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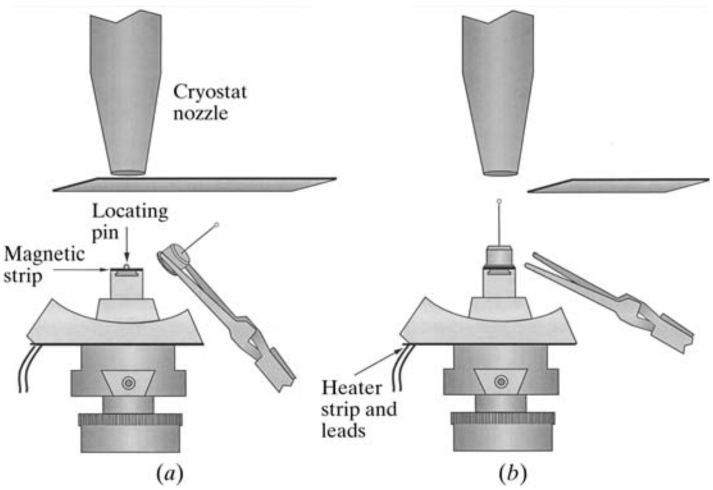

Fig. 10.2.4.1. Flash cooling a crystal in the cold gas stream from a cryostat. (*a*) The cold gas from the nozzle is blocked and the loop assembly placed on the goniometer. A locating pin on the goniometer ensures reproducible positioning of the loop assembly, which is held in place by a magnetic strip. (*b*) The gas stream is then unblocked to rapidly cool the crystal. A heating element in contact with the goniometer keeps it from icing during data collection. Based on a diagram by Rodgers (1997).

10.2.4. Flash cooling

Once mounted in the loop, the crystal must be cooled rapidly to prevent ice formation. A simple and often effective approach (see Hope, 1990; Teng, 1990) is to flash cool the sample in a cryostat gas stream (most frequently nitrogen, but also helium) right on the X-ray camera. This technique has the added advantage of leaving the crystal in position for immediate analysis and data collection. As shown in Fig. 10.2.4.1, the gas stream from the cryostat nozzle is temporarily deflected while the loop assembly is placed on the goniometer of the X-ray camera. The stream is then unblocked, allowing the cold gas to flow over the crystal. Deflecting the cold stream before placing the loop assembly eliminates the risk that the sample will cool slowly and form ice in the warmer outer layers of the gas stream. The arrangement of the cryostat nozzle shown in Fig. 10.2.4.1, with the gas stream coaxial to the loop assembly, is particularly effective. The cooling gas (usually at around 110 K for nitrogen cryostats) flows across both surfaces of the loop, maximizing the rate and evenness of cooling. Other orientations of the nozzle are frequently used, and in those cases the loop should be aligned with one edge pointing at the incoming gas. Note that a heating element, as shown in Fig. 10.2.4.1, is required to prevent icing of the goniometer with the nozzle in the coaxial position.

When handling the loop-mounted crystal before flash cooling, care must be taken to avoid drying the sample. The same characteristics that make the loop mount so effective for flash cooling, a large surface area and a small amount of surrounding solution, also promote a rapid loss of water and any other volatile component. The resulting change in solute concentration can damage the crystal or result in non-isomorphism between crystals. For this reason, every effort should be made to reduce the time required to flash cool the crystal after it is mounted. One key to avoiding delay when flash cooling in the cold stream is a rapid and reliable method of attaching the loop assembly to the goniometer. A magnetic mounting system (Fig. 10.2.4.1) developed by Rodgers (1994, 1997) is frequently used. Here, either a portion of flexible magnetic strip or solid magnet is affixed to the goniometer to hold the ferromagnetic base of the loop assembly. The base is positioned reproducibly by a small locating pin protruding from the goniometer, which mates with the centred hole in the loop base (Fig. 10.2.3.1*d*). A second pin or key can be used to specify the orientation of the loop assembly about its axis if necessary.

