Sublimation of SA CHCA DHB

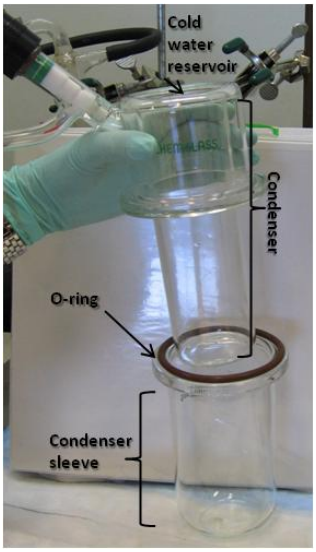

Figure 1. Sublimation apparatus.

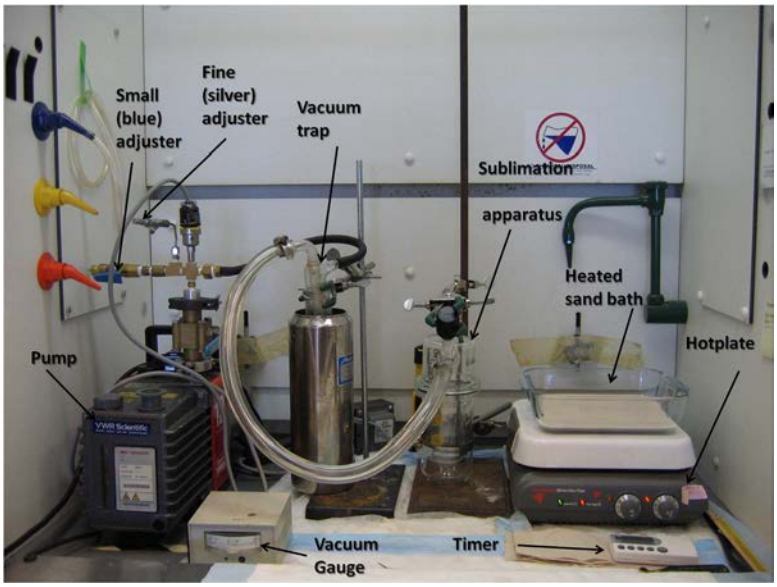

Figure 2. Sublimation equipment setup.

Health and Safety:

Wear nitrile gloves, laboratory coat and safety glasses. The process should be done with fume hood ventilation.

Equipment and Materials:

Equipment

1. Rotary vane mechanical pump [Edwards Model E2M12]
2. Vacuum trap [Sigma Z174211]
3. Vacuum measurement gauge
4. Sublimation apparatus, small [Chemglass Life Sciences CG-3038-01]
5. Sublimation apparatus, large [Chemglass Life Sciences VU-0904-281MS]
6. Hot plate [Corning 6795-200]
7. Thermometer [VWR 61019-025]
8. Thermally conductive adhesive tape [3M PN 8820]

9. Support stands and clamps for stabilization during operation.
10. Timer

Chemicals

MALDI Matrices

Alpha-cyano-4-hydroxycinnamic acid (CHCA) [Sigma 2020]

2,5-Dihydroxybenzoic acid (DHB) [Acros Organics 11481025]

3,5-Dimethoxy-4-hydroxycinnamic acid (SA) [Fluka 85429]

Procedure:

