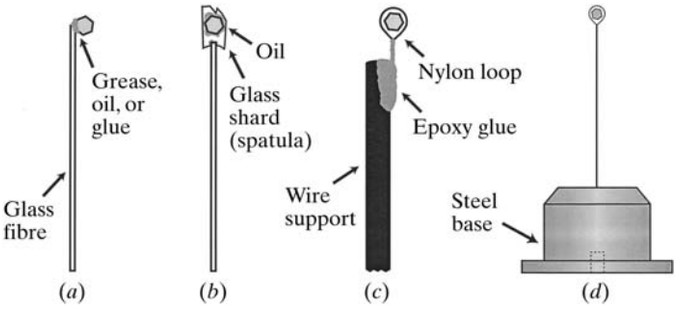

Figure 10.2.3.1
Different crystal mounts for flash cooling and cryogenic data collection. (*a*) Crystal mounted on a thin glass fibre with adhesive, grease, or oil. (*b*) Crystal placed in a hydrocarbon oil and then scooped onto a thin glass shard. (*c*) Crystal suspended in a film of aqueous solution within a nylon loop. The loop is attached to a thin (∼0.25 mm diameter) wire support. (*d*) A diagram of the entire loop-mount assembly. The base is made of plain steel or a magnetic alloy and has two holes, one for the wire post and one for a locating pin, which reproducibly positions the assembly on the goniometer.

stress and should result in a relatively compact sample that can be immersed in the narrow gas stream used to maintain the temperature during data collection. The glass capillary tubes conventionally used to mount macromolecular crystals are not well suited to flash-cooling procedures since they insulate the sample, reducing cooling rates, and their bulk interferes with cryogenic equipment. A number of alternative mounting methods used for flash cooling are shown in Fig. 10.2.3.1. Crystals can be affixed directly to thin glass fibres with cement or grease (Haas & Rossmann, 1970; Dewan & Tilton, 1987), or they can be scooped up on thin glass spatulas, a procedure first used in conjunction with the oil-coating method described in Section 10.2.2 (Hope, 1988). A loop-mounting technique introduced by Teng (1990) has proven the most generally applicable, however, and has become the method of choice. Here, the crystal is held suspended in a thin film of cryosolvent formed in a small loop. The technique is quick and straightforward, remarkably gentle to the crystal, and provides a large surface area for cooling.

The loops are generally formed from nylon fibre, although glass wool is useful for larger versions because its rigidity keeps them from collapsing under the surface tension of the suspended film. Both types of fibres should have a diameter of approximately 10 μm. This small cross section reduces absorption and scattering from the material itself and also minimizes the thickness of the film in the loop. Several methods of making the loops have been described in detail (Rodgers, 1997; Garman & Schneider, 1997), and nylon loops of different sizes are available

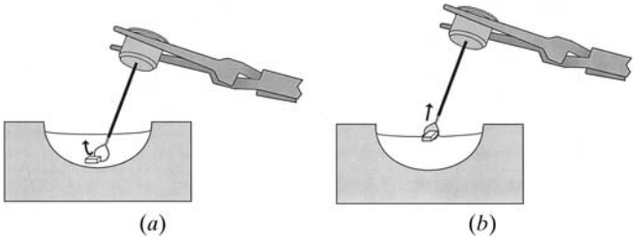

Figure 10.2.3.2
Mounting a crystal in a loop. (*a*) While viewing with a stereomicroscope, the crystal is teased to the surface of the liquid using the loop. (*b*) It is then drawn through the interface and into the loop. The sizes of the loop and crystal have been exaggerated. Reproduced with permission from Rodgers (1997). Copyright (1997) Academic Press.

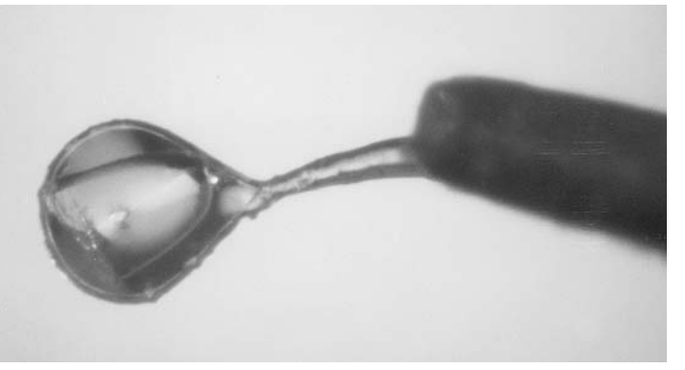

Figure 10.2.3.3
Photograph of a flash-cooled crystal mounted in a nylon loop. The wire post holding the loop is visible on the right. Reprinted from Rodgers (1994) with permission from Elsevier Science.

commercially. The loop is usually glued to a thin metal wire or other heat-conductive post. The ability to conduct heat rapidly is required to minimize ice formation at the point where the wire or post exits the cold gas stream of the cryostat, which occurs in some orientations of the loop assembly. This post is in turn attached to a steel base, which is used with the magnetic transfer system described below.

Crystals are placed in the loop as shown in Fig. 10.2.3.2. They can be mounted directly from the crystallization drop or after harvesting into any convenient container. Under a stereomicroscope, the crystal is teased to the surface of the solution, usually with the loop itself. Once at the surface, the crystal is carried through the interface by first resting it on the bottom of the loop and then moving the assembly vertically to pull it out of the solution. A practiced experimentalist can usually capture the crystal in the first few tries. The plane of the loop should be kept near the vertical to increase the chance of catching the crystal and to minimize the amount of liquid drawn up with it. An alternative technique is to use a small pipette to place the crystal and a drop of cryosolvent into the loop and then draw off the excess solution with filter paper. In either case, it can be difficult to form a film in

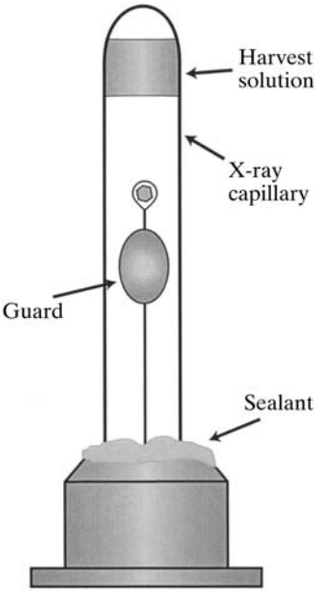

Figure 10.2.3.4
Arrangement for using the loop-mounting technique at non-cryogenic temperatures.