CRITICAL POINT DRYERS
THE CPD PROCESS

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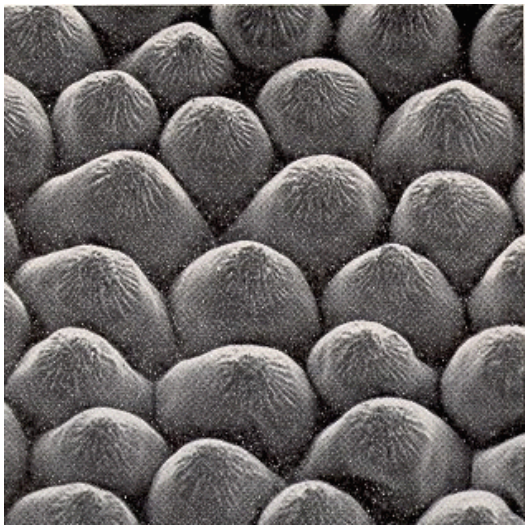
SPI-DRY™ Critical Point Dryers

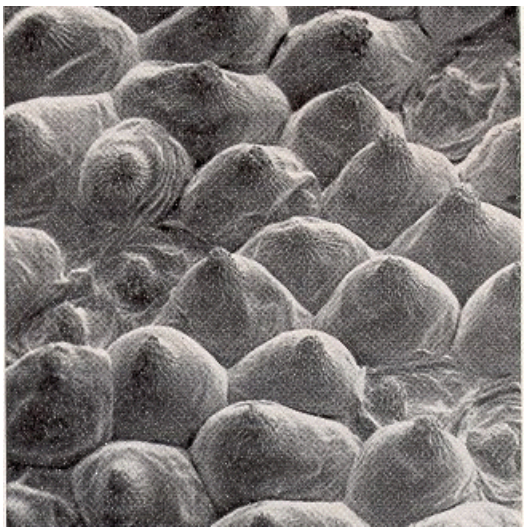
An Introduction to Critical Point Drying

The Technique

Critical point drying (CPD) is a method of drying tissue without collapsing or deforming the structure of wet, fragile specimens, generally as part of the sample preparation process for scanning electron microscopy (SEM). Although it has some applications in transmission electron microscopy (TEM), its major application, at least up until now, has been in tissue preparation for SEM. It is well known that allowing tissue to dry in air or under vacuum (during the metallization process, for example) causes damage to the surface which one wishes to examine in the SEM. An example is shown in the micrographs shown below:



SEM image (850X) of rose petal surface, CPD



SEM image (850X) of rose petal surface, fixed and air dried

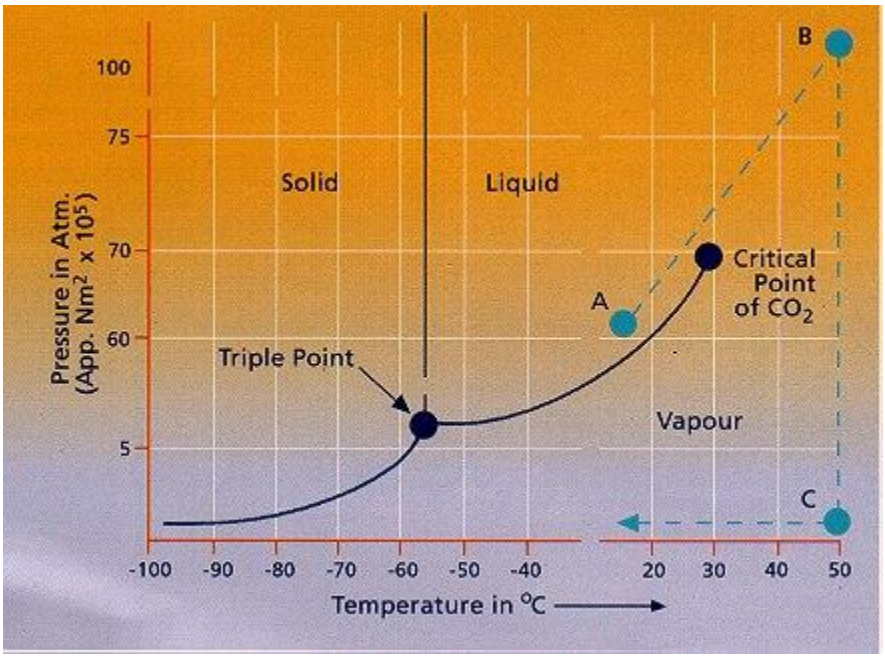
In some cases, this deformation is acceptable during routine experiments, but for the most part, it is not. At one time, researchers tried to "turn the other way" and pretend these drying artifacts did not exist. But today's researchers, pretty much worldwide, with their new high resolution SEMs, will not tolerate drying artifacts. And therefore, there is a universal acceptance of the need for a CPD unit is virtually all life science laboratories and even in some circumstances, depending on the nature of the samples being examined, a growing number of materials science laboratories.

