. It may be necessary to cycle the samples on and off the hot plate until the reaction is reasonably confined to the beaker. Then leave the samples on the hot plate to react and dry down.

**23.** Repeat the aqua regia reaction step, after preparing fresh aqua regia: add 3 mL aqua regia to each sample. Cap the samples and place them on the hot plate for a minimum of 8 hours. Remove from the hot plate and allow to cool.

**24.** For each sample, prepare a 15 mL Teflon beaker. Pour the 6M HCl from the clean beaker into the acid waste jar, then label the beakers with sample number and "AR-S." Weigh the empty beakers.

**25.** Carefully transfer the samples from the Teflon beakers to the 15 mL centrifuge tubes, reserving the beakers for use in step 27. Centrifuge the samples for ~ 5 min. Pipette the aqua regia solution into the beakers labeled "AR-S." Cap the beakers and set them aside.

**26.** To each beaker, add 10 mL hot MQ  $\text{H}_2\text{O}$ . Mix on a vortex mixer to suspend all of the remaining solid. Place in hot water bath for 10 minutes. Centrifuge the samples for ~ 5 min. Pipette the  $\text{H}_2\text{O}$  solution to the AR-S beakers. Cap the beakers and allow the solutions to cool. When cool, weigh the AR-S beakers.

**27.** Using a minimum of MQ  $\text{H}_2\text{O}$ , transfer the remaining solid residue back into the beakers used in step 25. Dry down the residues. When dry, remove from hot plate, cap and allow to cool. When cool, weigh the beaker+residue. Go to step 31 to treat residue.

**28.** For each sample, prepare a 15 mL Teflon beaker for IC and ID. Empty 6M HCl from clean beaker to waste jar. Label beakers with sample number followed by “AR Pb IC” or “AR Pb ID.”

**29.** To the IC beaker, pipette 0.5 mL of the AR sample solution. Add 1 mL of concentrated HNO<sub>3</sub> (2-9-11 batch!) and place the beaker, uncapped, on the hot plate. *The purpose of this step* is to destroy the organic compounds that may remain in solution.

**29a.** Dry down the AR – nitric acid solution.

**29b.** To each sample, add 0.2 mL conc HNO<sub>3</sub>. Then add 0.1 mL 30% H<sub>2</sub>O<sub>2</sub> (Seastar, not Suprapur), place on hot plate, and allow to react. When the reaction has died down, add another 0.1 mL aliquot of H<sub>2</sub>O<sub>2</sub>, and allow to react. Then add another 0.1 mL aliquot of H<sub>2</sub>O<sub>2</sub> and allow the sample to dry. At the end of this step, the samples should be free of organics.

**29c.** When the sample is dry, add 2 mL 6M HCl. Cap the beaker and allow to reflux on hot plate for at least 2 hours. After ~ 2 hrs, dry the samples down. They are now ready for processing through Pb ion exchange columns.

**30.** Take the ID beakers into the weighing room. Tare each beaker, open, on the balance. Add 2 drops of the <sup>208</sup>Pb spike (kept in hood in zircon lab). Record spike weight. Seal spike bottle and return to zircon lab.

**30a.** Tare each beaker, open, with spike. Pipette 0.1 mL of the AR sample solution into the beaker. Record sample weight.

**30b.** In the Sr lab, add 0.5 mL conc. HNO<sub>3</sub> to each beaker. Place the beakers, open, on the hot plate to dry down.

**30c.** When dry, process with 30% H<sub>2</sub>O<sub>2</sub> and the HCl conversion steps, as the IC samples were processed in steps **29b** and **29c** above.

### **E. HF Dissolution**

**31.** To the residue of each sample, pipette 0.5 mL conc. HNO<sub>3</sub>, then add 3 mL conc. HF. Cap the beakers and place them on the hot plate for a minimum of 8 hours. The purpose of this step is to dissolve all of the remaining residue.

**32.** Remove the beakers from the hot plate. Uncap them, carefully, preserving all of the solution. Place the open beakers back on the hot plate to dry down (ideally to “wet-dryness”).

