Table 3.4.1.1. *Single-crystal and powder mounting, capillary tubes and other containers*

Material	Temperature range (K)	Comments
(*A*) Capillary tubes		
Glass Lindemann glass Vitreous silica	< 773 < 773 < 1373	Lindemann glass scatters less, but is moisture sensitive Thinner walled tubes that are less sensitive to atmospheric influences can be obtained using other types of glass
Collodion Polyvinyl methylal resin (*e.g.* Formvar) Cellulose acetate	93 to 343 < 323 < 373	These capillaries can be made by coating a copper wire with a solution of the polymer in an appropriate organic solvent. When dry, the metal core may be removed by stretching, to reduce its diameter
Polyethylene	< 373	Tubes may be drawn from the molten polymer using a glass tube and a slow stream of air. The polymer gives a distinct diffraction pattern
(B) Other containers		
Gelatin capsules	< 303	Vessels with very thin, 20 μm, windows can be made
Methyl methacrylate resin (*e.g.* Perspex)	< 338	
Mica	< 1073	Mica windows useful in vessels for small-angle scattering, but the wall size is generally thicker, ∼0.3 mm, and there are discrete lines at 10.00, 3.34 and 2.60 Å in the diffraction pattern
Regenerated cellulose film (*e.g.* cellophane)	Ambient	

For optimum results, tube diameters should be between 0.3 and 0.5 mm with wall thicknesses of 0.02 to 0.05 mm. The materials listed above, except where stated, give diffuse diffraction patterns. If necessary, control diffraction patterns, recorded only from the capillary or other container, should be taken.

(1993) have developed a mirror furnace working at up to 2300 K and suitable for polycrystalline or single-crystal samples.

A comprehensive account of cryogenic studies pertinent to both polycrystalline and single-crystal samples is given by Rudman (1976). Nieman, Evans, Heal & Powell (1984) have described a device for the preparation of low-temperature samples of noxious materials. The device is enclosed in a vanadium can and is therefore only suitable for neutron diffraction studies. Ihringer & Küster (1993) have described a cryostat for powder diffraction, temperature range 8–300 K, for use on a synchrotron-radiation beam line at HASYLAB, Germany (Arnold *et al.*, 1989).

3.4.1.3. *Single crystals (small molecules)*

3.4.1.3.1. *General*

Small single crystals of inorganic and organic materials, suitable for intensity data collection, are normally glued to the end of a glass or vitreous silica fibre, or capillary (Denne, 1971*b*; Stout & Jensen, 1968). A simple device that fits onto a conventional microscope stage to facilitate the procedure of cementing a single crystal to a glass fibre has been constructed by Bretherton & Kennard (1976). The support is in turn fixed to a metal pin that fits onto a goniometer head. For preliminary studies, plasticine or wax are useful fixatives, since it is then relatively easy to alter the orientation of the support, and hence the crystal, as required. For data-collection purposes, the support should be firmly fixed or glued to the goniometer head pin. The fibre should be sufficiently thin to minimize absorption effects but thick enough to form a rigid support. The length of the fibre is usually about 10 mm. Kennard (1994) has described a macroscope that allows specimens to be observed remotely during data collection and can also be used for measurement of crystal faces for absorption correction. Large specimens can be directly mounted onto a camera or onto a specially designed goniometer (Denne, 1971*a*; Shaham, 1982). A method using high-temperature diffusion to bond ductile single crystals to a metal backing, for strain-free mounting, has been described by Black, Burdette & Early (1986).

Prior to crystal mounting, it is always prudent to determine the nature of any spatial constraints that are applicable for the proposed experiment. Some diffractometers have relatively little translational flexibility, and the length of the fibre mount or capillary is critical. For some low-temperature devices where the cooling gas stream is coaxial with the specimen mount, the

Table 3.4.1.2. *Single-crystal mounting – adhesives*

Adhesive	Temperature range (K)	Comments
Durofix, Duco cement *etc.* (celluloid composition dissolved in organic solvent)	93 to 373	* Dries rapidly
Shellac dissolved in alcohol	<423	* Correct amount of solvent is critical
Fish glue (*e.g.* Seccotine)	<423	* Unsuitable for humid atmospheres
Dental cement	93 to 573	Adheres well to glass or asbestos, but not metals
Epoxy resin (epichlorohydrin, *e.g.* Araldite)	93 to 373	* Permanent fixing, fast (minutes) and slow (hours) available. 'Uncured' adhesive, *i.e.* minus hardener, useful for cryogenic mounting
Vacuum grease (*e.g.* Apiezon)	<473	Can protect crystal from moisture
Silicone high-vacuum grease	<373	Can protect crystal from moisture
Vaseline		Low temperatures down to liquid helium
Canada balsam	<333	† Dilute with xylene.
Mixture of wax and resin, ~1:1	93 to 303	†
Aluminium	<873	Large crystals set in molten metal, irradiate only protruding part of crystal
Aluminium cement	<1973	Irradiate only protruding part of crystal

* These glues tend to pull in setting and may require adjustment during the drying process. † Useful adhesives if the crystal requires grinding to shape after fixing.

orientation of the fibre (and crystal) on the goniometer head may also need careful alignment.

