Sediment Desalting and Drying

IMPORTANT SAFETY INFORMATION

You must wear **safety glasses** and **gloves** when working in the lab. All work with solvents must be done in a **fume hood**. As with all chemistry labs, you need to wear appropriate clothing, including close-toed shoes, long pants, long sleeves (or a lab coat), etc.

Don't forget to write down all your steps (and/or mistakes) in the lab notebook!

1.1 Desalting sediment samples:

1. Prepare frozen samples for desalting by either placing in fridge overnight to thaw, or at room temperature for a few hours.
2. Once samples are thawed, mix sample thoroughly and transfer the desired amount to a 50 mL Falcon Tube. Filling the tube about ¼ full with sediment is usually sufficient.
3. To each tube, add 20 mL of Milli-Q Water (this comes from the milli-q machine in the clean zone).
4. Place centrifuge tubes into the centrifuge located in Jodi Young's lab.
    a. Set the centrifuge to 10,000 g (9,653 rpm) for 30 min at 15°C using rotor TA-14-50.
5. After centrifuge program is finished, remove samples and decant and discard the overlying water.
6. Add 20 mL milli-q water to each sample and vortex-mix for about 30 seconds. If the samples are still stuck on the bottom, vortex-mix until all sediments are re-suspended.
7. Repeat steps 4-6 at least two more times to effectively rinse your sample.
8. After centrifuging each sample three or more times, decant the final water from the sample.
9. Label 20 mL glass vials with appropriate samples names. Write directly on the vial, do not use tape.
10. Using a clean scoopula, fill the 20 mL glass vial about 2/3 full with sediment. Freeze the remaining wet sample, if desired.
11. Cover vial opening with aluminum foil.
12. Repeat steps 10-11 for each sample.
13. Place samples in the freezer (at least -20 °C) for at least 24 hours before proceeding to next step. Samples need to be very frozen before proceeding with freeze drying.

1.2 Freeze-drying sediment trap samples:

Freeze-drying samples removes about 99% of the moisture in the sample. Your samples MUST be frozen (< -40° C) in order for the freeze-dryer to work correctly. The process is completely product dependent, generally taking 24-72 hours.

Please ask Jaqui for assistance if this is your first time using the freeze-dryer. The manual is located next to the freeze-dryer log and can be of great help if you need to trouble-shoot the system. Check the operation summary for their step by step instructions.

1. Locate freeze-dryer in the Sachs lab on the first floor of OSB. Make sure no-one is using it, and that the drain line tube is removed from the front of the freeze-dryer.
2. Fill out your name, PI and number of samples you plan to freeze dry in the log. Record the start date and time.
3. Check that the unit can hold a vacuum, that all the black ports (quickseal valves) are closed and that the seals are correct. Make sure the unit is clean and free of any moisture.
4. Turn on the freeze-dryer, the switch is located in the back of the unit.
5. Press the condenser button to begin cooling condenser. Wait 20-30 min until condenser has reached at least -40 °C, usually the system will reach around -60 °C.
6. While you are waiting on the freeze dryer to cool, collect the materials you need to prep your samples. Wait to take your samples out of the freezer until the last possible second.
    a. You will need kim-wipes, aluminum foil, tweezers, your samples and rubber bands.
7. If your samples have lids on them, take the lids off and cover the vial mouth tightly with aluminum foil. If the vial is already covered with aluminum foil, make sure the foil is tight enough on the mouth of the vial.
8. Pierce three holes into the aluminum foil with the tweezer edge.
9. Fold a kim-wipe in fourths and cover the aluminum foil with the kim-wipe.
10. Secure with the rubber-band.
11. Quickly repeat steps 7-10 for all remaining samples. Place samples back in freezer if any signs of thawing have occurred.
12. When the freeze-dryer condenser temperature is low enough, retrieve your prepared samples from the freezer and place them evenly on the stand inside the freeze-dryer.
13. Turn on the vacuum by pressing the vacuum button on the front. Watch the vacuum readings, which must go below 200 millitorr and should hover around 100 millitorr. If the vacuum readings do not go low enough, break the vacuum seal and check all the connections that could leak. Retry vacuum until it reaches the desired pressure.
14. Once the vacuum is around 100 millitorr, periodically check on your samples during the process. You may need to release the vacuum and defrost the condenser if too much ice has built up.
15. Once your samples have been completely removed of water, they should be flaky or granulized in consistency.
16. If your samples are done, release the vacuum to the system by slowly opening up one of the quickseal valves on the top of the manifold. If you open this too fast, you can knock over your samples from the change in pressure, which is bad.
17. Once the vacuum has reached standard pressure (760 torr), turn off the vacuum by pressing the vacuum button.
18. Switch off the condenser.
19. Remove your samples.

20. Insert drain line, and then defrost condenser by pressing condenser button and the set up button at the same time.
21. Defrost condenser for 5-10 minutes, then switch off the defrost setting by pressing the set down button.
22. Turn off the freeze dryer, but leave in the drain tube. Mop up any extra water from the condenser and clean the freeze dryer.
23. Record the end time and date on the freeze-dyer log. Record the number of hours the freeze-dryer was in use.
24. Take your samples, scoopula, methanol, calculator and lab notebook into the weight room.
25. Remove coverings from the mouth of the vial and discard.
26. Wash scoopula with methanol and then crush sample in vial until it is homogenized. Scrape any bits of the sample stuck on the vial into the rest of the sample.
27. Weigh the sample and subtract the vial weight, recorded in section 1.1 step 16. If you do not have the vial weight for some reason, transfer sample into a new tarred vial. Make sure to record this vial's weight in your lab notebook before tarring the vial. Record your sample's dry weight in your lab notebook.
28. Replace covering of sample with a new bit of aluminum foil.
29. Repeat steps 25-28 for all your samples.
30. Place samples in appropriate section of the trap sample cupboard for further analysis.