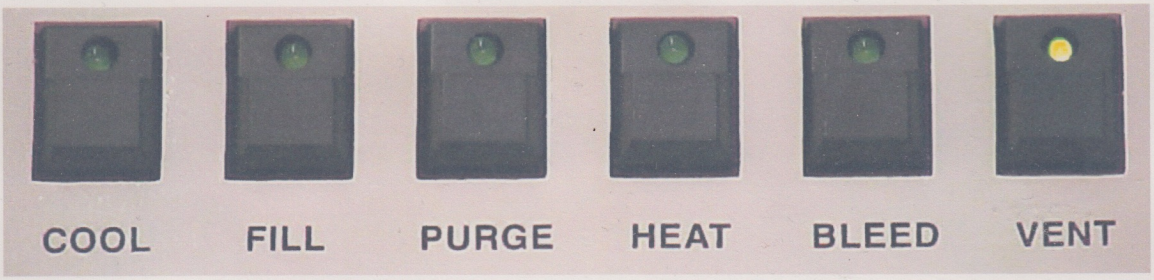
5-10-05 Change Tank after 10 runs

Full cycle 55-60 mins

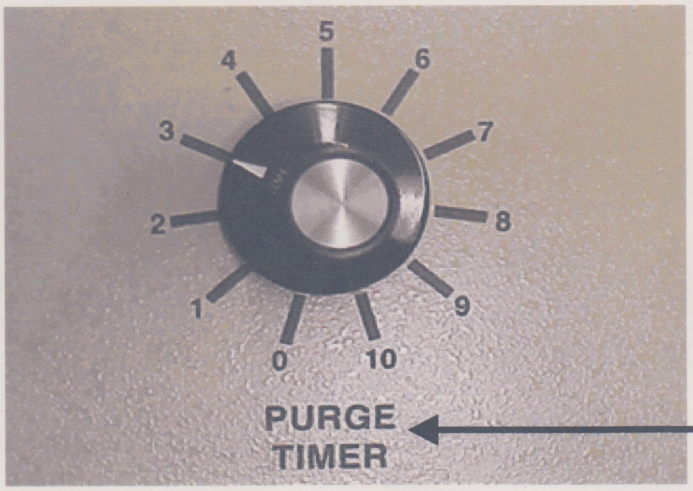
OPERATION

2.1 OPERATING SEQUENCE

(1) Turn on unit with ON/OFF switch on right hand side. Green LED on VENT button will illuminate. This indicates that the Samdri®-795 is in the VENT mode and there is power to the unit.



(2) At this point, you will need to adjust the PURGE timer to your desired PURGE time. The PURGE timer has a timer range of 0-45 minutes in increments (linear) of 5 minutes each.



10 minutes (Pos. #2) is sufficient time for most sample processing.

PURGE Timer:
#1 ... 5 min
#2 ... 10 min
#3 ... 15 min
Etc. to 45 min at the #9 position.
Each position refers to a 5-minute increment.

(3) Let the Samdri®-795 warm-up for 3-5 minutes prior to use. This will stabilize all plumbing components and the Metering Valves and Static Pressure Control module.

(4) Fill chamber with sufficient ultra-pure alcohol (i.e. Ethanol) to cover sample upon transfer into the chamber.

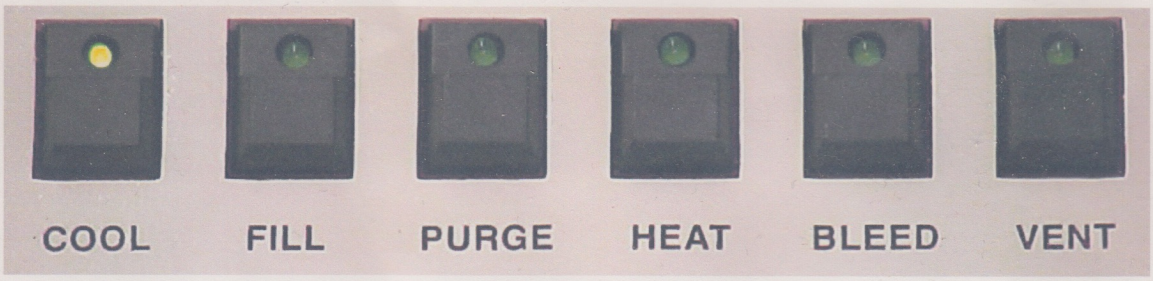
(5) Transfer sample (in proper sample holder) into process chamber. Your sample should never be exposed to air, before Samdri®-ing (See various sample holders, p. 26).

