3. PREPARATION AND EXAMINATION OF SPECIMENS

concentration of the metal ions in solution; it is often worth adding a few drops of an exhausted solution to a fresh batch to remedy this. Secondly, solutions become exhausted, at least partially, as a result of selective evaporation, concentration of a metal-ion species, and contamination, particularly by water. Quite apart from the hazards of long-term storage, it is unwise to use solutions once these processes occur.

3.5.3. Polymers and organic specimens

The major difficulty with polymers and organic specimens is that they are rapidly degraded by the electron beam. The specimens become amorphous and sometimes volatilize (Fryer, 1987; Fryer, McConnell, Zemlin & Dorset, 1992). The rate of degradation can be reduced by encapsulating the specimen between two evaporated carbon films or by cooling the specimen to near liquid-nitrogen temperatures in the microscope (Fryer & Holland, 1984). When the specimen is encapsulated with carbon films, the structure can be preserved long enough to obtain electron-diffraction patterns. Therefore, a thin covering layer of carbon should be evaporated on top of the specimen.

Close contact of the specimen with the supporting carbon film is necessary to reduce electron-beam damage. Simple dusting of powdered specimens on carbon film is rarely satisfactory. A drop of the specimen in suspension often gives agglomerated specimens. Therefore, the powdered specimen should be dispersed in a solvent (2-propanol has proved satisfactory for aromatic compounds) with the aid of a low-power vibratory ultrasonic bath. The dispersion is then sprayed with a fine aerosol spray onto the carbon-covered grid.

For specimens where the original morphology is not important, a solution of the compound may be crystallized directly onto the carbon-covered grid. Low concentrations (1–2%) are necessary for good dispersions. Features of the crystal-growth morphology, *e.g.* spiral growth in paraffins, can be highlighted by heavy-metal shadowing. The morphology is often solvent dependent so that needles, platelets, monolayers or multilayers can be obtained as required.

3.5.3.1. *Cast films*

Thin films of polymeric compounds can be obtained by casting solutions of the polymer in a volatile, non-polar solvent onto a water surface and collecting a specimen by bringing a carbon-coated grid up though the film (Porat, Fryer, Huxham & Rubinstein, 1995). This technique is used for specimens of Langmuir–Blodgett monolayer films (Fryer, McConnell, Hann, Eyres & Gupta, 1990; Fryer *et al.*, 1991). The crystallinity of the film is often poor with this method of preparation; better crystals can be obtained by crystallizing the polymer from solution directly onto a carbon film. For many polymers, an ordered array is obtained when crystallization is performed on a cleaved alkali halide single-crystal surface. The monomer can be cast onto the crystalline substrate and polymerization performed

thermally or by UV irradiation. After crystallization, the polymeric specimen is coated with carbon and floated off on a water surface. Specimens from bulk polymeric materials can be prepared also by microtome sectioning (see Subsection 3.5.1.1).

3.5.3.2. *Sublimed films*

Most organic compounds can be sublimed under vacuum to give an expitaxic layer on a suitable substrate. Specimens of compounds ranging from paraffins to polynuclear hydrocarbons (Fryer & Smith, 1982; Fryer & Ewins, 1992) and porphyrins (Fryer, 1994) have been prepared in this way. A small amount of material is placed in a molybdenum boat with a perforated cover and sublimed under high vacuum onto a heated substrate. Potassium chloride crystals cleaved in air (100) provide successful substrates. Crystal size and order increase with substrate temperature, however, and a high temperature leads to re-evaporation of the compound. Sometimes, the temperature difference between film deposition and re-evaporation is as small as 30 K. An empirical guide to the optimum substrate is one-third of the boiling-point temperature of the compound. Normally, the crystalline film produced is 10–15 nm thick and is discontinuous.

Following the compound sublimation, carbon is evaporated onto the compound and the film is floated off the KCl substrate onto a water surface. The carbon film can prevent disintegration of the organic compound. Specimens of the film are then picked up on grids.

Organic crystals easily undergo phase changes, so that the crystal modification of the evaporated epitaxic film may not be that of the bulk material. The structure may also vary between preparations on different substrates or between different temperatures on the same substrate.

3.5.3.3. *Oriented solidification*

Long-chain compounds, paraffins, phospholipids, *etc.*, can be prepared epitaxially from solution in molten naphthalene or benzoic acid (Fryer, McConnell, Dorset, Zemlin & Zeitler, 1997; Wittman & Lotz, 1990). For example, a dilute solution of a compound in naphthalene is alternatively solidified and liquified within a few degrees of the melting point to order the long-chain material relative to the naphthalene. When finally solidified, the compound is ordered along the (110) plane of the naphthalene. In practice, the final solidification is carried out on a carbon film and the naphthalene is removed under vacuum. The crystals are lath shaped and are aligned with the long-chain major axis on the carbon film across the lath and hence normal to the electron beam. Crystals of the same compounds prepared from normal organic solvents as described in Subsection 3.5.3.2 have the long-chain axis normal to the carbon-grid plane and thus parallel to the electron beam. The advantage of the normal orientation is that the large interplanar spacing along the chain axis is more accessible to direct imaging in the electron microscope.