1. Inspect the cold trap. Clean the cold trap with methanol and acetone if there is noticeable accumulation of matrix. Reapply vacuum grease to the ground glass portion of the cold trap sleeve to ensure vacuum tight seal. Ensure vacuum tight fit by rotating ground glass portions as they are attached to each other.
2. Prepare the cold trap by combining 100 mL of crushed dry ice in the bottom of the dewar with 50 mL of acetone. This ensures complete contact of the dry ice with the curved bottom of the dewar. Place the cold trap into the dewar and pack the entire outer length with crushed dry ice.
3. Prior to sublimation, adjust the sand bath to the desired temperature. At 50 mTorr, DHB sublimates well at 110 °C, sinapic acid sublimates well at 145°C, and CHCA sublimates well at 180°C. Shake the sand bath to disperse sand evenly, allowing uniform heating of sand.
4. Prepare ice water by mixing 300 mL water with 200 mL of ice.
5. Prepare the matrix. For the smaller sublimation apparatus, add 50 mg matrix to the bottom of the condenser sleeve. For the larger apparatus, add 100 mg of matrix or enough to evenly coat the bottom of the condenser sleeve. Avoid getting matrix on the sides of the sleeve. Tap the side of the condenser sleeve to evenly disperse matrix. Uneven dispersal of matrix results will result in uneven coating of the sample.
6. An alternative way of placing the matrices on the bottom of the condenser sleeve: after adding the matrices, add 5 mL of acetone, shake the condenser sleeve for a few times to make sure all the solid matrices mixing well with acetone----no need to dissolve all the solid-----then, use the air or nitrogen in the hood to blow the acetone gently till the acetone is evaporated resulting a relative homogeneous coating of matrices on the bottom of condenser sleeve. The advantage of doing this is that the entire matrix crystals are stuck on the bottom of condenser sleeve very nicely, so these crystals will not be attached to the slide during the vacuum start/off step.

7. Prepare the sample for sublimation. Mark fiducials using a silver metallic pen for the background and a fine pointed black Sharpie to create crosshair fiducials at four corners bracketing the sample. Record an image of the marked plate. Place small pieces (0.5-1 cm^2) of 3M thermally conductive adhesive copper tape onto the four corners of the slide or plate, clear side of the tape facing outward.

8. Tape the sample onto the bottom of the condenser. Remove the clear sided liner from the tape and adhere the sample to the center bottom of the condenser sleeve.

9. Inspect the condenser O-ring for matrix deposition or cracks. Clean the O-ring with ethanol if necessary.

10. Place condenser inside the sleeve, ensuring snug fit with rubber O-ring. If necessary, a metal clamp built for the apparatus may be used to maintain the snug fit.

11. Apply the vacuum. Check that the small blue lever on the left upper side of the pump is in a vertical position. Slowly turn the large blue lever on the right side of the pump to the vertical position. Use the fine adjuster (silver knob above large blue lever) to adjust the vacuum to 10 mTorr.

12. Place the ice water inside the condenser sleeve. Allow vacuum to stabilize at 30 mTorr for about 2 minutes. (stable vacuum number can be different for different pump)

13. Once the vacuum has stabilized, lower the apparatus onto the heated sand. Ensure that the bottom of the condenser is level and evenly submerged in the heated sand.

14. For DHB, about 8 min at 110°C, for SA, 16 min at 145°C, for CHCA, 22 min at 180°C are needed to obtain ~0.15mm/cm^2.

15. Remove the apparatus from the heated sand bath.

16. Slowly release the vacuum by turning the large blue lever to the horizontal position. The small blue lever may be used for further venting. Rapid release of vacuum will deposit particles on top of the sublimated matrix. This will interfere with data collection in these areas.

17. Pour off the ice water.

18. Remove the sample.

19. Upon removal, place the sample in a plastic culture dish for protection. Store in desiccator until analysis.

20. Turn the pump off. Leave the hotplate at 110°C or preferred temperature. Leave the cold trap assembled with dry ice.

Cleaning the Sublimation Apparatus

1. Wash the bottom of the condenser with acetone to dissolve sublimated matrix.
2. Dry the glassware to ensure that streaks of matrix are not left in the apparatus.

3. Wipe the O-ring with ethanol.
4. Reassemble the condenser inside the sleeve after cleaning. Ensure that the rubber O-ring is in place.

Expected Outcome:

After sublimation, a thin coating of matrix should be uniformly deposited across the sample.

References: Sublimation as a Method of Matrix Application for Mass Spectrometric Imaging Joseph A.Hankin Robert M.Barkley Robert C.Murphy J Am Soc Mass Spectrom 2007, 18, 1646–1652