(6) Place the Lid on top of the Chamber making certain the O-Ring is in place. Tighten the 3-Knurled Nuts by hand making certain that all are evenly finger tight.

Open 1 CO_2 Tank

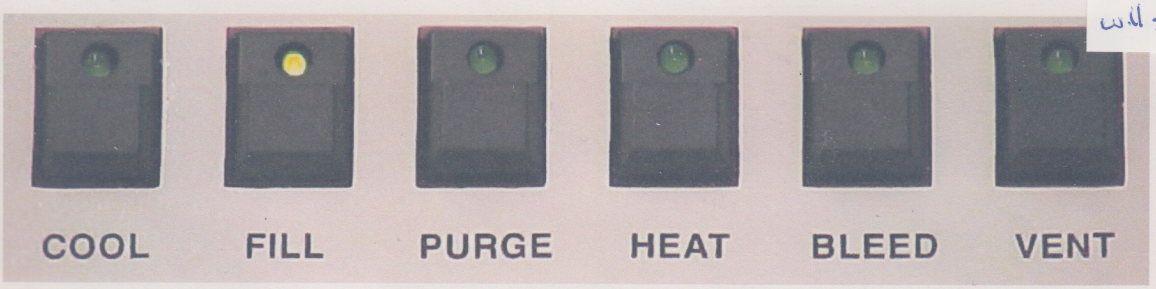
Pressure will read zero.

(7) Press the COOL button. Green COOL light will go on (VENT light will go off). The Samdri®-795 will go on cooling by itself until a reaching 0°C (± 5°C) – when it will stop by itself. Once cooled with sample in Chamber, proceed to the next step.

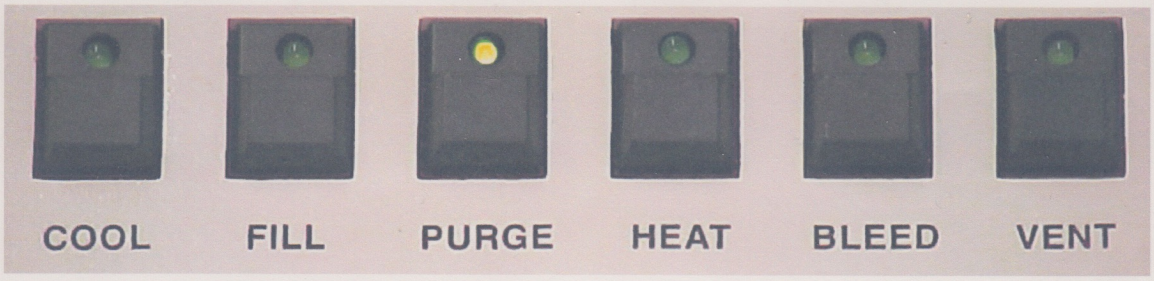


(8) Now, press the FILL button and the Samdri®-795 will start filling the Chamber with LCO_2. The Fill Metering Valve is factory-set for rate of flow. During the FILL Mode, the cooling works so that it will not interfere with the constant rate of fill. After 2 minutes, the FILL mode will shut off automatically.

Pressure guage will show tank pressure *



(9) The Samdri®-795 will next automatically advance to the PURGE Mode (i.e. the alcohol(s) are exchanged with liquid CO_2 under pressure in the Chamber). Collect the alcohol coming out of the short tubing into a beaker. At the end of the PURGE TIME, there should be no alcohol coming out of the Chamber. The Samdri®-795 will now advance to the HEAT MODE. The PURGE LED will go OFF and the HEAT LED will go ON.

