and remains in the controlled room for three days. At that time the desiccator vacuum is released by allowing air to enter after passing through three gas-washing bottles filled with concentrated sulfuric acid, which removes water from the air. This process takes approximately 1.5 hours. The desiccator is then reopened, and the weighing bottles are capped and reweighed. The loss in sample weight divided by the initial sample weight and multiplied by 100 yields the percent moisture (wt/wt) in the original sample.

### *Karl Fischer Method*

Iodine in the presence of pyridine, sulfur dioxide, and methanol reacts quantitatively with water. Karl Fischer (50) developed this quantitative method for water determination (51) in 1935. In the coulometric method iodine is electrically generated at the surface of the electrode emersed in pyridine, sulfur dioxide, and methanol to react with water. Coulometric Karl Fischer measurements (52) are conducted in a Plexiglas glove box that is located in a chemical fume hood. A low relative humidity is maintained in the glove box with anhydrous phosphorus pentoxide. The relative humidity is monitored by a portable hygrometer. The Karl Fischer instrument (Aquatest 8 Coulometric Moisture Analysis System, Photovolt, Indianapolis, Indiana, U.S.) is placed on top of the dry box (Fig. 5) to minimize corrosion of the electrical wiring. Custom elongated wires (Photovolt) connect the titration vessel inside the dry box to the instrument outside through rubber-stoppered ports in the Plexiglas. Samples are vortexed to render the freeze-dried cake into a powder; vials are scraped free of labels and glue. The vial is placed inside the dry box at approximately 15% to 20% relative humidity, and the vial's vacuum, if present, is released by quickly opening and then closing the stopper. Not releasing the vacuum could cause a significant erroneous weight due to buoyancy when the vial or ampoule contains vacuum

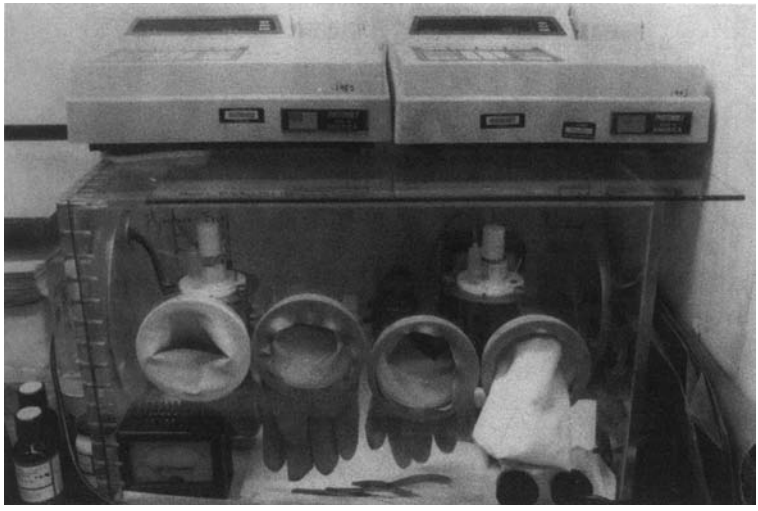

**FIGURE 5** Karl Fischer dry box configuration with coulometric processing unit outside and titration vessels inside the dry box. Elongated wires connect the instrument to electrodes in the titration vessel in the dry box. Unit on the right uses pyridine solvent; unit on the left uses pyridine-free solvent.