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Ultrathin Carbon Support Films for Electron Microscopy

Abstract. *Carbon support films only 4 to 6 angstroms thick have been made for use in electron microscopy. The determination of their thickness is based on geometrical calculation, electron scattering measurements, and elemental microanalysis.*

A method has been developed for the routine production of electron microscope specimen-support films believed to be only 4 to 6 Å thick. Such films are valuable in reducing background scatter in the examination of biological macromolecules by bright- or dark-field electron microscopy and by transmission electron diffraction.

Kleinschmidt and Vasquez (*1*) have reported the use of support films 30 to 40 Å thick, as measured by ellipsometry, while Crewe *et al.* (*2*) have deduced that films used by them were 20 Å thick. This conclusion was based on a comparison of measured electron scattering (*3*) with the scattering from films of different thicknesses calculated from equations derived by Lenz for a shielded Coulomb potential (*4*). Use of a carbon film 7 Å thick in dark-field microscopy has been reported (*5*), but it is not clear that such a film could be used as a specimen support.

The thin carbon films described here are deposited on freshly cleaved mica surfaces in a standard evaporating apparatus. A rotating shutter (240 rev/min) provided with a radial slot is placed 10.5 cm below the carbon arc; one piece of mica is positioned 0.5 cm below the plane of the shutter and another the same distance above it. The dimensions and shape of the slot are such that most of the lower mica surface should receive 5 percent as much carbon as does the companion upper one, but a small "tab" region at the outer edge of the lower mica piece receives 25 percent as much. The carbon arc is operated for about 10 seconds.

After deposition, the carbon film is floated off the mica by first inserting the thicker tab portion into a clean water surface. This portion is readily visible and helps to locate the almost invisible remainder of the film as it floats on the water. Portions of the film are then picked up by touching them from the air side with a reticulated collodion film on a 200-mesh copper grid (*6*). Films calculated as only 4.5 Å thick (5 percent of the companion film, measured as 90 Å thick) can be reliably manipulated in this manner.

The thickness of carbon deposited on the piece of mica above the rotating disk is determined by measuring the lengths of shadows cast by edges of broken fragments after they have been shadowed lightly with uranium. The fragments are obtained by floating the thick film on water and breaking it into small pieces, which are then picked up on a collodion support film. Polystyrene spheres are used to determine the local shadow angle. Film thicknesses ranging from 90 to 160 Å were measured with an estimated accuracy of ± 10 Å.

Measurements of the relative intensity of electron scattering by films of different thicknesses were made by dark-field electron microscopy at ×4000. In some instances portions of films were found to be folded (double thickness), and scattering from these was recorded. The electron microscope was operated at 80 kv and was equipped with one cold finger (decontaminator) located between the specimen and the objective aperture and a second one located above the specimen airlock. The intensity of primary illumination was measured and adjusted to a fixed value by use of a lithium-drifted silicon detector located in the final image plane (*7*). The dark-field images were recorded by use of tilted-beam illumination and an axially positioned,

50-μm objective aperture. Alternation between bright-field conditions (used for monitoring the illumination intensity) and dark-field conditions of operation was facilitated by dual power supplies for the coils controlling the condenser lens beam alignment. In this way, a series of dark-field images from different specimens could be photographed under identical conditions of aperture position, illumination intensity, and exposure time. Photographic densities were measured on a Joyce-Loebl microdensitometer. The results are shown in Fig. 1, a and b. The curves are distinctly nonlinear, a result similar to that obtained by Johnson and Parsons (*5*) in experiments involving the use of a central beam-stop technique of dark-field microscopy. It is seen that the scattering obtained from the ultrathin (5 percent) films implies thicknesses that are consistent with those calculated on the basis of slot geometry as indicated above.