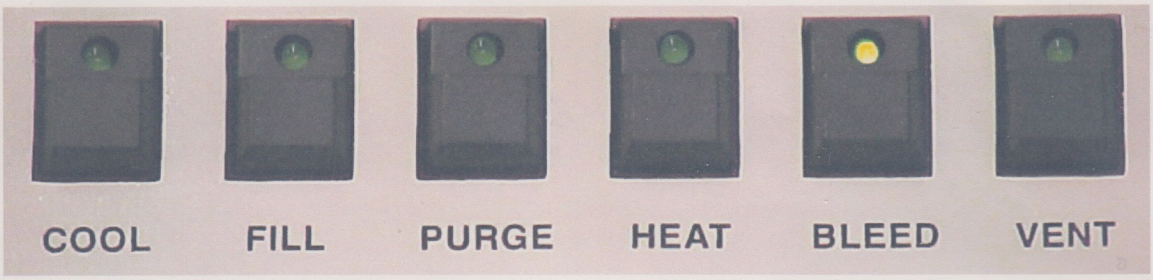


NOTE:

Now if you wish, you can start recording every minute, the corresponding pressure and temperature directly from the gauges. Please consult the Check-Out Data Sheet furnished with your Instrument in the Appendix at this User Manual. When the pressure reaches and goes beyond 1072 psi (it will stabilize at a factory predetermined pressure which in most cases is 1350 psi) and the temperature is above 31° C, and then the tousimis equilibrium®1 cycle starts.

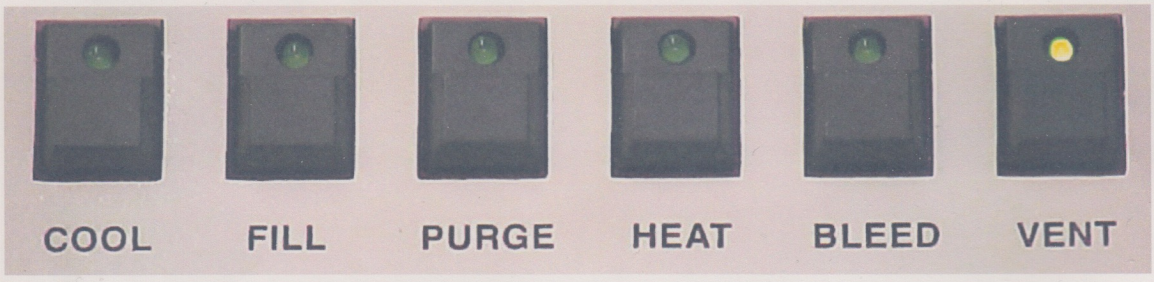
(10) At the end of the tousimis equilibrium® [1] period, press the BLEED switch whereupon the LED of the BLEED Switch will be ON. At this point, you can measure the Bleed Rate by attaching the Flow Meter to the outlet of the PURGE / BLEED / VENT exhaust which should read between 3-4 SCFH at the onset of the BLEED mode. This setting should average approximately 100psi/min reduction in pressure from 1200 psi to 400 psi. During the BLEED Cycle, the Samdri®-795 automatically maintains the chamber temperature above 31°C.

15mins



(11) Once the chamber pressure reaches 400 psi, press the VENT button. This will advance the process into the VENT mode.

5mins



It is not necessary to readjust the PURGE / VENT Metering Valve flow rate. The Chamber should come to atmospheric pressure in 3-5 minutes. The sample should be removed from the Chamber; it is now ready for further processing. Seal the Chamber with the Lid to keep it clean and moisture free. Turn the instrument off with the Power Switch on the right side of the cabinet. You will also note that there is a delay of the LED of the VENT Switch of several seconds before the LED light goes off.

Close LCO_2 Tank

[1] "tousimis equilibrium" refers to the period of time (4 minutes) that the unit sites above the critical point prior to initiating the BLEED cycle.

SECTION III

OPERATIONAL NOTES

3.1 SAFETY FEATURES

The specimen chamber is a metal vessel with viewing ports designed to withstand the pressures used in the critical point drying process with minimum 3x safety pressure rating.

