

3. PREPARATION AND EXAMINATION OF SPECIMENS

(1927). Standardized solutions or mixtures from one list in Table 3.2.2.1 may be used as calibrating drops in gradients made from those of the other.

For rapid preparation of mixtures from stock solutions of the basic compounds, a nomogram is very useful, such as is given in Fig. 3.2.2.1 for the system bromobenzene–xylene at room temperature. In the construction of the nomogram, it has been assumed that the volumes of the liquids are additive. In general, this assumption is not valid, but it is a sufficiently good approximation for the purpose.

3.2.2.1.3. *Sensitivity*

The range of density covered by a column, and thus the accuracy of the determination, is controlled by the liquids or liquid mixtures chosen for the top and bottom components. A precision of about $\pm 0.002 \text{ g ml}^{-1}$ can easily be obtained without any special precautions. If narrow-range columns are carefully protected from temperature changes and vibration, the accuracy of the measurement may be increased 10- to 100-fold.

3.2.2.2. *Flotation method*

Although historically used much earlier, this technique is essentially an approximation to the gradient-tube method. The specimen is immersed in a liquid, and a denser or less dense liquid miscible with the first is added until the sample neither rises nor sinks in the solution (Wulff & Heigl, 1931). The density of the immersion medium is then determined immediately by standard techniques such as pycnometry, by the Westphal balance, or by refractive index (Midgley, 1951). The method is reported as capable of a probable accuracy as great as 0.02%.

The compounds listed in Table 3.2.2.1 are also useful in this method. With slurries or with specimens smaller than 1 mm^3 , a centrifuge must be used to achieve a reasonable rate of settling. As little as 0.05 mg of material has been used with good results (Bernal & Crowfoot, 1934). A modification of this method has been described in which the density of the immersion medium is varied by altering the temperature (Reilly & Rae, 1954; Wunderlich, 1957).

3.2.2.3. *Pycnometry*

This is one of the most demanding of the available techniques. A previously calibrated pycnometer containing the sample is weighed. A liquid of known density is then introduced, air bubbles are removed by reducing the pressure, and the filled bottle is reweighed. The volume of the sample and its mass may thus be determined. With care, a probable accuracy of 0.02% may be achieved (Johnston & Adams, 1912). Contrary to many published statements, the accuracy of this technique is not dependent to any significant extent on the use of immersion media of high density.

Liquids with low surface tension will facilitate the removal of air bubbles. In some cases, it is advantageous to fill the bottle with the mother liquor from which the crystal grew. Powders or many small crystals may be used as well as large single specimens. There is no restriction on the density of the materials for which this technique is suitable.

A micropycnometer for use with samples of total volume as small as 0.01 ml has been described (Syromyatnikov, 1935). An accuracy of better than 1% has been achieved with this instrument.

3.2.2.4. *Method of Archimedes*

The specimen is weighed in air and again in a liquid of accurately known density. From the apparent loss of weight the volume is computed, and thence the density (Reilly & Rae, 1954). The technique requires little special equipment and is capable of great accuracy when used with large, well formed crystals. The accuracy is maximized by using immersion liquids of density as close to that of the crystals as possible. For precise work, correction must be made for the interfacial tension between the supporting wire and the upper surface of the suspending medium.

A torsion microbalance has been adapted to the determination of crystals as small as 25 mg (Berman, 1939). A probable accuracy of better than 1% may be achieved with this micro-method.

A densitometer based on Archimedes principle with control of the composition of the gas phase and a wide temperature range has been described by Graubner (1986). The method is not suitable for finely divided materials.

3.2.2.5. *Immersion microbalance*

Some crystals, such as those of globular proteins grown from alcohol–water mixtures, rapidly change their composition, and thus their density, when removed from the mother liquor in which they were grown. The density may then be computed from the weight of the crystal immersed in its mother liquor, the density of the latter, and the volume of the crystal (Low & Richards, 1952*b*, 1954; Richards, 1954).

A horizontal quartz fibre, free at one end, is mounted in a glass case that can be filled with liquid. After calibration, the deflection of the fibre gives the weight of an immersed crystal suspended on the free end. The volume is computed from the crystal dimensions as determined from two photomicrographs of the immersed crystal taken at right angles to each other. The density of the mother liquor is measured by one of the standard techniques for liquids.

The method is suitable for single, well formed crystals having a volume of about 0.1 mm^3 or greater. The accuracy is related inversely to the difference in density between the crystal and its mother liquor.

3.2.2.6. *Volumenometry*

This is the only technique not requiring immersion of the sample in a liquid medium. The technique is therefore used in instances where the specimen would be attacked by the customary immersion media, or where one wishes to work over a temperature range where liquid media would be inappropriate.

The gas-pressure change caused by altering the volume of a calibrated vessel by a given amount is determined when the vessel is empty, and again after the weighed specimen has been introduced (Reilly & Rae, 1954).

Any gas inert to the crystal may be used. Powders and crystal fragments may be employed. A probable accuracy as great as 0.1% may be attained. Samples with an aggregate volume as low as 0.01 ml have been measured with a probable accuracy of 1% (Hauptmann & Schulze, 1934).

3.2.2.7. *Other procedures*

A novel procedure that may be useful in special circumstances is based on measuring the frequency of a vibrating string of the material in question. If the length of the string is fixed and the transverse deformation is small, the various harmonic frequen-