Explanation of drying artifacts

The reason why tissue samples became damaged by normal air drying is that very large surface tension forces are created in cavities of small dimensions when there is a liquid/gas interface. As tissue dries, the liquid/gas interface travels through the surface of the material collapsing the cavities between projecting structures. In the case of delicate liquid- containing samples, which become hollow when dried, complete collapse often results. The critical point drying method of drying avoids these effects by never allowing a liquid/gas interface to develop; in this way the tissue is not exposed to surface tension forces.

The critical point:

The critical point of a liquid/gas system (e.g. water/steam, liquid CO_2/CO_2gas) is its critical temperature and the pressure associated with this temperature, that is, it is a point T_c, P_c smaller on the T,P phase diagram. Above the critical temperature, T_c the system is always gaseous and cannot be liquefied by the application of pressure. The transition from liquid to gas at the critical point takes place without an interface because the densities of liquid and gas are equal at this point. If tissue is totally immersed in a liquid below its critical point and the liquid is then taken to a temperature and pressure above the critical point it is then immersed in gas (i. e. dried) without being exposed to the damaging surface tension forces.



In order to carry out this procedure at a convenient temperature and pressure, it is normal to replace the water (which has a very high critical point) with some other liquid before carrying out the drying. More than one substitution is usually necessary if the final liquid is not miscible with water, e.g. if the final liquid is liquid carbon dioxide, the water in the tissue is first replaced with acetone and then the acetone is replaced with liquid CO_2.

Fixation with glutaraldehyde and osmium tetroxide followed by the substitution of acetone or Freon 113 is carried out before transferring the tissue to the critical point drying apparatus. Final substitution with liquid CO_2 and the drying run are carried out inside the apparatus. After the drying run, the pressure is released and the dried tissue can then be metallized before being inserted into the SEM for observation and imaging. Usually, the metal used is gold and this is done in a sputter coater or osmium coater.

The comparison micrographs shown above shows an example of tissue which has been fixed in gluteraldehyde and osmium tetroxide, substituted with ethanol, and finally critical point dried from liquid CO_2. The difference between the two micrographs shows the obvious advantages of using the technique of critical point drying on these kinds of samples.

The Apparatus Itself

The main body of the apparatus is a pressure vessel with integral water jacket for heating and cooling. The normal operating range of the pressure chamber is 0-2000 psi and 10-50°C. As can be seen in the photo of the unit, one can see various control valves, a thermometer, a pressure gauge and a support stand are all attached to the vessel. At one end of the cylindrical chamber is a demountable window for viewing the process and in the opposite end a removable access door for the specimen holder. The viewing window is an indispensable part of the design of the SPI CPD unit because it is so important to make sure that there is no turbulence when the CO_2 is being run through the unit.

There are four pressure control valves. Built into the support column is an over-pressure safety valve, sometimes called also a rupture disc, set at 2000 psi. Should this pressure be exceeded by overheating the chamber, the value opens and reduces the pressure to ambient. This rupture disc must be replaced before the unit can be used further.

The manual value at the top rear of the body is used for admitting the liquid gas to the chamber. A transfer pipe with couplings is provided for connecting the apparatus to a suitable siphon cylinder. The manual valve at the top front of the body is used for venting trapped air when filling the chamber. The valve at the bottom rear of the body is used for draining transfer fluid after filling with liquid gas.

Both the vent and drain valves are used for causing a thorough mixing action when substituting the transfer fluid with the liquid gas. It is important that all transfer fluid be removed from the tissue and flushed from the chamber if efficient drying is to be carried out. Turbulence and flushing are achieved by opening the inlet and drain (or vent) valves simultaneously.

Care must be taken to ensure that the tissue remains below the level of the liquid during this operation. And one must avoid turbulence if their samples are especially fragile in order to avoid artifacts and other damage.

The tissue holder consists of a boat shaped liquid holder in which are placed tissue baskets with lids. There is an automatic drain in the boat which acts when the access door is closed with the holder inside the chamber. This design fulfills two requirements: (a) that the tissue remains wet during transfer to the apparatus and (b) that the transfer liquid can be totally removed before the drying run is started.

After replacing the transfer liquid (e.g. acetone, ethanol) with the critical point drying liquid (e.g. liquid CO_2) the drying run can be started. All the valves are closed and hot water is circulated through the water jacket. In the case of liquid CO_2 raising the chamber temperature to 32°C causes a pressure rise from about 800 psi to about 1150 psi. At this point, the liquid/gas meniscus becomes diffuse and then disappears. The chamber now contains only gas. The vent valve can be opened slightly and the gas bled off to leave dry tissue. To ensure that recondensation of the liquid does not occur, we recommend that the temperature should be taken at least 5 C° above the critical point.

Safety precautions

Should any pressure leaks develop during the use of the apparatus, these are easily fixed as nearly all the seals are standard EPDM O-rings or bonded seals. For use with aggressive solvents and acetone, we recommend the use of special EPDM bonded seals. They cost a bit more but they can be expected to last longer as well.

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