To ensure additional operator safety, the following features are incorporated:

- A mechanical Automatic Pressure Control System (APCS) which will both vent and maintain the chamber at a pressure between 1200 psi and 1500 psi (see factory check-out data sheet for your unit).
- A rupture disc, which will be activated (2,000 psi at 20°C) if the mechanical APCS should fail (see factory check-out data sheet for the setting(s) of your unit.)
- Quartz ports, which will crack, rather than shatter, should they become unduly stressed or abused.
- Metal-alloy (sintered stainless steel) filters placed internally along both the inlet and exhaust paths, preventing solid particles down to 0.5 micron from fouling the gas lines/valves/fittings.
- An electronic heater which is governed by two thermostats: one to maintain the chamber during critical point passage at temperatures 37°C ± 2°C (see factory check-out data sheet for your unit) and a second safety thermostat to shut off all heating should a failure bring the chamber temperature near 49°C ± 3°C.
- A bulb and a translucent diffuser below the specimen chamber which illuminate chamber contents.

3.2 SEMI-AUTOMATIC OPERATION

The SAMDRI®-795 critical point drying apparatus and its electromagnetic valving system will self-maintain in each mode of operation until such time as the operator, on the basis of temperature and pressure display, chooses to activate the next mode. Metering valves are preset as desired (note the typical factory setting on the "Check Out data Sheet", in the appendix of this manual).

3.3 CHAMBER SAMPLE LOADING

A sufficient amount of high purity* intermediate fluid (i.e. Ethanol) to cover the sample and holder should be introduced carefully into the chamber before placing the sample holder into the chamber. This will minimize surface evaporation of the dehydrating intermediate fluid (ethanol) from the sample.

3.4 CHAMBER COOLING

The patented cooling capability of the SAMDRI®-795 may be exercised at any time, for it is an entirely independent operation. The chamber and contents are brought down to a temperature of approximately 0°C or below (see factory checkout data sheet for your unit). The CO_2 cylinder should have more than 25 lbs net weight of CO_2 for proper cooling and operation of the unit.

* Always use minimum 99.5+% purity alcohol.

Chamber cooling is necessary to ensure rapid filling with the liquid phase of the transitional fluid (LCO_2). Cooling also insures expulsion of the gas phase ahead of the liquid phase in the pressure hose connecting the transitional fluid tank to the SAMDRI®-795 inlet circuit. CO_2 passing through the exhaust hoses may appear in solid flakes as it encounters the lower pressure environment.

3.5 SOLVENT EXCHANGE (PURGE MODE)

Transitional fluid (CO_2) may now be allowed to enter and leave the chamber, filling it completely to the top port, and purging the specimen of intermediate fluid (ethanol). Characteristics visible to the operator during the solvent exchange step are as follows:

- The intermediate fluid will appear as "oily currents" (better known as schlieren patterns) within the chamber, and
- The intermediate fluid will appear as a liquid or slurry when exiting through the exhaust hose, towards the end of the PURGE mode. Once thoroughly exchanged, the chamber liquid will be perfectly clear; the exiting material will be cold and white. During the heat mode, the CO_2 may appear as schlieren "currents" due to the heat causing variations in LCO_2 densities and thus the *schlieren* pattern appearance.

The purge exchange rate is manually adjustable with the PURGE/VENT valve, and should be set by the operator to allow for a steady flushing of transitional fluid (without letting the fluid level drop away from the top window). *NOTE: The PURGE / VENT meter valve has been factory pre-set during final check-out.*

A warming trend will be noted during the PURGE mode since the incoming fluid originates at room temperature. The operator should occasionally check the chamber temperature, and turn on (the COOL switch) as necessary, ensuring that the purging continues in the liquid state at a temperature range of +10°C to 0°C

The purge time required for complete solvent exchange is not standardized, *but is dependent upon the size, density, and cellular characteristics of the sample*. Generally, 24 specimens of a size 4mm x 6mm x 2mm accommodated by a Sample Holder (tousimis® catalog #8763) will require about 10 minutes. Purge time is only suggested; however, and should be established in each case by the individual operator.

SUGGESTED TEST TO VERIFY COMPLETION OF PURGE:

Since more adiabatic cooling usually takes place near the exhaust orifice of the unit (evidenced by white flakes of CO_2, which will form at -79°C), and since intermediate fluids such as pure ethanol have even *lower* freezing points, these fluids will be flushed out as *liquids*. A soft, dry coated paper held momentarily in the exhausting stream will catch the flakes of CO_2, and when shaken out, will show "wet" spots and give off the odor of any ethanol which remains to be purged.

