

3.2. Determination of the density of solids

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3.2.1. Introduction (By P. F. Lindley)

The measurement of the density of a crystal has become a neglected art, and yet, in combination with an accurate knowledge of the unit-cell dimensions, it can provide vital information regarding the total molecular weight of the unit-cell contents. From this quantity, it is usually possible to determine the number of molecules in the unit cell and their individual molecular weights. The equation relating the crystal density (ρ), unit-cell volume (V), and the overall molecular weight is

$$\rho = Mm_a/V,$$

where m_a is the atomic mass unit (1.66057×10^{-24} g) and V is expressed in mm^3 . Alternatively,

$$M = 0.602206V\rho,$$

where V is in units of \AA^3 . The mass per asymmetric unit can be determined by dividing M by the number of asymmetric units, Z (dependent on the space group), and this will normally correspond to the molecular weight. However, the quotient can either be a fraction of the molecular weight (normally 1/2) when the molecular symmetry permits the molecule to lie on a special position such as a centre of symmetry or a symmetry axis, or a multiple if the asymmetric unit contains more than one molecule. In either case, a special examination of the choice of unit cell and space group should be undertaken to ensure that the *correct* ones have been chosen. Normally, the measured and calculated densities should agree within at least 1.5%; discrepancies greater than this may indicate an incorrect molecular formula (not unknown in preparative chemistry) or the presence of solvent molecules or other additives. Incorrect choice of space group, inappropriate choice of unit cell, and incorrect asymmetric unit contents can all have profound effects on the success of a structure analysis and on the refinement of the resulting structure.

The classical techniques of density measurement are described by Tutton (1922) and by Reilly & Rae (1954). An excellent and detailed review of both the standard and the less common methods is given by Mason (1944), but, because this work can be difficult to obtain, some of the references compiled by this author are cited herein.

3.2.1.1. General precautions

Meticulous temperature control is essential for the highest precision. The allowable temperature fluctuation will depend on the thermal coefficient of expansion of the material and on the required accuracy of the measurement. The utmost care must be taken to avoid air bubbles and inclusions. In those techniques that require immersion of the solid in a liquid, it is assumed that no chemical or physical interaction occurs between the liquid and the solid, and that the volume of the liquid displaced represents the true volume of the solid. For most hard crystalline materials, liquids can easily be found for which these assumptions are valid. However, for amorphous powders, porous structures such as zeolites, crystalline proteins, and natural and synthetic fibres, the measured 'density' may depend markedly on the particular liquid chosen and on the details of the method applied. In these cases, *penetration* or *swelling* of the solid will depend on a variety of factors such as interfacial tension, the relation of pore size to molecular dimensions, adsorption, and electrostrictive forces. The structural unit to which the measured density applies

may be very difficult to specify. Even with materials not subject to these difficulties, variability in the measured density is frequently found. Such variations may arise from differences in trace impurities or in the previous history of the sample (Johnston & Adams, 1912).

3.2.2. Description and discussion of techniques

(By F. M. Richards)

The discussion here will be limited to six general methods, of which at least one may be adapted to the requirements of almost any problem. The method of choice will depend to a large extent on the nature of the material under study. The merits and disadvantages of each method will be discussed.

3.2.2.1. Gradient tube

This technique is simple, versatile, and capable of the greatest sensitivity. It is the method of choice except in those cases where immersion liquids with an appropriate density and chemical inertness cannot be found.

Originally devised by Linderstrom-Lang (Linderstrom-Lang, 1937; Linderstrom-Lang & Lanz, 1938) for the determination of the density of aqueous solutions, the procedure has been adapted for the measurement of crystal densities by Low & Richards (1952*a*). For the original solution measurements, a precision of $0.000001 \text{ g ml}^{-1}$ was obtained, although no attempt has been made to attain that precision with solids. This technique was apparently developed and used quite independently in the sugar-cane industry [see, for example, Guo & White (1983) and earlier references contained therein].

3.2.2.1.1. Technique

When one liquid is layered over another of greater specific gravity, with which it is miscible, a linear gradient of density develops near the interface. Manipulation of a plunger-type stirrer in a vertical tube can extend the gradient over the greater part of the column. In the absence of convection, the process of diffusion in a column of this type is so slow that the gradient will be maintained virtually unchanged for many months.

A crystal introduced into the tube falls until it reaches a level corresponding to its own density, where it will remain stationary. The density gradient may be calibrated either by introducing immiscible liquid drops of known density, or by the use of a micro-Westphal balance designed for the purpose (Richards & Thompson, 1952).

With an adequate thermostat, measurements may be made at any temperature between the freezing and boiling points of the mixtures involved.

Powders and crystals with cavities or inclusions may be ground to a slurry with the lighter column liquid, subjected to reduced pressure to remove trapped air bubbles, and then introduced into the gradient tube. With hygroscopic materials, these operations are carried out in a dry atmosphere. Finely divided material settles rapidly if the tube is centrifuged. Although centrifugation does not markedly affect the gradient, the column should be calibrated after this step.

