Protocol for Acid-Base-Acid Pre-treatment of Pika Pellets in preparation for graphitization

1. **Purpose:**
   The intent of this protocol is to provide instruction on how to prepare pika pellets for graphitization by cleansing the sample of all fecal substances except digested plant fibers.

2. **Application:**
   This method is used to prepare scat samples for graphitization prior to radiocarbon analysis. This method may also be used for the treatment of charcoal or wood samples and is the preliminary step to cellulose extraction of wood samples.

3. **Summary of Method:**
   The sample is subjected to hydration with 1N HCl, where carbonates are removed, followed by removal of humic acids with several 1N NaOH washes, removal of carbon gases with a second 1N HCl wash and a final rinse with nanopure water to a neutral pH.

4. **Interferences:**
   - **4.1** As in any radiocarbon lab contamination from ^{14}C poses a constant concern. Do not accept samples from labs that have not passed a swipe test. Prepare the work area with a fresh layer of foil.
   - **4.2** At times the pellets contain sand grains. Their presence will greatly bias the weight of the sample, and if included also bias the radiocarbon results.
   - **4.3** Sulfur compounds may be present in the sample. This is dealt with at the time the sample is weighed by adding silver powder.

5. **Apparatus/Equipment:**
   - **5.1** block heater (VWR 12621-104)
   - **5.2** 20-hole block module (VWR 13259-130)
   - **5.3** thermometer
   - **5.4** vortex
   - **5.5** 13 x 100 mm borosilicate tubes
   - **5.6** glass stir rod
   - **5.7** drying oven
   - **5.8** 3 mL transfer pipettes (Samco Scientific 225)
   - **5.9** aluminum foil
   - **5.10** 2 150 mL beakers
   - **5.11** beaker for waste
   - **5.12** secondary containment for reagents
   - **5.13** timer

6. **Reagents:**
   - **6.1** 1N HCl (Fisher Scientific SA48-1, or other equivalent. CAS# 7647-01-0).

6.2 1N NaOH (BDH 3222-1, or other equivalent. CAS# 1310-73-2).
6.3 nanopure water (18.2 M-ohm)
6.4 ethanol

7. Safety Precautions:

7.1 Familiarize yourself with the MSDS for HCl and NaOH and be prepared should any spills occur.

7.2 Nitrile gloves, safety goggles (or other appropriate eye protection), close-toed shoes, and a lab coat must be worn while performing this procedure. Although the concentrations of hydrochloric acid and sodium hydroxide are low, direct contact with these chemicals can still cause skin irritation, severely burn the eyes, and damage clothing. The reagents are brought to a near-boiling temperature causing fumes to escape, and possible burns may result from the high temperature should the sample splash out or spill from the tube.

7.3 Neutralize the waste generated by adding sufficient sodium bicarbonate to the waste beaker. It can then be sewered with copious amounts of water. This step mitigates potentially harmful personal contact.

8. Procedure:

8.1 Oven dry Pika samples at 50°C for 24 hours.

8.2 Have prepared sufficient borosilicate disposable culture tubes, size 13 x 100 mm. Prepared tubes have been baked in the muffle furnace at 400°C for 2 hours. Keep wrapped in foil and stored in humid Secador (i.e. containing a tray of 1N NaOH to absorb nitrates) until ready for use.

8.3 Wipe clean the weighing area and place a fresh layer of aluminum foil on working surface.

8.4 Label a borosilicate tube with sample number and tare it.

8.5 Weigh out between 35 - 50 mg of sample into the tube (approximately 4 pellets) using fine-tipped forceps to pick up the pellets.

8.6 Clean forcep tips with ethanol between each new sample.

8.7 Begin ABA pre-treatment as follows:

8.7.1 Set block heater temperature to 95°C.

8.7.2 Set all labeled sample tubes in block heater

8.7.3 Using a 3 mL transfer pipette add approximately 2 mL 1N HCl to each tube. It is important that the acid come in complete contact with the sample. After a few moments the sample will begin to hydrate and small gas bubbles will form on the surface of the pellets.

8.7.4 Gently mash the sample using a small, clean glass rod. **Note:** *It is important not to cross contaminate any of the samples. The glass rod must be cleaned with ethanol between each sample*.

8.7.5 Vortex each sample lightly. This will cause much of the sample to adhere to the sides of the tube.

8.7.6 Add approximately 1 mL of 1 N HCl with the transfer pipette to wash sample down.

8.7.7 Set timer for 20 minutes. During this time unwanted carbonates and some fulvic acids are removed.

8.7.8 Aspirate off the HCl with a transfer pipette and discard aspirant in a beaker designated for liquid waste. ***Note***: *each sample will have its own disposable transfer pipette for aspiration.*

8.7.9 Add 3 mL of 1N NaOH to each sample.

8.7.10 Set timer for 25 minutes. **Note:** *The first wash in the 1N NaOH will greatly reduce the sample size, as will be evidenced by the solution turning near-black, or coffee-like. With each subsequent repeat of the 1N NaOH wash the solution becomes clearer as base-soluble humic acids are separated out.*

8.7.11 Aspirate off the NaOH with a transfer pipette and discard aspirant in a beaker designated for liquid waste. ***Note:*** *When aspirating this supernatant, it is difficult to see the remaining sample. Be sure that you allow sample to completely settle at bottom of tube and then tip it slightly. Gently insert the pipette, so as not to stir up the sample, and aspirate off the solution. It is important not to cross contaminate any of the samples. Each sample will have its own disposable transfer pipette for aspiration.*

8.7.12 Repeat steps 8.7.9 through 8.7.11 until supernatant remains clear.

8.7.13 Make note on the 'sample treatment card' for the sample the number of NaOH washes performed. Expect up to 5 washes.