WARNING: FOR HEALTH REASONS, WE DO NOT RECOMMEND THE USE OF AMYL ACETATE OR ACETONE AS INTERMEDIATE FLUIDS!!

3.6 CRITICAL POINT PASSAGE

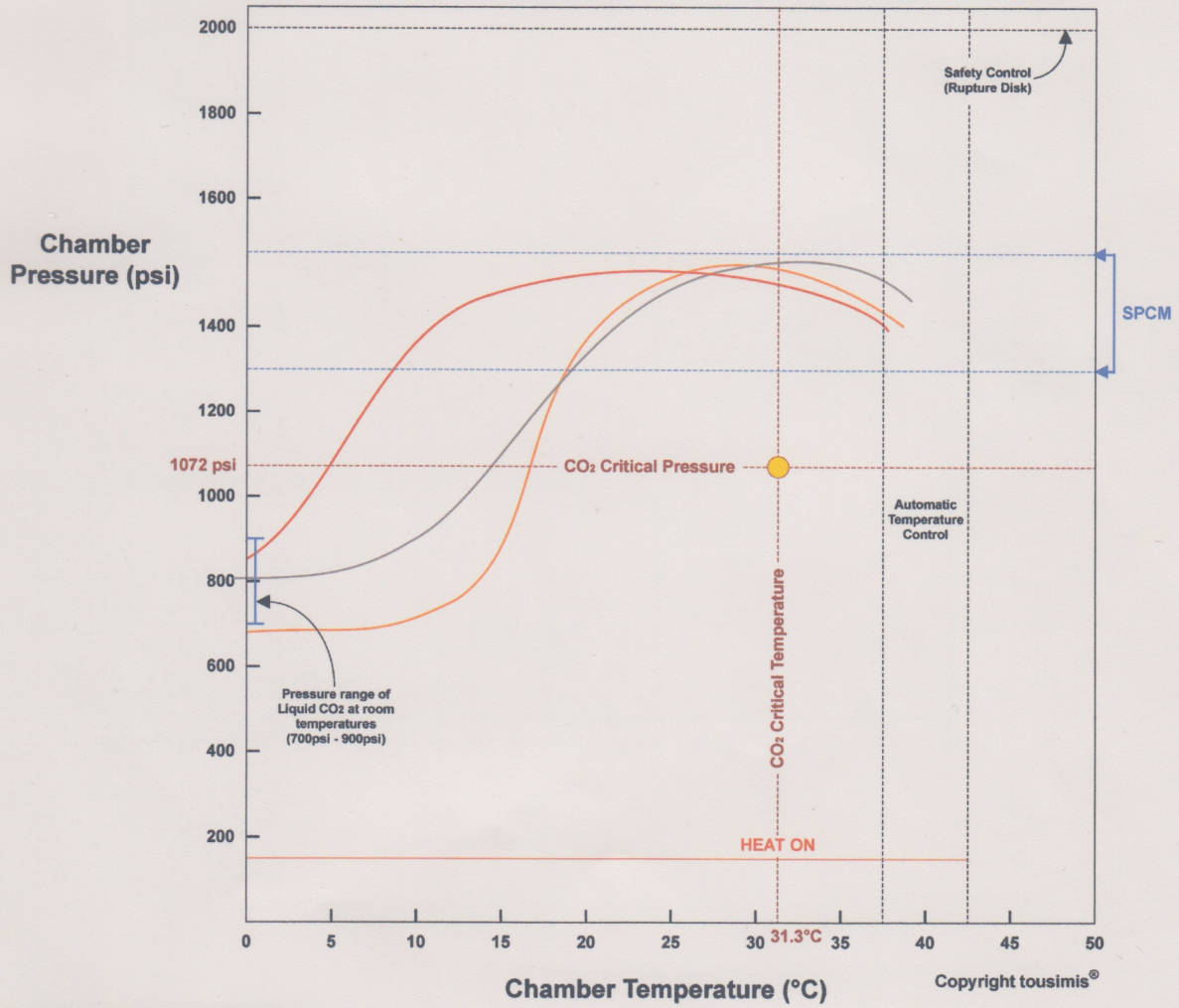
When all the intermediate fluid permeating the sample has been replaced by LCO_2, flushing and cooling are terminated. Make sure the chamber is completely filled with LCO_2. Return to FILL again, if necessary. Heat is now applied to the chamber, elevating the transitional fluid to above its critical temperature and pressure.

