Table 4.1.2.1. *Factors affecting crystallization*

Physical	Chemical	Biochemical
Temperature variation	pH	Purity of the macromolecule or impurities
Surface	Precipitant type	Ligands, inhibitors, effectors
Methodology or approach to equilibrium	Precipitant concentration	Aggregation state of the macromolecule
Gravity	Ionic strength	Post-translational modifications
Pressure	Specific ions	Source of macromolecule
Time	Degree of supersaturation	Proteolysis or hydrolysis
Vibrations, sound or mechanical perturbations	Reductive or oxidative environment	Chemical modifications
Electrostatic or magnetic fields	Concentration of the macromolecules	Genetic modifications
Dielectric properties of the medium	Metal ions	Inherent symmetry of the macromolecule
Viscosity of the medium	Crosslinkers or polyions	Stability of the macromolecule
Rate of equilibration	Detergents, surfactants or amphophiles	Isoelectric point
Homogeneous or heterogeneous nucleants	Non-macromolecular impurities	History of the sample

macromolecule to be crystallized together with buffer, precipitant and additives is equilibrated against a reservoir containing a solution of precipitant at a higher concentration than that in the drop (Fig. 4.1.2.1*c*). Equilibration proceeds by diffusion of the volatile species until the vapour pressure of the drop equals that of the reservoir. If equilibration occurs by water (or organic solvent) exchange from the drop to the reservoir (*e.g.* if the initial salt concentration in the reservoir is higher than in the drop), it leads to a volume decrease of the drop, so that the concentration of all constituents in the drop increase. The situation is the inverse if the initial concentration of the crystallizing agent in the reservoir is lower than that in the drop. In this case, water exchange occurs from the reservoir to the drop. Crystallization of several macromolecules has been achieved using this 'reversed' procedure (Giegé *et al.*, 1977; Richard *et al.*, 1995; Jerusalmi & Steitz, 1997).

Hanging drops are frequently deployed in Linbro tissue-culture plates. These plates contain 24 wells with volumes of ∼2 ml and inner diameters of 16 mm. Each well is covered by a glass cover slip of 22 mm diameter. Drops are formed by mixing 2–10 µl aliquots of the macromolecule with aliquots of the precipitant and additional components as needed. A ratio of two between the concentration of the crystallizing agent in the reservoir and in the drop is most frequently used. This is achieved by mixing a droplet of protein at twice the desired final concentration with an equal volume of the reservoir at the proper concentration (to prevent drops from falling into the reservoir, their final volume should not exceed 25 µl). When no crystals or precipitate are observed in the drops, either sufficient supersaturation has not been reached, or, possibly, only the metastable region has been attained. In the latter case, changing the temperature by a few degrees may be sufficient to initiate nucleation. In the former case, the concentration of precipitant in the reservoir must be increased. A variant of the hanging-drop procedure is the HANGMAN method. It utilizes a clear, non-wetting adhesive tape that both supports the protein drops and seals the reservoirs (Luft *et al.*, 1992).

Sitting drops can be installed in a variety of different devices. Arrangements consisting of Pyrex plates with a variable number of depressions (up to nine) installed in sealed boxes were used for tRNA crystallization (Dock *et al.*, 1984). Drops of mother liquor are dispensed into the depressions and reservoir solutions with precipitant are poured into the bottom sections of the boxes. These systems are efficient for large drop arrays and can be used for both screening and optimizing crystallization conditions. Multi-chamber arrangements are suitable for the control of individual assays (Fig. 4.1.2.2). They often consist of polystyrene plates with 24 wells which can be individually sealed. Sitting drops can also be placed on microbridges (Harlos, 1992) or supported by plastic posts in the centres of the wells. Reservoir solutions are contained in the wells in which the microbridges or support posts are placed. Plates with 96 wells, sealed with clear sealing tape, are convenient for large-matrix screening. Most of these plates are commercially available and can often be used for a majority of different vapour diffusion crystallization methodologies (hanging, sitting or sandwich drops, the latter being maintained between two glass plates). A crystallization setup in which drops are deployed in glass tubes which are maintained vertically and epoxy-sealed on glass cover slips is known as the plug-drop design (Strickland *et al.*, 1995). Plug-drop units are placed in the wells of Linbro plates surrounded by reservoir solution and the wells are then sealed as usual. With this geometry, crystals do not adhere to glass cover slips, as they may with sandwich drops.

Vapour phase equilibration can be achieved in capillaries (Luft & Cody, 1989) or even directly in X-ray capillaries, as described for ribosome crystallization (Yonath *et al.*, 1982). This last method may even be essential for fragile crystals, where transferring from

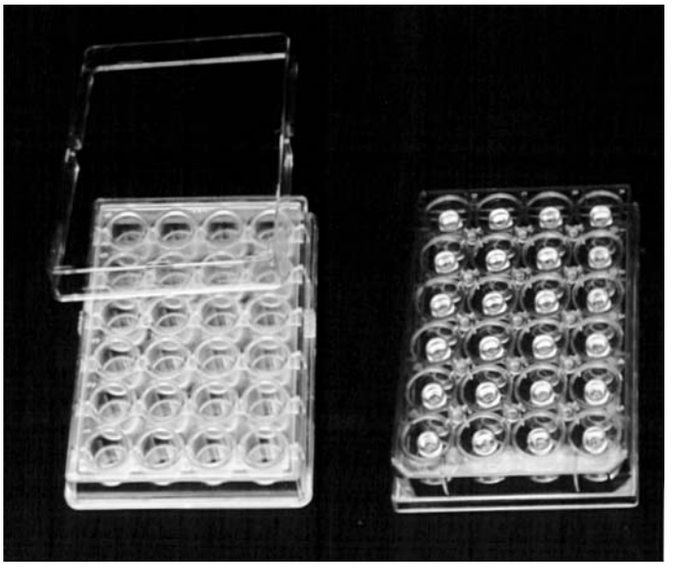

Fig. 4.1.2.2. Two versions of boxes for vapour diffusion crystallization. On the left, a Linbro tissue-culture plate with 24 wells widely used for hanging-drop assays (it may also be used for sitting drops, dialysis and batch crystallization). On the right, a Cryschem multichamber plate, with a post in the centre of each well, for sitting drops.

references