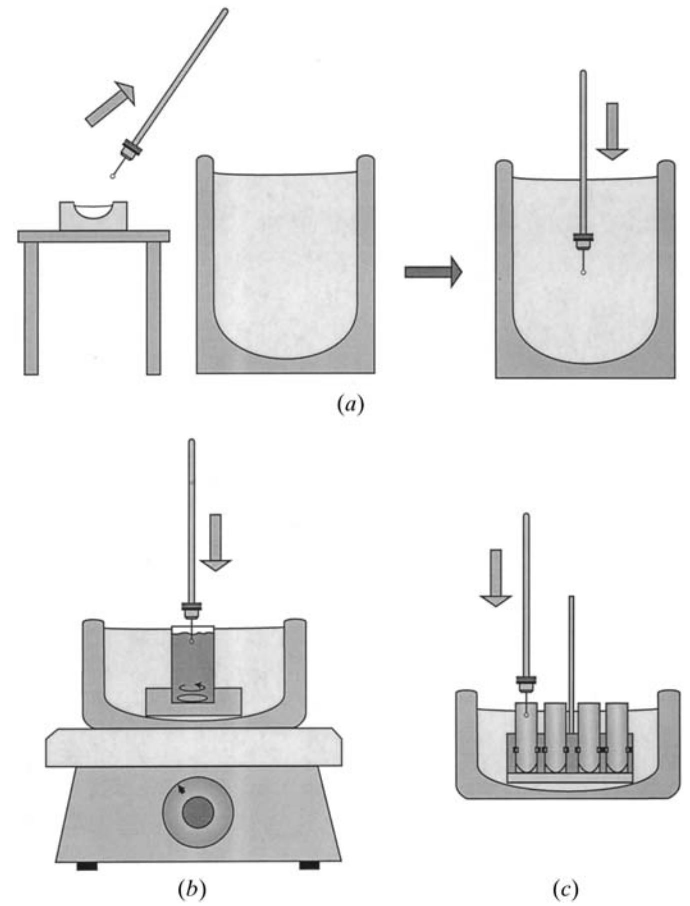

Fig. 10.2.4.2. Flash cooling in a liquid cryogen. (*a*) Cooling in liquid nitrogen. The loop assembly is attached, *via* a magnet mount, to a short rod and the crystal is captured. It is then quickly plunged into a nearby Dewar of liquid nitrogen. (*b*) One method of flash cooling in a liquid cryogen such as propane. The cryogen is placed in a weighted container, which itself stands in a Dewar of liquid nitrogen. The Dewar rests on a stir plate, which mixes the liquid cryogen to ensure a uniform temperature. When the temperature of the cryogen is just above its melting point, the loop assembly is plunged into the liquid. (*c*) A variation on cooling in propane or a similar cryogen. The cryogen is placed into small plastic vials designed for cryogenic storage. Just before the cryogen freezes, the loop assembly is plunged directly into a vial. A holder for the vials allows multiple samples to be prepared sequentially.

While flash cooling in the cold stream is convenient, an alternative method, rapidly plunging the crystal into a liquid cryogen, offers several advantages. This technique generally results in more even cooling of both sides of the loop-mounted sample, which may decrease damage due to thermal stress (Haas & Rossmann, 1970). It also reduces the time between mounting the crystal and flash cooling, and it can be used easily in any location – a cold room, for example. Another possible advantage of the liquid-cryogen method is that it produces a higher cooling rate than the cryostat gas stream, at least over much of the temperature range traversed during cooling (Walker *et al.*, 1998; Teng & Moffat, 1998). With increased cooling rates, the percentage of cryoprotectant necessary to prevent ice formation is lower, an advantage when benign cryoprotectant conditions prove difficult to find. Changes in solution dielectric or other parameters may also cause less damage. On the other hand, although cooling may be more even in a liquid cryogen, the overall increase in cooling rate could result in even greater thermal gradients, and therefore greater thermal stress, across the crystal. Systematic studies are needed to assess the effect of cooling rate on the quality of flash-cooled crystals, but in practice the liquid-cryogen technique has proven effective and is widely used.

Common cryogens for flash cooling are liquid nitrogen, propane, and, to a lesser extent, ethane and some types of Freon. (Another potentially useful cryogen, liquid helium, has not yet been explored for flash cooling macromolecular crystals.) There is some disagreement about relative cooling rates in liquid nitrogen *versus* liquid propane for samples the size of loop-mounted crystals (Walker *et al.*, 1998; Teng & Moffat, 1998), but both cryogens are known to work well for flash cooling. Since liquid nitrogen is simpler to use and safer than propane, it should be considered for initial trials with a new type of crystal. A diagram showing flash cooling with liquid nitrogen is presented in Fig. 10.2.4.2(*a*). The crystal is captured in the loop and quickly plunged into a Dewar filled with liquid nitrogen. Attaching the loop assembly to a short rod equipped with a magnetic mount allows it to be plunged deeply into the liquid nitrogen, which may increase the cooling rate by preventing the build-up of insulating gas around the crystal. To minimize drying of the sample during transfer, the crystal container and the Dewar are located as close as possible. If necessary, drying can be further reduced by using a portable humidifier to add moisture in the area.

Other cryogens, such as propane, can be tested if results with liquid nitrogen are not satisfactory. Two methods for flash cooling in these other cryogens are illustrated in Figs. 10.2.4.2(*b*) and (*c*). In the first (Fig. 10.2.4.2*b*), the liquid cryogen is held in a small container with a weighted base, which is placed in a Dewar of liquid nitrogen to cool the cryogen. The cryogenic liquid is mixed using a magnetic stir bar to ensure a uniform temperature throughout the sample. Since the boiling points of these cryogens are well above their melting points, it is possible in the absence of stirring to have relatively warm, and therefore less effective, cryogen near the top of the container. When a temperature probe indicates that the cryogen is just above its melting point, the crystal is mounted and plunged quickly into the liquid. A variant of this technique (Fig. 10.2.4.2*c*) calls for plunging the loop assembly directly into cryogen-filled plastic vials, which are used for low-temperature transfer and storage of the crystals (see Section 10.2.5). The cryogen is then allowed to solidify around the crystal before it is placed on the X-ray camera or stored for later use. With this technique, it is more difficult to ensure that the temperature of the cryogen is uniform throughout the container. Other mechanisms for flash cooling in liquid cryogens have been described (Hope *et al.*, 1989; Abdel-Meguid *et al.*, 1996), and devices for combining xenon derivatization with flash cooling (Soltis *et al.*, 1997) are available commercially.