**33.** When dry, add 1 mL conc. HNO<sub>3</sub> and allow to dry down (“wet-dryness”). Repeat twice (i.e., 3 mL total HNO<sub>3</sub>). The purpose of this step is to break down all of the fluoride salts that may have formed in step 31.

- 34.** When the samples are dry, add 3 mL 6 M HCl. Cap the samples and allow them to reflux on the hot plate for a minimum of 8 hours. The purpose of this step is to effect final dissolution of all solids, and to convert the sample to chloride salts.
- 35.** Remove samples from hot plate and allow to cool. When cool, weigh beakers.
- 36.** For each sample, prepare a 15 mL Teflon beaker for ID. Empty 6M HCl from clean beaker to waste jar. Label beakers with sample number followed by “HF Pb ID.”
- 37.** Take the ID beakers into the weighing room. Tare each beaker, open, on the balance. Add 2 drops of the  $^{208}\text{Pb}$  spike (kept in hood in zircon lab). Record spike weight. Seal spike bottle and return to zircon lab.
- 37a.** Tare each beaker, open, with spike. Pipette 0.1 mL of the HF sample solution into the beaker. Record sample weight. Make sure that all of the spike is mixed with all of the sample solution.
- 37b.** In the Sr lab, place the beakers, open, on the hot plate to dry down. When dry, these samples are ready for ion exchange chemistry.
- 38.** Relabel the beakers with the remaining solution with sample number and “HF IC.” Place the beakers, uncapped, on the hot plate to dry down. When dry, these samples are ready for ion exchange chemistry.

## **F. Pb Ion Exchange Chemistry**

**39.** To each sample (products of steps **9c**, **10c**, **18c**, **19c**, **29c**, **30c**, **37b**, and **38**), add 0.5 mL 1.1 M HBr. Allow the samples to sit overnight at room temperature.

**40.** For each sample (IC and ID separately), prepare a Pb ion exchange column. Rinse the column with MQ H<sub>2</sub>O multiple times, then add ~ 1 mL of AG1-X8 anion exchange resin suspension to each column and allow it to settle. When the resin is settled, adjust the resin height in the column by pipetting the excess resin to waste. Allow the columns to drain.

**41.** Cleaning the resin: Sequentially, add 1 mL 6 M HCl, 1 mL 0.1 M HCl, and 1 mL 6 M HCl to each column, allowing the acid to drain from the column before adding the next aliquot. Capture all acid from the columns in waste containers.

**42.** Conditioning the resin: To each column, add 0.5 mL 1.1 M HBr, and allow it to drain.

**43.** Loading the sample: To each column, add the 0.5 mL 1.1 M HBr solution containing the sample (use a clean pipette tip for each sample!). IF the sample contains undissolved solids, transfer the sample to a 1.5 mL centrifuge tube and centrifuge for ~ 5 min, then load the solution, only, onto the ion exchange column. Once the sample beakers are empty, add 2 mL 6 M HCl to each beaker, cap it and place it on the hot plate to reflux until it is needed in step **47**.

**44.** Drop Rinse: Add 1 drop of 1.1 M HBr to each column and let it drain. Repeat until you have added a total of 5 drops HBr.

**45.** Bulk Rinse: Add 0.5 mL 1.1 M HBr to each column and let it drain.

**46.** Bulk Rinse: Add 0.5 mL 2 M HCl to each column and let it drain.

**47.** Remove waste containers from under columns, and empty them to the acid waste jar. Then, pour the 6 M HCl from the sample beakers into the waste jar and place the beakers under the respective columns to begin collecting the Pb solution. To each column, add 1 mL of 6 M HCl, and allow it to drain.

**48.** When the 6 M HCl solution is completely drained from the column, remove the beakers from under the columns, add 1 drop of 0.1 M H<sub>3</sub>PO<sub>4</sub> and place the beakers on the hot plate to dry. When dry, these samples are ready for mass spectrometry.



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