

# Use of the Lyophilizer

by Scott Hart

## I. Description and Purpose

The Labconco Lyophilizer is a freeze-dry system used to remove solvent from frozen samples, typically collected HPLC fractions, but essentially any mostly aqueous or DMSO solution.

For amounts that will fit in a few Eppendorf tubes, it is probably more efficient to use the Speed-Vacs. The lyophilizer is ideal for amounts that fit more conveniently in 15 or 50 mL Corning tubes (plastic) or in any glass round bottom flask.

### A. How does freeze-drying work?

Lyophilization works via sublimation. The sample therefore must be frozen throughout the process. Samples that melt while on the system will bump, splatter, and otherwise make a mess of your vial/flask as well as the system itself. If your sample does melt, you should remove it promptly and deal with the problem (see below).

### B. Operation

The lyophilizer is maintained by the lyophilizer Czar. Other users should not need to worry about defrosting the system or changing pump oil. The RC5 Hybrid vacuum pump on this system is very durable and will not need a regular oil change. But the pump should NOT be shut down, as this defeats the mechanism by which the pump continually clears itself of 'inhaled' organics and water. The lyophilizer Czar will shut the pump off for short times while defrosting the drying chamber (without allowing the oil to cool significantly), but any other need to shut the pump off should be cleared by the Czar.

#### *1. To add a sample*

First confirm that the temperature is below  $-40^{\circ}\text{C}$  and the pressure is AT LEAST registering a value on the control panel. Ideally, samples should only be added when the green indicator light is blinking or solid. In situations where many samples have been added to the system, this may be impractical, as the vacuum in the system may not be capable of getting this low. In these cases, as long as the vacuum has stabilized, it should be okay to add your sample. If the control panel does not show a numerical pressure (i.e.,  $100 \times 10^{-3}$  mbar), but instead reads "HI", do not add samples, as the vacuum is not sufficient for lyophilization.

Add your sample (pre-frozen on dry ice) by connecting either your flask or the Labconco container containing your vials to the chamber with the appropriate fittings. To open your sample to the vacuum system, turn the grey knob **slowly**  $180^{\circ}$ . The 'vent' position (flask closed to system) is when the flat portion of the grey knob is lined up with the hole in the black seal. Turning the grey knob  $180^{\circ}$  opens the flask to the vacuum chamber. At this point any residual liquid in your sample may bubble or otherwise cause your sample to shift in your flask. After you have opened the flask to the vacuum chamber, observe the sample a few moments to ensure that any shifting or bubbling does not upset your sample too much, and to make sure your sample stays solid initially.

## 2. To remove your sample

Reverse the process outlined above by turning the grey knob 180° to the 'vent' position. Beware that turning the grey knob to the vent position (flat surface lined up with the hole in the seal) will allow room air into your sample flask. This flask is under high vacuum, and the air will rush in very vigorously. Take care to turn the knob **slowly**, or your dry sample will blow around in your flask, possibly flying *out* of your flask. Many a sample has been violently blown into the drying chamber in this way.

## C. Problems/Fixes

For efficient lyophilization, your sample *must* be frozen. Unlike the Speed-Vac, the lyophilizer will not reduce liquid samples in a desirable way. If you attempt to dry a wet sample, you will learn why the Speed-Vac uses a centrifuge system (and you will never again take the fact that your sample stays *inside* your flask for granted).

### 1. If your sample thaws prematurely, or will not freeze:

- ⌚ Refreeze your sample more thoroughly,
- ⌚ Use the rotovap to remove some of the organic solvent (acetonitrile is often removed in this way... beware of foaming peptide-water-acetonitrile solutions while rotovaping),
- ⌚ Add more water, since high concentrations of organic solvents (CH<sub>3</sub>CN, MeOH, etc.) or salts will hasten thawing of the sample, and/or
- ⌚ Use a drying flask that allows a higher surface area of the frozen sample, with the hope that the sample will sublime prior to warming enough to melt the mixture (this is a last resort that will work less than half the time).

In cases where you use a round bottom flask directly attached to the system, **it is normal for frost to form on the outside of the flask**. This will also occur with the Labconco glassware if the contents (vials, etc.) are in contact with the outer glass container. Keep in mind that this frost melts, so you may want to place a paper towel under the flask to absorb the water.