The HEAT mode is thermostatically controlled to maintain a temperature at a safe margin above the critical values, yet low enough that thermal destruction (above 55°C) of the sample does not occur.

The chamber pressure increases relative to the increase in temperature. It is likely that the critical pressure will be reached before the critical temperature if the chamber was completely filled with LCO_2 when heat was applied. The APCS is factory-set for 1200 psi - 1500 psi (see factory check-out data sheet). Variations in LCO_2 tank net weight and room temperature will affect the speed of attaining critical point passage (NEVER use a LCO_2 tank if it is kept outside in the winter. Bring it to room temperature for 2-3 days before use; otherwise, due to low tank temperatures, the tank will not supply sufficient tank pressure to properly operate the tousimis® Samdri®-795).

FIGURE 1

TYPICAL RELATIONSHIPS BETWEEN CHAMBER PRESSURE AND TEMPERATURE

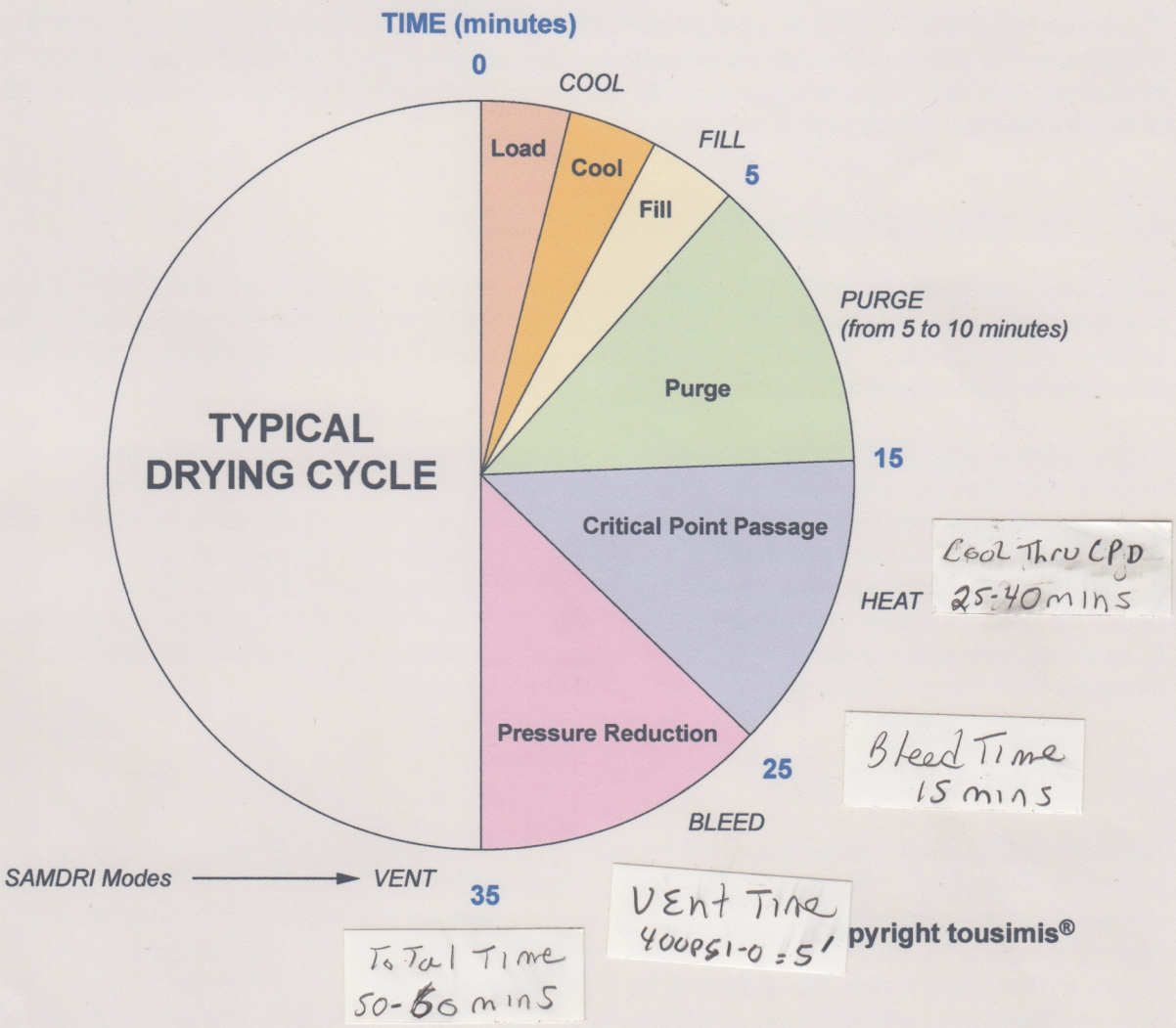


3.8 TYPICAL CYCLE

50-60 min's

A typical cycle, including 10 minutes of purge time, might run for approximately 35 minutes, as shown in Figure 3, and may use up to two pounds (~1.0kg) of LCO_2.

FIGURE 3



3.9 CARE OF DRIED SAMDRI® SAMPLES

After Samdri®-ing, care should be taken to prevent damage to the sample surfaces. SAMDRI®-ed biological samples are receptive to a metal coating in a vacuum and do not require any preparatory carbon coating. If the specimen cannot be further processed immediately after drying, it should be stored in an isolated and desiccated environment. After coating, specimens should be maintained in a dry environment to prevent changes as a result of water absorption or possible cracking of the coating layer.

SECTION IV

THEORY OF CRITICAL POINT DRYING

4.1 THE SAMDRI®-795

The tousimis® SAMDRI®-795 is a critical point drying apparatus for sample preparation in both the MEMS and the Electron Microscopy laboratory. Through a series of stages, the specimen is processed by a technique, which preserves and stabilizes the delicate three-dimensional structure of MEMS devices and biological tissues.