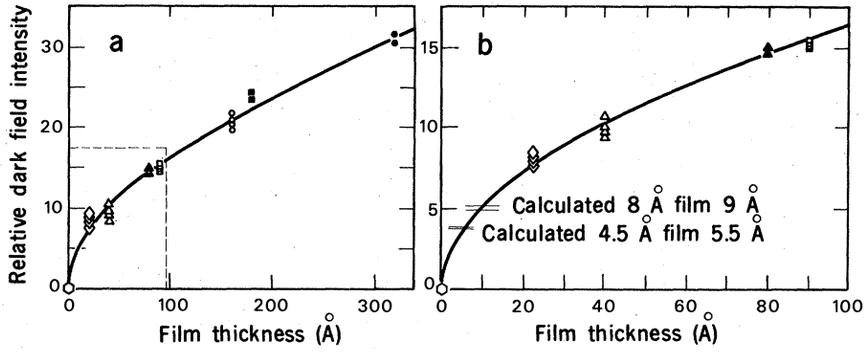

Fig. 1. (a) Relative dark-field intensities from single and folded carbon films. A film and its folded counterpart are designated by the same geometrical symbol, shown solid for the folded film. Films 90 and 160 Å thick are those measured by the metal shadowing technique, while the films 22.5 and 40 Å thick are their (25 percent) tab portions. The point at the origin is an experimental point, which shows that the background intensity attributable to spherical aberration and other sources is negligible. (b) The graph, redrawn on an expanded scale, includes the relative dark-field intensities of two ultrathin films, indicated by the horizontal lines. Their thicknesses derived from the curve are close to the thicknesses calculated geometrically as 5 percent of 90 and 160 Å, respectively.

An independent determination was made of the thickness of a film in the ultrathin region in order to check the validity of calculating the thickness of a "5 percent" film. A microanalysis was made by the gas chromatography method (*8*) for the carbon, hydrogen, and nitrogen contents of a carbon film deposited on an aluminum foil and calculated as 12 Å thick (5 percent of a film measured as 240 Å thick). Triple determinations gave a value of $4.1 \pm 0.35 \times 10^{-7}$ g/cm^2 for carbon, with hydrogen and nitrogen below the level of detection (0.3×10^{-7} g/cm^2). Blank aluminum foils placed under the rotating shutter, but covered by a fixed shield 4 mm above them, yielded no detectable amounts of carbon, hydrogen, or nitrogen above the background level recorded for blank foils that had not been placed in the evaporator. The density of a similarly deposited film, measured as 200 Å thick, was ascertained in order to allow conversion of the mass determination to thickness. The weight of this film, deposited on an aluminum foil 25 cm^2 in area, was 155 μg. Hence, its density was 3.1 g/cm^3 and the thickness of the microanalyzed film was 14 Å. The agreement between the calculated and measured thicknesses is close enough to allow us to conclude that calculated thicknesses of "5 percent" films are valid and that the thinnest films we have made are not thicker than 6 Å.

The low magnification, dark-field images were obtained at low electron beam intensities, and the specimen area was efficiently cold trapped. It is believed, therefore, that specimen contamination occurring in the electron beam had a negligible effect on the electron scattering, even for the thinnest films. Adsorbed layers of water, oxygen, pump oil, and other kinds of uniformly spread contaminants seem not to remain on the carbon films (at least, not after brief electron illumination) to an extent greater than the equivalent of 1 to 2 Å of carbon.

The nonlinearity of the curve relating dark-field intensities with film thicknesses suggests that the physical characteristics of the "average" scattering process change somewhat with film thickness. This would be expected, for example, if the differential scattering cross sections associated with carbon atoms in the surface layers were noticeably different from those associated with carbon atoms in the bulk phase. For very thin films the scattering characteristics would then be dominated by the behavior associated with surface atoms, while for thicker films the behavior associated with bulk-phase atoms would be more important.

The ultrathin support films exhibit a mechanical stability that is comparable to that of carbon films of conventional thickness, a remarkable strength inasmuch as their average thickness is only three to four carbon atoms. Specimen materials such as viruses and protein molecules may be deposited by droplet spraying without signs of breakage. The films also have a stability in the electron beam quite comparable to that of conventional support films. The total area covered by the ultrathin film is normally at least half of the open area of the copper-mesh grid on which it is mounted, with individual areas often as large as 100 to 200 μm^2.

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