If such samples are homogeneous, they will form a thin layer after centrifuging. If, on the other hand, some air bubbles or

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Table 3.2.2.1. Possible substances for use as gradient-column components

Hydrophobic components			Hydrophilic components	
Liquid	Approximate density at 298 K (g ml ⁻¹)	α	Solute	Approximate maximum density of concentrated aqueous solution at 298 K (g ml ⁻¹)
Isooctane (2-methylheptane)	0.69	-0.8	Sodium chloride	1.20
Kerosene	0.79	-0.5 -0.8	Potassium chloride	1.40
<i>m</i> -Xylene	0.86	-0.85	Potassium iodide	1.63
Chlorobenzene	1.10	-1.1	Iron(III) sulfate	1.80
Bromobenzene	1.49	-1.3	Zinc bromide	2.00
Carbon tetrachloride	1.60	-1.9	Zinc iodide	2.39
Methyl iodide	2.28	-2.7	Thallium(I) formate	3.5
Bromoform	2.89	-0.9	Thallium(I) formate-malonate	4.3
<i>s</i> -Tetrabromoethane	2.96	-2.2		
Methylene iodide	3.32	-2.6	Ficoll* (60% w/w in water)	1.25

The density at temperature T K can be computed by substituting the values of the density at 298 K and α in the formula $d_T = d_{298} + 10^{-3}\alpha(T - 298)$. *Trade name for a synthetic high-molecular-weight polysaccharide derivative.

inclusions still remain, or if the sample is truly a mixture, a stable distribution of material will be observed. The density of the material of interest can then usually be obtained by measurement of the appropriate layer, generally the most dense, without further treatment of the sample. This is the only technique by which the homogeneity of the sample can be tested simply. All other methods provide an average density value. A satisfactory technique for removing crystalline powders from the gradient column has not been devised. If a precision of

± 0.002 g ml⁻¹ is adequate, it is simplest to prepare a new wide-range column for each determination in a 10 ml test tube.

Detailed specifications for the preparation of large density-gradient columns are contained in the records of the British Standards Institution (1964). In the experience of the author, for ordinary laboratory use, the procedures described are unnecessarily complicated as is the large scale of the system. The large columns are not suitable for centrifuging and the settling times tend to be many hours. However, if extreme sensitivity (*i.e.* use of a shallow gradient) is required, the large column may be useful, as it was in the original studies of Linderstrom-Lang (Linderstrom-Lang, 1937; Linderstrom-Lang & Lanz, 1938).

In the specific application of this technique to protein crystals, where a gradient of organic liquids is used, it is necessary to have available crystals sufficiently large that they can individually be quickly wiped free of adhering mother liquor with dampened filter paper before insertion. The uncertainty of successful cleaning combined with rapid evaporation of liquid from the pores within the crystal always affect the estimated accuracy of the measurement. An important improvement in the technique has been made by Westbrook (1976, 1985) through the use of concentrated aqueous solutions of the water-soluble polymer Ficoll. This very high molecular weight polysaccharide can be dissolved in water to concentrations of at least 60% by weight. The solutions are very viscous but do provide satisfactory water-based gradient columns. The polymer is both too large to enter the solvent-filled pores of the protein crystals and too high in molecular weight to develop a significant osmotic pressure. An aqueous suspension of crystals can be added directly to the column. This procedure has been adapted for measurements of protein-crystal density under hydrostatic pressures from 1 to 2000 atm (1 atm \equiv 101 325 Pa) (Kundrot & Richards, 1988). The general principle of using high-polymer-based gradients can presumably be extended to other porous materials.

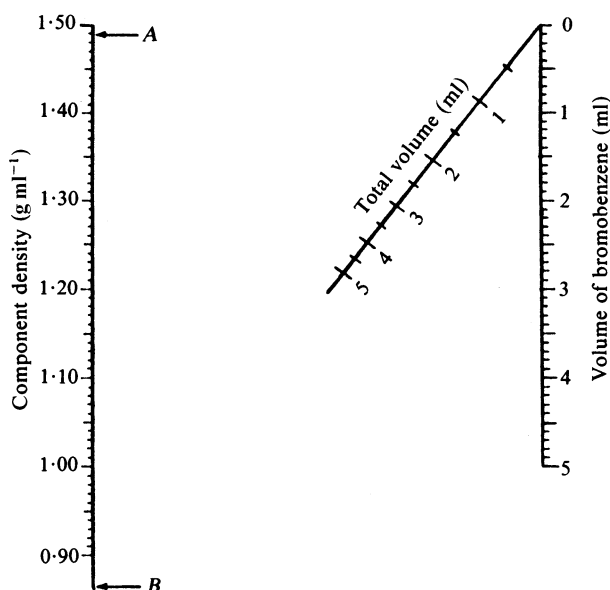


Fig. 3.2.2.1. Nomogram for the preparation of bromobenzene-xylene gradient column components at room temperature. From the desired component density and total volume, the required amount of bromobenzene is read from the chart, the volume difference being made up with xylene. To adapt this chart to any other pair of liquids, it is only necessary to change the component density scale. A uniform scale is drawn up such that the density of the heavy liquid lies at the point *A* while that of the light liquid is at *B*. The volume scales may be multiplied by any constant factor in order to change their range.

3.2.2.1.2. Suitable substances for columns

Some representative liquids are listed in Table 3.2.2.1; all are readily available. For further information, see Meyrowitz, Cuttitta & Hickling (1959), and for very heavy liquids Sullivan