Many proprietary adhesives can be used (see Table 3.4.1.2), but it should be remembered that adhesives such as epoxy resins are often permanent, and attempts to dismount specimens lead to crystal damage. Some adhesives contain organic solvents that may react with the sample, and others may be X-ray sensitive and deteriorate with exposure. In low-temperature work, some adhesives shrink or become brittle. Ideally, the adhesive should have the same thermal characteristics as the crystal and its mount. An account of how strong stresses on adhesives, typically used to mount single crystals, induced by low and high temperatures is given by Argoud & Muller (1989*a*). The stresses appear to cause anisotropic modifications to secondary extinction, leading to discrepancies in the intensities of symmetry-related reflections. Beeswax and paraffin wax were found to be free from such stresses. Crystals that are sensitive to air can be mounted inside capillary tubes or other containers, as listed in Table 3.4.1.1. A useful summary of the methods available has been provided by Rao (1989). All adhesives and containers will give diffraction patterns, typically comprising diffuse bands, that contribute to the general background, and that may change with ageing. Minimal amounts of adhesive and thin-walled capillaries should be used. If the background diffraction is critical, it is highly recommended that diffraction patterns of the container and/or adhesive are recorded separately as controls.

The morphology of a given crystal will normally dictate the way that it is mounted, particularly for data-collection purposes. Thus, prismatic crystals and needle-shaped crystals are usually mounted with the longest dimension parallel to the fibre, in order to minimize systematic errors due to absorption. Jeffery (1971) and Wood, Tode & Welberry (1985) have described devices for shaping crystals into spheres and cylinders, respectively. A solvent lathe whereby a string moistened with solvent is used to shape the crystal is described by Stout & Jensen (1968).

3.4.1.3.2. *Non-ambient conditions*

As in the case of polycrystalline samples, a number of devices have been described to study single crystals at elevated pressures and at a range of temperatures. The mounting of the sample is very dependent on the device and radiation used. In recent years, the field of high-pressure crystallography has expanded significantly, and several sample holders based on the diamond-anvil cell have been reported for pressures up to 10 GPa. Recent papers include those by Alkire, Larson, Vergamini, Schirber & Morosin (1985) for neutron diffraction, and Malinowski (1987) and Leszczynski, Podlasin & Suski (1993) for X-ray diffraction.

Various types of furnace have been designed for high-temperature studies of single crystals. These are based either on radiative heat transfer mechanisms [*e.g.* Swanson & Prewitt (1986); temperatures up to 1400 K], electrically heated gas streams [*e.g.* Tsukimura, Sato-Sorensen & Ghose (1989); temperatures up to 1600 K], or flame heaters [*e.g.* Miyata, Ishizawa, Minato & Iwai (1979); temperatures up to 2600 K]. Furnaces specific to Weissenberg geometry (Adlhart, Tzafaras, Sueno, Jagodzinski & Huber, 1982) and Laue diffraction (Bhat, Clark, El Korashy & Roberts, 1990) have also been reported. There are many techniques available for mounting single crystals for high-temperature diffraction (Hazen & Finger, 1982), and a detailed account using an MgO-based ceramic cement is given by Swanson & Prewitt (1986). A recent paper by Peterson (1992) summarizes previous work in the design of high-temperature furnaces and describes a flame-heated gas-flow furnace operating in the range 373 to 1573 K. For this system, the crystals can be mounted either directly onto the thermocouple bead with a paste of fine platinum particles and oil, particularly useful if the crystal is to be exposed to a gas mixture that controls oxygen fugacity, or sealed under high vacuum in an ampoule made from 0.2 or 0.3 mm diameter silica capillary. In the latter case, the main support for the crystal is a 0.2 mm Pt wire threaded through a 0.3 mm diameter silica glass capillary. A 0.05 mm Pt/13Rh lead is welded to the end of this support to form the thermocouple bead. The wire is then wound around the outside of the capillary. A 0.05 mm Pt lead is welded to the other end of the 0.2 mm Pt wire and is threaded through a hole in the capillary near the base. The whole assembly can be mounted on a goniometer head that has only translational adjustments. For neutron diffraction, Lorenz, Neder, Marxreiter, Frey & Schneider (1993) have described a mirror furnace operating upto 2300 K. The sample support is normally a thin ceramic tube or rod of Al_2O_3 or ZrO_2 to which the sample may be glued with a ceramic cement. Neder, Frey & Schulz (1990) have described a versatile holder for high-temperature neutron studies. One part of the crystal is ground away to leave a stem, which is then fixed to an alumina rod with a ceramic glue based on zirconia. The ceramic glue is surrounded by a cylinder of BN to minimize spurious scattering.

