

UCI KCCAMS Facility

Acid / Base / Acid (ABA)

Sample pre-treatment

Dec 26, 2011

SUMMARY

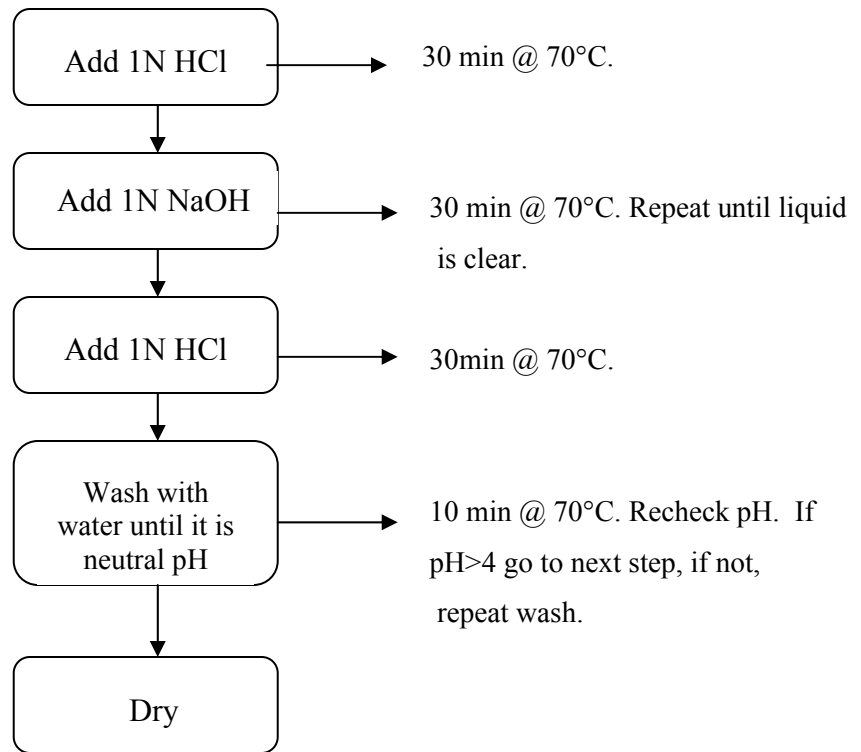
Setup:

Assign blanks and standards, assign UCIG#'s, print index cards

Set up sample tubes and pipettes

Turn on the heat block, check the cold trap on the vacuum oven (if required).

ABA flow chart



I. Introduction

To ensure that a sample is free of residues that may accumulate from the natural environment, pretreatment of the sample must be carried out before combustion. This pretreatment can involve physical, chemical or both procedures, depending on the actual conditions of the sample.

The first step for all samples submitted to the laboratory is a physical examination and cleaning. Obvious extraneous materials (roots, surface dirt, etc) are removed, using forceps, scalpels, etc. Some samples can also be sieved if a specific size fraction is required. In some cases, the sample can be crushed or split to increase the surface area. After the physical examination, the sample undergoes chemical pretreatment.

Most organic samples receive a standard acid-base-acid (ABA) treatment (Olsson, 1986). An initial acid wash dissolves any contaminating carbonate from dust or soil. The sample is then treated repeatedly with base (alkali) to remove soil humics, until the base solution does not turn brown but remains clear. Another acid wash is carried out to remove any atmospheric CO₂ absorbed during the alkaline washes. The sample is then washed with Milli-Q water to remove chloride that could potentially corrode combustion tubes, and dried on a heating block or in a vacuum oven.

II. Pre-ABA setup.

1. All samples entering the lab are assigned a unique lab number (UCIG #), and a numbered index card that records processing information. To log in samples and make cards, enter the pertinent information into the Master List spreadsheet on the Irvine6 computer, then open the **20 Index-Cards** desktop icon. This Excel file has instructions on how to print cards, and there is also a copy of the directions on the side of the computer. Blanks and secondary standards, chosen according to sample size, type and expected age, must be assigned to each set of samples, and these also require UCIG#'s and cards. **If you need help with any of this, ask a lab assistant.**
2. Samples are processed in 13mm culture tubes that are covered with ventilated caps and are labeled with the UCIG#. To avoid cross contamination, each sample has an individual

ultra-fine disposable pipette that is used to discard the ABA liquids. Set up the required numbers of 13mm tubes in test tube racks, cover with the ventilated caps, add an extra line of tubes to each rack to act as pipette holders, and label tubes and pipettes with the UCIG#'s using a Sharpie.

