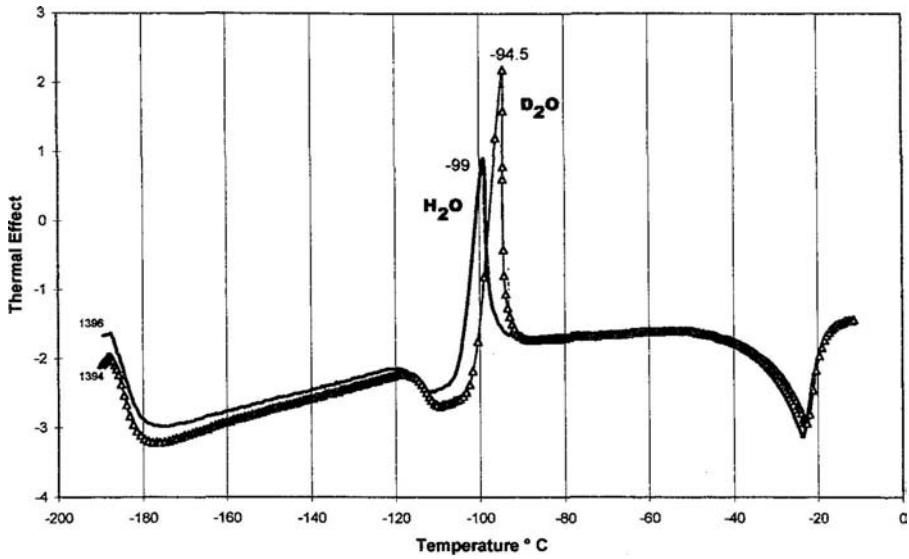

FIGURE 12 Differential thermal analysis of 50:50/100 mixtures of glycerol and D_2O/H_2O. Both normal and "heavy" water-glycerol mixtures demonstrate the same behavior but the transition temperatures for D_2O are shifted upward by 4.5°C.

secure a steady and successful freeze-drying operation. In the example depicted in Figure 13A and B, it was discovered that a refined biochemical (recombinant glycoprotein-Mol-2 from the Center for Molecular Immunology in Havana, Cuba) needed to be stabilized by thermal treatment to raise the softening temperature (as witnessed by the evolution of the electric impedance) from −32°C to −19°C and allow an easy freeze-drying.

In other instances—as this will be developed later in this book by several authors (Randolph, Searles, Carpenter, and Pikal)—it appears essential to safeguard the glassy state, which is indeed an absolute prerequisite to secure the tertiary structure of active proteins. Here again a precise knowledge of the boundaries and sensitivities of this glass is definitely needed.

Finally, let us mention the interesting case of the sucrose/water systems, which after drying gives a very sensitive porous cake susceptible to undergoing adverse transformations at *above-zero* temperatures and *in the dry state* because of the existence of a rather high T_g (57°C). It is, thus, compulsory to keep these products at temperatures low enough to prevent the shrinkage of the pellet by what has been called a "rubber behavior."

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This is, indeed, the heart of the lyophilization process: the step during which the frozen product is dried—from a solid phase—by direct sublimation of ice. During that phase the drying boundary sinks into the frozen material from top to bottom or else from outside to inside leaving an upper layer or surrounding shell of fine porous material. The resulting dry matrix is thus carved from the frozen mass under vacuum. To achieve this operation this frozen mass has to keep a structure when ice disappears and water vapor moves away. To that end,