**Critical Point Drying**

Critical point drying is the simplest, most reliable and most rapid method for drying biological samples free of distortion caused by forces of surface tension. The tissue is placed in a high pressure bomb with a sufficient quantity of ethanol or acetone to insure that the tissue remains wet until liquid CO2 is admitted. The tissue is alternately soaked in CO2 in the sealed bomb and/or flushed by a flow of liquid CO2 through the bomb is sealed and heated until the critical temperature and critical pressure are balanced in such a way that the specific gravity of the liquid and its vapor are the same. No boundary exists between the gas and liquid. Surface tension drops to zero and the tissue is dry the moment this critical point is passed. Above the critical temperature, the gas can no longer exist as a liquid regardless of any increase in pressure. If the gas is bled off slowly to prevent adiabatic cooling (which could cause the temperature to fall below the critical temperature and cause condensation of gas to a liquid) the tissue is dried without exposure to any tension forces.

**Method**

This method uses the Balzers 020 Critical Point Dryer.

1. Be sure all three valves on the Critical Point Dryer (CPD) 020 are closed.
2. Unscrew the chamber lid. Take the perforated discs and stir bar out of chamber.
3. Fill the chamber with enough of the transferring liquid, i.e. amyl acetate or ethanol so that the baskets containing the specimens are just covered.
4. Place the specimen basket at the bottom of the chamber into the transferring liquid.
5. Rapidly transfer the baskets containing the dehydrated samples to the CPD 020 chamber.
6. Place the large perforated disc inside the chamber with the stir bar on top. Screw the lid hand tight.
7. Turn on the MAINS switch (make sure the machine is plugged in). Open the valve all the way on the cooling gas (CO2) tank (i.e. the gray tank) by turning the knob counter-clock wise.
8. Set the chamber cooling to +10 oC. This is the temperature you want the specimen to be, but the "set" temperature must be much lower at approx. -17 oC to get the chamber cooled.
9. Depress the TEMP button. The CPD 020 will begin automatically cooling the specimen chamber to the selected temperature. When the selected temperature is reached, exchange of the transferring agent (amylacetate, ethanol) with the drying agent (CO2) can begin. \*\*\*Turn off TEMP when the read out temp is at +13. The temperature will continue to drift down to the desired +10 degrees. When temperature increases as the process continues, depress TEMP button again to maintain a temperature between +9 and +13 degrees.
10. Slowly open the gas inlet valve (i.e. turn knob on the LHS of CPD 020 CCW). Observe the filling of the specimen chamber with liquid. \*\* Fill the chamber very slowly so that the 2 liquids don’t mix on the first fill and you can see the meniscus between the liquids. When you empty the first fill, you can get rid of most of the alcohol. Close gas inlet valve.
11. Open gas outlet valve several times by turning the first knob on the RHS, CW. Carefully open the metering valve and slowly drain liquid to just above specimen baskets.
12. Repeat step 10.
13. When the specimen chamber is full, close the gas inlet valve and press the STIRRER button. This will ensure the mixing of the drying agent with the transferring liquid. Let the liquid mix for about 5 minutes. Shut off the magnetic stirrer.
14. Repeat step 11.
15. Repeat step 12 to 14 several times, until all traces of the transferring liquid are gone. Note: It is very important that the drying agent completely replace the transferring liquid. Otherwise, the samples will not dry properly.
16. When the transferring liquid has been completely washed out, fill the chamber halfway, or to the point where liquid just covers the specimen baskets, whichever is greater.
17. Make sure that all valves are closed.
18. Set the TEMPERATURE SELECTOR to +44 oC. Depress TEMP button. The CPD will now automatically begin warming the specimen chamber. Turn off TEMP when temperature hits +38 oC. Notice also that the pressure within the chamber begins to rise. If the pressure begins to exceed 120 bar, reduce the pressure to 80-85 bar with the gas out and metering valves.
19. As the critical temperature and critical pressure are approached, the drying agent will begin to go from a liquid to a gaseous state.
20. To be assured that complete drying has taken place, wait until the critical temperature and pressure are exceeded (i.e. keep temperature at about 40 oC).

Critical Values:

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| CO2 | 31.1 oC | 73.9 bar |
| Freon (Frigen 13) | 28.9 oC | 38.7 bar |
| NO2 | 36.5 oC | 72.7 bar |

For example, for CO2, wait until the temperature is +40 oC and the pressure is between 80-85 bar.

1. Begin pressure reduction by opening the GAS OUT valve and slowly opening the METERING valve. Pressure reduction should take place over a period of 15 minutes. The METERING valve will allow you to precisely regulate the rate of pressure reduction. If the gas within the chamber begins to recondense, you are reducing the pressure too quickly. Do not let this happen!
2. When the pressure in the chamber is 0 bar, the cover can be opened and the samples removed.
3. Close all valves, and release all buttons. Shut off MAINS. At this point the samples are very hygroscopic. Care should be taken to prevent them from absorbing water. They can either be stored in a dessicator containing a drying agent, or they can be coated with a thin layer of metal using our SCD 040 Sputter Coating Device.
4. Sign the Supplies Log book.