The *sublimation phase or primary drying* will follow when the frozen material, placed under vacuum, is progressively heated to deliver enough energy for the ice to sublimate. During this very critical period a correct balance has to be adjusted between heat input (heat transfer) and water sublimation (mass transfer) so that drying can proceed without inducing adverse reactions in the frozen material such as back melting, puffing, or collapse. A continuous and precise adjustment of the operating pressure is then compulsory to link the heat input to the "evaporative possibilities" of the frozen material.

The *desorption phase or secondary drying* starts when ice has been distilled away and that a higher vacuum allows the progressive extraction of bound water at above-zero temperatures. This, again, is not an easy task since overdrying might be as bad as underdrying. For each product an appropriate residual moisture has to be reached under given temperatures and pressures.

Final conditioning and storage begins with the extraction of the product from the equipment. During this operation great care has to be taken not to lose the refined qualities that have been achieved during the preceding steps. Thus, for vials, stoppering under vacuum or neutral gas within the chamber is of current practice. For products in bulk or in ampoules, extraction might be done in a tight gas chamber or an isolator by remote operation. Water, oxygen, light, and contaminants are all important threats and need to be monitored and controlled.

Ultimate storage has to be carried according to the specific "sensitivities" of the products (at room temperature, $+4^{\circ}C$, $-20^{\circ}C$). Again uncontrolled exposures to water vapor, oxygen (air), light, excess heat, or nonsterile environment are major factors to be considered. In that context the composition and quality of the container itself, (type of glass, elastomers of the stoppers, plastic, or organic membranes) have to be considered.

At the end, we find the *reconstitution phase*. This can be done in many different ways with water, balanced salt solutions, or solvents either to restore the concentration of the initial product or to reach a more concentrated or diluted product. For surgical grafts or wound dressings, special procedures might be requested. It is also possible to use the product as such, in its dry state, in a subsequent solvent extraction process when very dilute biochemicals have to be isolated from a large hydrated mass, as this is the case for marine invertebrates.

Figure 1 summarizes the freeze-drying cycle and indicates for each step the different limits that have to be taken into consideration. Figure 2 gives an example of a typical freeze-drying cycle.

INSIGHT INTO THE BEHAVIOR OF PRODUCTS AT LOW TEMPERATURE

A Sensitive Issue: The Freezing Step

This initial operation in the freeze-drying process is, of course, a critical one since, if the product is impaired ab initio, there is obviously no interest to go further. We will see in the following paragraph what are the governing parameters, thermal, electric, and structural, but before it is worthwhile to make a few comments on the way freezing is performed.