Table 5.2.6.1
Organic liquids for density determinations

Name	Density (g ml^{-1})
Carbon tetrachloride (tetrachloromethane), CCl_4	1.5940
Bromobenzene, CH_5Br	1.4950
Chloroform (trichloromethane), $CHCl_3$	1.4832
Methylene chloride (dichloromethane), CH_2Cl_2	1.3266
Chlorobenzene, CH_5Cl	1.1058
Benzene, CH_6	0.8765
m-Xylene, 1,3-$(CH_3)_2C_6H_4$	0.8642
Iso-octane (2-methylheptane), $C_5H_{11}CH(CH_3)_2$	0.6980

Table 5.2.6.2
Inorganic salts for density determinations

The densities are approximate values for aqueous solutions at 20 °C.

Solute	Density (g ml^{-1})
Sodium chloride	1.20
Potassium tartrate	1.40
Potassium iodide	1.63
Iron(III) sulfate	1.80
Zinc bromide	2.00
Zinc iodide	2.39

method can be tedious, so its practitioners rarely achieve an accuracy better than 0.2–1.0% (Low & Richards, 1952*a*).

5.2.6.6. Tomographic crystal-volume measurement

Recently, a new method for density measurement which is specific for protein crystals has been reported (Kiefersauer *et al.*, 1996). The crystal volume is calculated tomographically from a set of optical-shadow back projections of the crystal, with the crystal in many (>30) orientations. This measurement is analogous to methods used in electron microscopy (Russ, 1990). The crystal is mounted on a thin fibre which is in turn mounted on a goniostat capable of positioning it in many angular orientations. The crystal must remain bathed in a humidity-regulated air stream to avoid drying. The uncertainty of the volume measurement improves asymptotically as the number of orientations increases (estimated to be 10–15%). The images are captured by a digital charge-coupled device camera, transferred to a computer and processed with the program package *EM* (Hegerl & Altbauer, 1982). This same crystal must then be recovered and subjected to quantitative amino-acid analysis (the authors used a Beckman 6300 amino-acid analyser). With a lower limit of 100 pmol for each amino acid, the uncertainty of this measurement was estimated to be 10–20% for typical protein crystals. The method appears to work for crystals with volumes ranging between 4–50 nl. Errors in the determined values of n ranged from 4–30%.

Implementation of the method requires complex equipment and considerable commitment (in terms of hardware and software) by the research laboratory. The accuracy of the method is sufficient to determine n unambiguously in many cases, but it is not as high as can be obtained with gradient-tube or flotation methods if care is taken. The method has the virtue that once established in a research laboratory, it might lend itself to a considerable degree of automation, thereby reducing the activation barrier to measuring crystal densities for members of the research group.

5.2.6.7. Gradient-tube method

This is the most commonly used method for measuring densities of macromolecular crystals. It is simple and inexpensive to implement. It can be used to measure densities of very small crystals and crystalline powders. Practised with care, the gradient-tube method is capable of measuring crystal densities with a precision and accuracy of ± 0.002 g ml^{-1}.

Although density gradients were used earlier for other purposes, the application of the gradient-tube method for crystal-density measurement was first described by Low & Richards (1952*a*). The gradient can be formed in a long glass column (preferably with volume markings, such as a graduated cylinder), in which case the crystal sample will settle by gravity in the tube; or in a transparent centrifuge tube, in which case the crystal's approach to its equilibrium density may be accelerated by centrifugation. The gradient may be made by two organic liquids (Table 5.2.6.1) with different densities, or it may be made by a salt concentration gradient in water (Table 5.2.6.2). In either case, formation of the gradient is simplified with a standard double-chamber 'gradient maker' – however, a *glass* gradient maker should be used if the gradient is made of organic solvents! Be aware that all these substances are toxic, particularly to the liver, and some are listed as carcinogens, so avoid prolonged exposure.

Desired upper and lower density limits for the gradient can be made by mixing two of these liquids in appropriate ratios. The sensitivity and resolution of the measurement can be enhanced by using a shallow gradient covering the expected density. These organic liquids have a nontrivial capacity to dessicate the crystal sample, so it is important that they be water-saturated before use. Also, when an alcohol is the precipitant of a crystal, organic solutions may be inappropriate for density measurements.

For aqueous gradients, the salts listed in Table 5.2.6.2 may be added to water to create a dense liquid.

A widely used variant of the method has been to form aqueous gradients with Ficoll, a sucrose polymer cross-linked with epichlorhydrin (Westbrook, 1976, 1985; Bode & Schirmer, 1985). Manufactured by the Pharmacia Corporation specifically for making density gradients used in the separation of intracellular organelles or intact cells, Ficoll is a large polymer ($M_r = 400\ 000$) which is very hydrophilic and soluble, and has chemical properties similar to sucrose. Since it is highly cross-linked, each Ficoll molecule tends to be globular and is so large that it is effectively excluded from the crystal. Ficoll precipitates protein from solution on a per-weight basis as effectively as polyethylene glycol and can prevent protein crystals from dissolving, even in the absence of other solutes. A 60% (w/w) solution of Ficoll has a density of about 1.26 g ml^{-1}, sufficiently dense that almost all protein crystals will float in this solution (nucleic acid crystals are usually too dense for Ficoll). Used with care (see below), Ficoll gradients seem to yield the most reproducible crystal-density measurements. Concentrated Ficoll solutions are quite viscous, so these gradients are usually made by manually overlaying small volumes (0.5 ml each) of decreasing density, rather than with a gradient maker. In a standard cellulose nitrate centrifuge tube of about 5 ml capacity, this procedure makes an almost continuous gradient which works satisfactorily.

The density column must be calibrated once it has been formed. This is performed by introducing small items of known density into the column and noting their vertical positions. The density of the gradient as a function of vertical position can then be defined by interpolating between adjacent calibrated points. Usually, the calibrating points are made from small drops of immiscible liquid. Thus, in an organic solvent gradient, the drops are made of salt water; in an aqueous gradient, the drops are