A comprehensive account of low-temperature diffraction is given by Rudman (1976). Procedures for the selection and transfer of crystals to diffractometers have been described by Boese & Bläser (1989) and Kottke & Stalke (1993). These procedures are applicable down to temperatures of 213 and 193 K, respectively. The latter authors do not recommend the use of capillaries, but describe a device employing the oil-drop mounting technique pioneered by Hope (1987, 1988). Lippman & Rudman (1976) have used a mechanically refrigerated gas stream to achieve temperatures down to approximately 150 K, and the use of liquid nitrogen extends the range to 77 K. Devices such as the Oxford Cryostream can be readily fitted to diffractometers and other types of camera. Closed-cycle refrigerators, liquid-helium-based devices (*e.g.* Henriksen, Larsen & Rasmussen, 1986; Argoud & Muller, 1989*b*; Zobel & Luger, 1990; Graafsma, Sagerman & Coppens, 1991; Toyoshima, Hoya & Ohshima, 1991) further extend the low-temperature limit to 5 K, but often involve substantial blind regions and collision zones. For sample mounting in these devices, it is essential to have good heat conduction to the crystal. Zobel & Luger (1990) describe a taper-formed sample holder made of special copper screwed to the cold head. A steel injection needle with a Be wire inside (0.3 mm diameter and exactly 2 mm in length) is fitted into a 0.5 mm bore hole. The crystal is glued with Araldite to the Be needle, which has little X-ray absorption but good heat conduction. In addition to diffractometer-based devices, Moret & Dallé (1994) have described an adaptation of the closed-cycle refrigerator for a precession goniometer, and various authors have reported systems utilizing Weissenberg geometry for both X-rays and neutrons (*e.g.* Hohlwein & Wright, 1981; Aldhart & Huber, 1982; Allen *et al.*, 1982). Reference should be made to the individual papers for methods of mounting, including spatial and any other constraints.

3.4.1.4. *Single crystals of biological macromolecules at ambient temperatures*

Crystals of biological macromolecules are normally grown from an aqueous solution (see Subsection 3.1.1.2), and when growth is complete are in equilibrium with the mother liquor. Changes in this equilibrium may often result in crystal damage, so the most important aspect of crystal mounting in this case is to preserve the crystal in its state of hydration. This is most readily accomplished by sealing the crystal in a thin-walled quartz or glass capillary tube (King, 1954; Holmes & Blow, 1966). The crystal adheres to the inside of the tube by surface-tension effects through a small droplet of liquid, and a further pool of liquid at one end maintains the required degree of hydration. The general principles involved are well described by Rayment (1985). D'Aprile & Moretto (1975) have described two simple devices, a small electric heater for melting the wax used for sealing the capillary and a refrigerating microcell to prevent heat affecting the wet crystal, which are very useful for mounting wet single crystals in capillary tubes.

Alternatively, crystals can be grown directly within capillary tubes (Phillips, 1985) or microdialysis cells such as those described by Zeppezauer, Eklund & Zeppezauer (1968). A further mounting device particularly useful for enzymatic studies is the flow cell (Wyckoff *et al.*, 1967), in which the specimen is immobilized while mother liquor, or buffer with substrates or inhibitors, is allowed to flow over the crystal. A useful account of this device is given by Petsko (1985). More recently, Edwards (1993) has described a yokeless flow cell, which uses a plastic cone fixed to a brass mounting pin with a wire harness to support the quartz capillary. Although the device was originally designed for Laue studies, its simplicity and practicality should make it useful for a wide range of diffraction experiments. Pickford, Garman, Jones & Stuart (1993) have designed a mounting cell that allows the humidity around a protein crystal to be varied in a controlled manner. This may be particularly useful for crystals where the solvent content is high and the molecular packing, and hence the diffraction intensities, highly dependent on the precise amount of solvent present.

The relatively short crystal lifetimes and large volumes of intensity data often dictate that crystals of biological macromolecules be mounted so that data collection can be accomplished in the most efficient manner, for example, with a symmetry axis parallel to the rotation axis of the collection device. Samples crystallizing in the form of thin plates that have to be aligned perpendicular to the capillary axis can be wedged using cotton lint fibres (Narayana, Weininger, Huess & Argos, 1982), or mounted on a fibre plug (Przybylska, 1988).

One of the key problems in collecting diffraction data from wet crystals is movement of the specimen within the capillary, *i.e.* crystal slippage. Numerous ways have been suggested to surmount this problem, including flattening of the capillary surface, surrounding the crystal with a thin film of plastic (Rayment, Johnson & Suck, 1977) and supporting the crystal with fibre plugs in contact with the mother liquor.