4.2 PRESERVATION OF HYDRATED MORPHOLOGY

It has been known for more than one hundred years that organic solvents could replace water in inorganic gels. It was subsequently shown that water could be similarly replaced in organic gels and biological materials. These observations form the basis for procedures involving dehydration and replacement with an embedding medium.

Critical point drying (SAMDRI®-ing): In order to preserve the three-dimensional structure, one must avoid the sudden (air-drying) change of densities that occurs when the fluid "embedding" the sample and permeating its ultra structure is converted to gas. Most liquids used to replace water in samples have a well-defined critical point -- a specific pressure, temperature and volume for each of them. At the critical point the densities of the gas and liquid phases of the suspension fluid are equal; therefore, a phase boundary does not exist. After the critical point transition from the liquid to vapor phase has taken place, the vapor phase can be removed (while above atmospheric pressure) and the dried sample preserved without structural damages.

4.3 SAMDRI®-ING* METHOD

SAMDRI-ING involves immersion of the sample in an intermediate fluid [an alcohol] and its substitution with LCO_2, under pressure. The LCO_2 is automatically converted to its gaseous phase at the critical point in the high-pressure chamber and removed at temperatures higher than 31.1°C, thereby avoiding the distortion resulting from evaporation boundaries or thermal expansion. Figure 4 (See p.15) shows the isotherms for CO_2, including the critical isotherm at 31.1°C. Superimposed is a path which an average critical drying cycle (SAMDRI®) can follow, divided here into four stages:

FIRST, the specimen and chamber are COOLED and FILLED with liquid CO_2 (LCO_2), and maintained between 10°C and 0°C;

SECOND, the sample in the alcohol under pressure is PURGED of the alcohol with cold LCO_2 until all traces of intermediate fluid [alcohol] are removed.

*SAMDRY®-ING = Sample drying at the critical point.