10.2.5. Transfer and storage

Crystals flash cooled in a liquid cryogen must be placed for data collection in the cold gas stream of a cryostat without any substantial warming. One common transfer method (Rodgers, 1994, 1997) is shown in Fig. 10.2.5.1. Once the loop assembly has been plunged into the Dewar of liquid nitrogen, it is inserted into a small plastic vial of the type normally used for cryogenic storage, ensuring that the sample remains below the liquid surface during the operation. There is then sufficient liquid nitrogen in the vial to keep the sample cold as it is transferred to the cryostat gas stream. Again, the magnetic mounting system is used to reduce the time required for transfer. For X-ray cameras with vertical spindles, as shown in Fig. 10.2.5.1, some means of pointing the magnetic mount downward is required to prevent the nitrogen from spilling out of the vial. The goniometer illustrated has a detachable arc extension (Engel *et al.*, 1996; Litt *et al.*, 1998) that provides this capability. When the loop assembly is attached to the magnet, the vial is quickly withdrawn, exposing the crystal to the gas stream. The arc slide can then be returned to the normal position and the arc extension removed.

Another device (Mancia *et al.*, 1995) for achieving the correct transfer geometry is shown in Fig. 10.2.5.2. This 'flipper' mechanism can be extended to permit transfer of the crystal. The device is then rotated about the hinge to reorient the loop assembly for data collection. The hinge is positioned so that rotation does not translate the crystal, keeping it in the cold stream during reorientation.

When cooling in other liquid cryogens such as propane, the same cryovial transfer system is used. Flash cooling in cryovials (Fig. 10.2.4.2*c*) permits direct transfer using the magnetic mounting system. Usually, the liquid cryogen has been solidified in the vial, and it is allowed to melt at least partially before placing the crystal on the goniometer. Any remaining solid then melts and drips away (although it is often necessary to remove the last drop on the crystal with filter paper). When cooling in a larger volume of cryogen (Fig. 10.2.4.2*b*), the crystal can be 'hopped' rapidly from the cooling cryogen to the surrounding vat of liquid nitrogen. A drop of cryogen transfers with the crystal, keeping it from warming. The loop assembly can then be placed in a cryovial and transferred to the goniometer.

Another device that does not use cryovials has been introduced (Parkin & Hope, 1998) to facilitate transfer from liquid nitrogen. The device consists of a split metal cup attached to handles that allow the cup to be opened and closed. When closed, the two halves of the metal cup form a cavity that can accommodate and grasp the loop assembly. As shown in Fig. 10.2.5.3, the loop assembly is inserted after first cooling the tongs in liquid

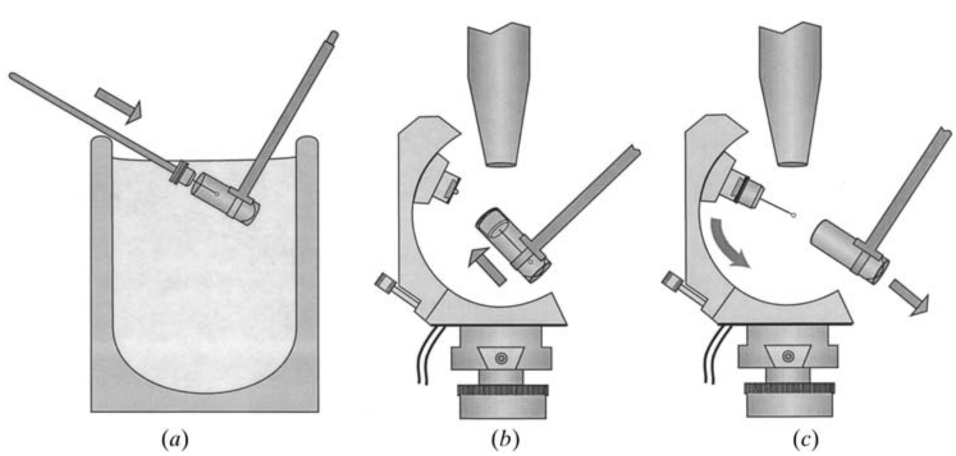

Fig. 10.2.5.1. Transfer of the flash-cooled crystal and loop assembly to a goniometer using a cryovial. (*a*) The loop assembly with a flash-frozen crystal is placed in the vial, which is held by a rod-shaped tool. The operation is carried out beneath the surface of the liquid nitrogen in a Dewar. (*b*) The loop assembly is transferred to the goniometer using the magnetic mounting system. (*c*) The vial is withdrawn, exposing the crystal to the cold gas stream. With this arrangement of goniometer and cryosystem nozzle, it is necessary to use a device that allows the magnetic mount to point downward. Here, a detachable arc extension provides this ability. After crystal transfer, the arc slide can be returned to the normal position and the extension removed.

references