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.

Table 3.4.1.3. *Cryoprotectants commonly used for biological macromolecules*

Protectant	Concentration (% by volume)
Glycerol	13–25
Ethylene glycol	11–30
Poly(ethylene glycol) 400	25–35
Xylitol	22
(2*R*,3*R*)-Butane-2,3-diol	8
Erythritol	11
Glucose	25
2,4-Methylpentanediol	28–45

Pressure cells. Tilton (1988) has described an attachment that can be used on conventional diffractometers for collecting X-ray data from biomolecular crystals under gas pressures up to 300 atm (30 MPa). The crystals are coated with mineral oil to minimize dehydration (see Subsection 3.4.1.5) and mounted in a quartz glass capillary between two layers of cotton fibres. These fibres give mechanical support to the specimen and protect it from shock during gas pressurization. No plugs of mother liquor or oil are used so that the gas flow is unimpeded. Kundrot & Richards (1986) describe an adaptation of the flow cell for hydrostatic pressure studies up to 0.2 GPa. More recently, Kroeger & Kundrot (1994) have described a gas cell that allows data sets at several partial pressures to be collected from the same crystal.

3.4.1.5. *Cryogenic studies of biological macromolecules*

Useful recent reviews on protein crystallography at low temperatures have been written by Hope (1990) and Watenpaugh (1991).

3.4.1.5.1. *Radiation damage*

Crystals of biological macromolecules are very susceptible to radiation damage, and this can severely limit the amount and quality of diffraction data that can be collected per crystal. There have been relatively few systematic studies of this phenomenon (Young, Dewan, Nave & Tilton, 1993; Gonzalez & Nave, 1994; Nave 1995), but one of the first effects of radiation damage is the deterioration of the high-resolution regions of the pattern, followed by increasing loss of crystallinity. Improvement of crystal lifetime in X-ray beams has been obtained by the addition of free-radical scavengers (Zaloga & Sarma, 1974) and the replacement of the mother liquor with solutions containing 10–20% polyethylene glycol 4000 or 20000 (Cascio, Williams & McPherson, 1984). The use of synchrotron radiation has also led to improved data-per-crystal ratios (Lindley, 1988). The high intensity allows fast collection of data, and the high collimation permits different sections of the same crystal to be used for data collection. This is particularly useful for prismatic crystals, which can be mounted along their largest morphological axis. An alternative method of surmounting this problem, however, is to freeze the protein crystal. As the temperature is decreased, the rate of diffusion of free radicals is reduced, with a corresponding reduction in radiation damage. Appreciable reduction in diffusion rate is achieved even at 250 K, and at 100 K diffusion essentially ceases. Cryogenic measurements not only minimize radiation damage but often lead to improved resolution owing to decrease in thermal motion in the crystal. Increasing the crystal lifetime may be particularly important with respect to multiwavelength anomalous-dispersion measurements in order to derive phase information. Since crystals of biological macromolecules contain substantial amounts of solvent, typically between 35 and 80% by volume, the technical problem is to force the solvent to cool in an amorphous glass-like state, rather than as crystalline ice. The latter normally degrades the crystallinity by expansion and gives rise to powder rings, which complicate data measurement.

3.4.1.5.2. *Cryoprotectants*

Cryoprotectants are normally required to avoid ice formation, and the choice of cryoprotectant will depend on the nature of the mother liquor from which the crystals have been grown. Crystals grown from high salt will usually require high salt concentration in the cryobuffer to avoid dissolution, although the addition of organic solvents may be a useful alternative. Table 3.4.1.3 lists commonly used cryoprotectants and their typical concentrations (Gamblin & Rogers, 1993).

The introduction of the cryoprotectant can be achieved through: (*a*) crystal growth in the cryoprotectant; (*b*) direct transfer of crystal from mother liquor into cryoprotectant buffer either in a single step or in steps of increasing cryoprotectant concentration; (*c*) dialysis, either direct or stepwise; or (*d*) exchange of liquor using a flow cell and a gradient maker.

3.4.1.5.3. *Crystal mounting and cooling*

Experience indicates that small crystals are better for cryogenic purposes, presumably because the rate of diffusion of small molecules and the rate of heat loss during rapid freezing is significantly faster than for large crystals. In most cases, there is an increase in the mosaicity (typically by a factor of 2–3), and in large specimens the increase may render the crystals useless for data collection. Successful freezing is often indicated by the crystal remaining transparent. Opacity usually indicates considerable breakdown in the crystallinity. Three commonly used methods for mounting crystals of biological macromolecules for cryogenic measurements are detailed below.

(i) *Coating methods*. Useful accounts of this method are given by Dewan & Tilton (1987) and Hope (1988). The crystal is first transferred to a hydrocarbon environment, mounted on a glass fibre attached to a brass pin on a goniometer head, and then fast cooled by introduction into a nitrogen-gas stream. The crystal adheres to the fibre by surface-tension effects, and the hydrocarbon also prevents loss of solvent during transfer into the gas stream. Paratone-N (Exxon) mixed with mineral oil (25–50% mineral oil) has a suitable viscosity, and excess oil should be removed by draining. This method has been successfully used for a number of biological macromolecules including crambin (Teeter, Roe & Heo, 1993) and the bovine eye lens protein, γB-crystallin (Lindley *et al.*, 1993). In the case of γB-crystallin, it was found that large crystals, $0.5 \times 0.5 \times 1.0$ mm, often became opaque after freezing, indicating gross damage to the crystallinity, or showed appreciable mosaic spread in the subsequent diffraction patterns, rendering them useless for data collection. Smaller crystals, $0.2 \times 0.2 \times 0.8$ mm, gave good diffraction patterns with an increase in the mosaic spread of only a factor of

references