Liquid Ethane for Rapid Freezing of Biological Materials
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Liquid ethane is an effective tool for use in the rapid freezing of biological materials. The method described for collecting liquid ethane was first developed in the Somlyo Laboratory, and has been used routinely in our experiments for rapid freezing of contracting cardiac muscle or isolated HL60 (promyelocytic leukemia) cells. Rapid freezing of cells or tissues in this manner limits redistribution of diffusible elements across membranes, a critical factor when using these materials in electron probe microanalysis studies. Ultrapure (>99%) ethane gas is chosen to prevent contamination of biological samples.

Tygon tubing is connected at one end to an ethane tank regulator and at the other end. The copper coil is placed in a 400 mL plastic beaker in a styrofoam box in a fume hood. The ultrapure ethane gas is turned on at the regulator to a pressure of 18 to 20 psi and liquid nitrogen is poured around the coil. As the copper and ethane start to get cold, the ethane will begin to liquefy and collect in the beaker. At this point the pressure of the gas should be turned down to about 8 to 10 psi to prevent splashing of the ethane. It takes about 2 to 3 minutes for the ethane to start to liquefy, and another 5 to 7 minutes to collect 200 to 300 mL.

Extreme caution must be exercised when working with liquid ethane. Beside the obvious (it’s extremely cold and will produce severe burns rather quickly), it is volatile if it comes in contact with liquid nitrogen. Thus, when a beaker of liquid ethane is immersed in a box containing liquid nitrogen, the potential for injury cannot be overstated. Hand (and preferably forearm) was well as eye protection is essential. For disposal, we simply leave the beaker containing the liquid ethane in a corner of a fume hood to evaporate (with a note nearby indicating that it is ethane and very cold!). It only takes about an hour for 300 mL of liquid ethane to completely dissipate. A conversation with our chemical safety officer and examination of the Material Safety Data Sheets for ethane and propane offer no conclusive evidence that one is “safer” than the other. They’re both highly flammable and quick to ignite, so I guess there really is no logical reason to use one over the other from a safety standpoint. I was also told by the same safety officer that our hoods are efficient enough to remove ethane vapor as it evaporates and that there is no chance of the vapor coming in contact with any source of spark which might ignite it. Other fume hoods may be different, and this should be checked.

I posed the question of why one might choose to use ethane instead of propane for rapid freezing of biological specimens to a colleague at NIH who has also been using ethane for cryo work for many years. Quoting from Dr. Brian Andrews, “I can think of at least two reasons to prefer ethane over propane, specifically for applications involving cryosectioning or cryo-electron microscopy. For these techniques, the temperature of the specimen should never rise above about -160°C, in which case propane will remain as a slushy liquid covering the specimen. This slushy stuff plays havoc with imaging and with sectioning. Ethane, on the other hand, vaporizes at these temperatures in vacuo, so the specimen stays ‘dry.’”

References:

Figure 1: Equipment set-up for making liquid ethane, topview.