

HMDS and Specimen Drying for SEM:

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Hexamethyldisilazane (HMDS) is an excellent method of chemical drying of hydrated specimens. There are several variables involved in its use, the most easily controlled being the number of transitional steps from 100% ethanol (EtOH) to 100% HMDS and the drying temperature.

Fixation and dehydration are the same for both HMDS and CPD. Once the specimen is in the final 100% ethanol, it must then be transferred to 100% HMDS through a graded series of ethanol-HMDS mixtures. This can follow one of four basic paths:

Ratio absEtOH : HMDS starting from 100% EtOH going to 100% HMDS

- 1) 100% E => 1:1 => 100% H
- 2) 100% E => 2:1 => 1:2 => 100% H
- 3) 100% E => 2:1 => 1:1 => 1:2 => 100% H
- 4) 100% E => 3:1 => 1:1 => 1:3 => 100% H

(Extra gradations may be added as needed, for instance between the 3:1 - 1:1 and 1:1 - 1:3 steps)

After the final transitional step, make 3 changes in HMDS (the last two steps can sometimes be skipped). Dry from the last 100% HMDS step, or exchange with new 100% HMDS one final time then dry. There should be just enough HMDS in the container to cover the specimen, any more just increases the drying time.

Time in these steps will usually be the same as that used in the final 100% EtOH steps. However, the time can apparently be extended with little ill effect on the sample. Incomplete transition from EtOH to HMDS is a worse source of problems than extended time in HMDS.

Choice of steps is basically determined by sample density, and the permeability (to HMDS and ethanol) of the least permeable structures in the sample. Microorganisms can usually be done with the first series, animal

tissues need the second or fourth series, and plants require the fourth series, or even more gradations because of their cell walls.

Drying is done at either:

25° C = room temperature 8 hr => overnight

37° C }

45° C } 1=>4 hr

60° C }

(Drying time by both temperature and volume of fluid.)

The higher the temperature, the shorter the drying time, *but* the quality of results may also vary. Microscopic unicellular algae did best at 60° C, fish skin at room temperature, bacteria equally well at room temperature and 60° C.

HMDS may have a significant time advantage over CPD. If more specimens are being processed that can fit in the CPD chamber, then the times in the transitional fluids and for drying will be much less than the time necessary for CPD. The greater the number of samples that can be batch processed, the greater the time advantage for HMDS.

HMDS has another advantage: *if* you can find containers that seal tightly enough (HMDS likes to evaporate given any chance at all), samples can be collected in the field, processed to 100% HMDS, then stored in vials filled with HMDS and transported long distances from remote sites - like from Antarctica to Chicago, Illinois. The samples are protected by the fluid, and at least for some specimens, so fewer artifacts than specimens stored in fixatives or alcohol. Dried specimens are of course fragile.

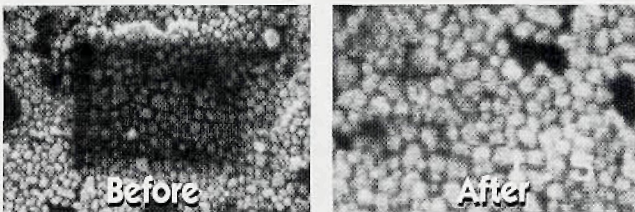
HMDS is not the cure-all for specimen drying. It can introduce its own distorting artifacts and some samples, biological ones in particular, still shrink after drying as they do with CPD or freeze-drying. Some specimens do poorly when dried from HMDS. Plant tissues in particular may not do well, and may be better off dried by CPD. Also, if the specimen is going to be studied for elements that are labile, or in solution, standard fixation and dehydration methods won't work. *Cryo techniques must be used, and if the specimen is to be examined in an unfrozen state, for example to look at structures and elements within cavities that would be obscured by ice if left hydrated, then the specimen must be dried by freeze-drying methods.*

All of this information is empirical. Theoretical explanations and any modifications for particular samples are welcome!

A final note: HMDS *must* be used in a fume hood! A sniff of it will clear the sinuses back to the *foramen magnum*. ■

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