8.7.14 Using a 3 mL transfer pipette add 3 mL 1N HCl to each tube. ***Note:*** *because the NaOH is hygroscopic and readily absorbs CO_2 from the atmosphere, the sample is again washed one time with 3 mL of 1N to remove any carbon gases that may have formed during the NaOH washes.*

8.7.15 Set timer for 20 minutes

8.7.16 Aspirate off the HCl, transferring waste to the beaker.

8.7.17 Turn off the heating block.

8.7.18 Rinse each sample with 3 mL of nanopure water (ASTM Type I, 18.2 MΩ).

8.7.19 Set timer for 2 minutes

8.7.20 Aspirate off the water.

8.7.21 Repeat steps 8.7.18 through 8.7.20 two times or until the water pH is neutral.

8.7.22 Set heating block temperature to 85°C .

8.7.23 Lightly cover all samples with a foil tent so moisture can escape and allow samples to dry in the heating block overnight.

The following chart outlines the ABA steps:

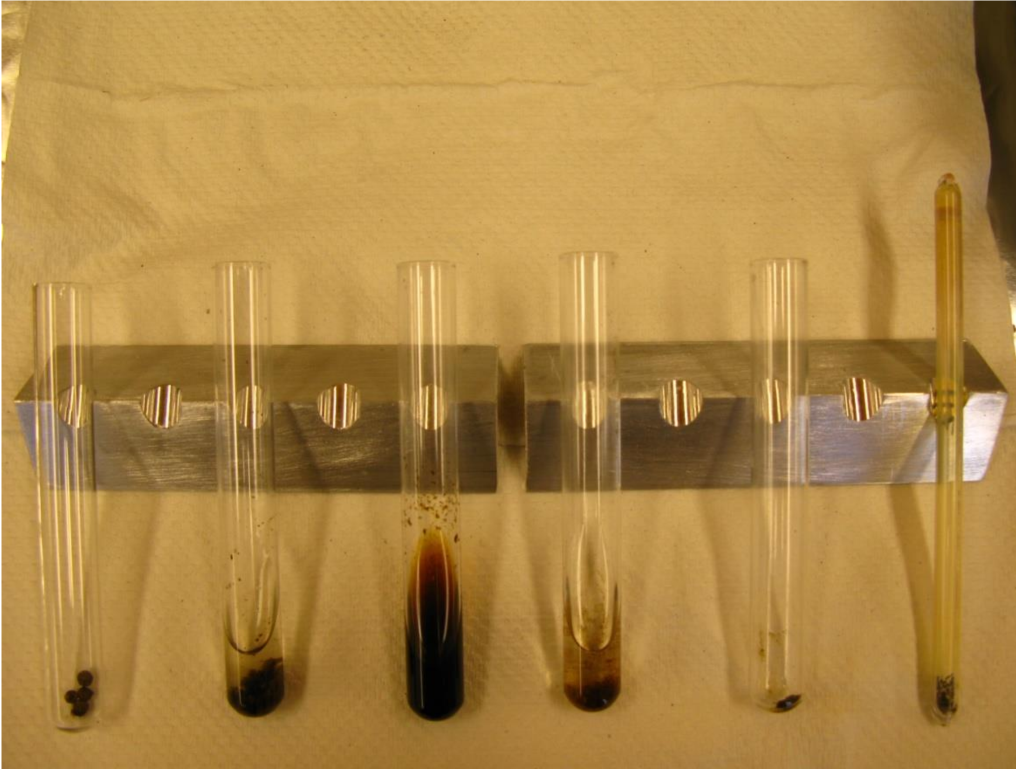
Treatment	Solution	Time (min)	Temp (°C)
Acid	1N HCl	20	95
Base	1N NaOH	25	95
Base	1N NaOH	25	95
Base	Repeat base treatment until supernatant is clear		
Acid	1N HCl	20	95

Rinse	nanopure H_2O	1-2	Room temp
Rinse	nanopure H_2O	1-2	Room temp
Dry	None	Until dry	85

The pre-treated sample is now ready for weighing.
Weigh out organic material only. Often there are mineralized crystals, or quartz granules in the pellets. These must be separated out. Pour the sample onto a sheet of weighing paper and inspect the sample for any unwanted substances. With forceps, select cleaned plant fibers for weighing.

Following is an image depicting the set-up for the ABA procedure:

An image showing how samples will appear after each step of the ABA treatment:



Carbon, Water, and Soils Research Lab
410 MacInnes Drive
Houghton, MI 49931