3. Place an appropriate amount of sample in each tube. For most reasonably well preserved materials, a few (<10) mg is enough to provide a full sized sample, and there is no point in using larger samples - they will only take longer to process. Some materials such as peat typically lose a lot of mass during the chemistry and may require more starting material, but except in special circumstances, avoid filling the tube with more than 1 cm of material. For faster processing, large chunks of charcoal or wood (>5 mm diameter) should be split into smaller pieces (but not powdered). Twigs or plant stems more than 2-3 mm diameter should be split lengthways
4. Set the heating block to 70°C (switch set to the low range, low temp. dial set to 7).

Note: If you are going to use the vacuum oven for drying the samples, make sure the refrigerated cold trap is turned on an hour prior to use. The cold trap should be cleaned out beforehand if necessary: see Section V below.

III. ABA Procedure

First HCl wash

1. Fill the tube about 2/3 full (about 2 finger widths below the top) with 1N HCl., replace the cap, place on the heat block @70°C for 30 min.. Write an "A" (= Acid) on the index card – this lets you keep track of how far along each sample is in the ABA process.
2. If sample is powdery and has not settled to the bottom of the tube, centrifuge it for 1-2 minutes at 3000 rpm. You may need to change the set time on the centrifuge – ask a lab assistant if you don't know how. Remember to keep the centrifuge balanced – set tubes opposite each other with the same approximate weight, and use extra dummy tubes if necessary.
3. Pipette off the liquid into a waste container. Try to pipette as much liquid as you can without losing sample.

NaOH washes

4. Fill the tube 2/3 full with 1N NaOH, replace the ventilated cap, place on the heat block, heat at 70°C for 30 minutes. Write a "B" on the card for each base wash..

5. Centrifuge if necessary, then pipette and discard the liquid.
6. Repeat steps 4 and 5 until the liquid is clear.

Final HCl wash

7. Fill the tube 2/3 full with 1N HCl, replace the ventilated cap, place on the heat block, heat at 70°C for 30 minutes. Write an “A” on the card.
8. Centrifuge if necessary, then pipette and discard the liquid

Sample neutralization – water washes

9. Fill the tube 2/3 full with MQ water, replace the ventilated cap, place on the heat block, heat at 70°C for 10 minutes. Write “W” on the card.
10. Centrifuge if necessary.
11. Check the pH by pipetting a little liquid on to a pH strip. Pipette and discard the water and repeat steps 9-11 until the pH is ≥ 4 (2 washes are usually enough, though peat and sediments will need more). Try to remove as much water as possible, particularly on the last step.

IV. Drying – heat block

1. Uncap the tubes to help water vapor to escape, place them in the heating block at 70°C. and cover them with an aluminum foil tent to protect them from dust, etc.
2. After they are dry (this will take several hours), cap the tubes with gas-tight storage caps to minimize exchange of carbon with the atmosphere and place the rack in the correct sample tray by the microscope.

V. Drying – vacuum oven

Setup: cleaning the cold trap (if required)

1. Make sure the cold trap is turned off. Remove the hoses and the foam ring. Carefully remove the glass cold trap. If you can't remove it, the methanol in the reservoir is full of water and is frozen. Let the trap warm up and then change the methanol.
2. Empty any liquid out of the trap. Rinse it twice with methanol, and let it drain very thoroughly on the 2nd rinse. Check that the alcohol level in the metal reservoir is up to the fill line about 1” from the bottom. If needed, top up the methanol to the fill line. If the methanol looks milky or if the liquid froze, sponge it out and replace with

fresh methanol.

3. Replace the dry glass cold trap in the reservoir, and replace the foam insulation and hoses. (do not overtighten the connectors - finger tight only).
4. Close the outlet valve on the vacuum oven (this is used to vent the vacuum system).
5. Turn on the roughing pump and check that the vacuum goes to <200 mT on the vacuum gauge (this will take several minutes). If the system does not pump down, check it for leaks and/or ask for help.
6. Turn off the pump and vent via the oven (open both oven valves).
7. Turn on the trap and wait at least one hour for it to cool down before using the vacuum oven.
8. Close the outlet valve on the oven and turn on the pump again a few minutes before you want to use the oven.

Drying

1. Place the samples with ventilated caps in a rack in the oven. The cold trap and vacuum pump should already be turned on. Turn on the oven and check that the temperature dial is set to 2 (50°C).
2. Close the oven vent valve and slowly open the oven outlet valve. The samples will be dry once the vacuum gauge has reached <200 mT, which typically takes ~30 minutes.
3. Close the oven outlet valve and open the vent valve, and remove the sample rack when the oven reaches atmosphere. Turn off the oven and the pump, and open the outlet valve to vent the trap. Turn off the trap unless you intend to use it again.
4. Replace the ventilated caps on the tubes with gas-tight storage caps to minimize exchange of carbon with the atmosphere, and place the rack in the correct sample tray by the microscope.

References

Olsson I (1986) Radiometric Methods. In: Berglund B (eds) Handbook of Holocene palaeoecology and palaeohydrology. John Wiley & Sons, Chichester, pp 273-312