4.4 SAMPLE PREPARATION BEFORE SAMDRI-ING

It is not the intention of this manual to present an extensive review of sample preparation techniques. Certain specimens present special problems in preparation and it is the responsibility of the investigator to determine the best procedures in each case. It is the general object of pre-critical point drying preparation steps to yield a sample in which the histological ultra structure is well stabilized and free of water.

Most biological sample preparation will include:

FIXATION - Fixation generally utilizes an aldehyde such as formaldehyde[2], glutaraldehyde[1], or acrolein[3] to provide rapid inter- and intra-cellular penetration and fixation, followed by osmium tetroxide for further fixation.

STAINING AND SPECIAL TECHNIQUES - A fixative/stain such as aqueous solutions of osmium tetroxide or silver is employed for SEM specimens prior to critical point drying.

DEHYDRATION - The fixed specimen is taken through a series of increasing concentrations of a liquid such as ethanol, ending in a 100% ethanol of the highest possible purity. Transfer to room temperature before transferring it in alcohol in the SAMDRI®-795 chamber, and seal. Then proceed with cooling the chamber.

4.5 WATER EXCLUSION

Any water or oils remaining on the specimen during critical point drying will not pass through the critical point with the transitional fluid; it will instead adhere onto the specimen surface, causing damage, or remain as a coating on the surface (oils), rendering sputtering or shadowcasting with a conducting element or alloy impossible. Therefore, the sample is first completely dehydrated, as mentioned above, with adequate precautions taken to avoid subsequent re-hydration from the possible water contaminated chamber.

Dehydration involves three conditions for the 100% dehydrating liquid. One is that this liquid should fill the vessel containing the specimen, in order to reduce contact with atmospheric water outside the SAMDRI®. The vessel should then be tightly stoppered. The last condition for the 100% fluid is that it be obtained from a newly opened bottle of ultra-pure ethanol or acetone.

Since 100% dehydration fluids are extremely hygroscopic, the specimen should be in the proper carrier to be accommodated in the SAMDRI® chamber previous to the final dehydration bath, so that it can be transferred quickly and without further manipulation.

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[2]High purity or ultra-pure fixatives are recommended.

[3]Acrolein is a very hazardous chemical. Avoid its use unless there is no other alternative, and then use as per special instructions in your laboratory and after careful reading of the MSDS.

4.6 TRANSITIONAL FLUID (CO_2)

LCO_2 filtered for oil, water and particulate matter (down to 0.5 µm) is the preferred transitional fluid in critical point drying, since it is easier to use, more economical, less noxious, safe in a well-ventilated laboratory, and provides more consistent results than any other transitional fluid. Only research grade or filtered (see tousimis® combination filter, catalog #8784) "bone dry" LCO_2 should be used. Use of LCO_2 requires a "dip" or syphon tube in the CO_2 cylinder tank, which provides liquid at the tank outlet. Tanks should be kept at room temperature for at least 2-3 days prior to use in case they are frozen. A ceramic heater is the recommended heat source to be used on the LCO_2 tank, if you do not have time to equilibrate the tank to room temperature.

Your local gas distributor ships LCO2 in steel cylinders to in your laboratory. Please note the tare weight (tw) and gross weight. You can only use about 50% of the net LCO_2 since the dip tube does not reach the bottom of the tank. Advise your supplier to *never* wash the empty steel cylinder with water or leave it "in the yard" to accumulate pollutants. Special cleaning of the CO_2 dip tube/steel cylinder should be requested and designed for your institution or specifically for your laboratory. ALWAYS KEEP AN EXTRA FULL TANK IN RESERVE AT ROOM TEMPERATURE.

As you receive the CO_2, it is under pressure typically between 750 to 900 psi depending on the tank temperature. While CO_2 is a respiratory stimulant, humans cannot breathe air containing more than 10% carbon dioxide (at this level one could faint). Use the SAMDRI®-795 in a well-ventilated room, or exhaust all CO_2 into a working hood or the outdoors. Please consult your laboratory regulations for exhaust requirements. Usually one to two pounds of LCO_2 is used for each critical point run. The critical temperature and pressure of pure CO_2 are 31.3°C (88.84°F) and 72.9 atm (1072 psi), respectively.