

CHEMICALLY CLEAVED GRAPHITE SUPPORT
FILMS FOR ELECTRON MICROSCOPY

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Today, high resolution electron microscopy is seriously limited by the noise which results from the irregularity of the substrates used. For example, this limitation is of particular significance in recent attempts (1) to elucidate by electron microscopy the nucleotide sequence of DNA by chemical attachment of base-specific, heavy metal markers. The following technique was originally developed in conjunction with efforts to improve the "visibility" of markers presently being employed in such studies.

Fernández-Morán (2) has pointed out that single crystal substrates have the advantage of being smooth and presumably regular. However, the preparation of appropriate films is difficult; previously adhesives have been used to cleave single graphite crystals (3,¹), and thin crystals of graphite have been grown epitaxially on single crystals of nickel (4). Here we report a chemical method involving alkali splitting of graphite oxide.

Graphite oxide is an unusual sandwich compound composed of alternate layers of carbon and oxygen with each carbon layer thought to be organized in the hexagonal lattice array characteristic of graphite (5). It is cleaved into thin lamellae by immersion in dilute solutions of NaOH. These lamellae, some of which are thought to be only one carbon atom thick, apparently remain complexed with oxygen but can be reduced to pure graphite by appropriate treatment (6). The extremely thin graphite sheets thus formed are then suspended on perforated films to serve as specimen-supporting substrates.

¹ Sprague, R. 1960. Personal communication cited in Beer and Highton (3).

In the initial preparation of graphite oxide, complete oxidation is necessary to obtain the thinnest possible graphite sheets. The most thoroughly oxidized preparations are canary yellow in color; successively less satisfactory samples appear golden yellow and brownish yellow. Early techniques for this oxidation (7, 8) required long periods of time and entailed substantial explosion hazards. The more recent procedure developed by Hummers and Offeman (9) successfully overcomes both of these difficulties and has been adapted for our purposes.

MATERIALS AND METHODS

Under a fume hood the following chemicals were thoroughly mixed with magnetic stirring in a 250 ml Pyrex Erlenmeyer flask:

- (a) 1 g of 325 mesh powdered graphite flake²
- (b) 0.5 g sodium nitrate
- (c) 23 ml of concentrated sulfuric acid

The Erlenmeyer flask was immersed in an ice bath and the contents were allowed to cool. 3 g of potassium permanganate were added slowly while stirring, and the temperature was maintained below 20°C to avoid explosion hazards. Constant monitoring of the temperature and continued stirring for 5 additional min to insure complete solution of the permanganate is recommended.

The flask was then transferred to a hot water bath and the temperature of its contents increased to 35°C ± 3°, which was maintained for 30 min. During the latter stages of this step a faint yellowish tinge was observed in the suspended graphite.

Next, the contents of the flask were diluted twofold

² Asbury Graphite Mills Inc., Asbury, N.J. A wide variety of other grades are available; further information may be obtained by contacting H. N. Riddle III, President.

by slow addition of 25 ml of distilled water. The temperature was then increased to 98° for 10–15 min. Although this step is exothermic, additional heating with a water bath was required. While the suspension was cooling, 10 ml of 3% hydrogen peroxide were added which left the contents of the flask turbid, with the suspended graphite oxide particles ranging in color from golden brown to bright yellow, depending on the thoroughness of the oxidation.

Oxidation of larger graphite flakes is often incomplete and the preparation was centrifuged at 500 rpm for 1 min in a desktop centrifuge to remove these particles, which were discarded. The fine particles were then sedimented, the supernatant was discarded, and the precipitate was washed by resuspension in the original volume of distilled water. This washing was repeated three times, during which the precipitate became reddish-brown. After the final wash the precipitate was resuspended in distilled water and the residual coarse particles were allowed to settle out for 8 hr in the refrigerator. The remaining aqueous suspension of fine graphite oxide particles constituted a stock suspension which could be stored indefinitely under refrigeration and used after resuspending the particles which had settled out. Since the concentration of graphite in this stock suspension varied from preparation to preparation, small samples were diluted with various amounts of distilled water for use in the next step.

3 ml of the stock suspension were mixed in a test tube with an equal volume of 0.02 N sodium hydroxide (0.8 g/liter). The suspension was then allowed to sit for 10 min, permitting the graphite oxide particles to cleave into thin lamellae; separation seems to be accelerated by ultrasonication.

Five drops of hydrazine hydrate were then added to the test tube and the contents were heated almost to boiling for 2 min. During this step the graphite oxide was reduced and the suspension became black, opaque, and nonparticulate. As the suspension cooled to room temperature, the graphite lamellae tended to reaggregate and very small, black plaques (about ½ mm in diameter) could be observed in the meniscus and fluid coating the inner surface of the test tube.

After the suspension had cooled, it was transferred to a 10 ml beaker. Grids covered with perforated collodion³ were coated with graphite simply by wiping them over the surface of the suspension. Surface tension helps to stretch the resulting graphite films over the holes in the supporting membrane. In an

³ We have employed perforated collodion films backed with evaporated carbon furnished courtesy of Dr. Paul Bartl (Hoffmann-La Roche Inc., Nutley, N.J.). Preliminary experiments indicate that similar Formvar films described by Hall (reference 10, p. 293) are equally satisfactory.

alternate technique⁴ the suspension was streaked on the surface of freshly cleaved mica or a carefully cleaned microscope slide and allowed to dry almost, but not quite, completely. The graphite film deposited was then floated off on distilled water in a manner analogous to that used for conventional, vacuum-deposited amorphous carbon films. During drying the tendency of surface tension forces to tear extremely thin films was minimized by placing the grids on filter paper saturated with distilled water and allowing them to dry overnight. However, satisfactory films were also obtained simply by placing the grids, graphite-streaked side up, on dry filter paper for a few minutes.

RESULTS

Fig. 1 shows a typical film coated with 880 A spheres. Photographs were made of a number of similar, torn films. These plates were examined with a microdensitometer and the relative opacities of the graphite films and 880 A spheres were compared by using the "straight through" area for reference purposes (10). If it is assumed that graphite and latex have equal electron densities,

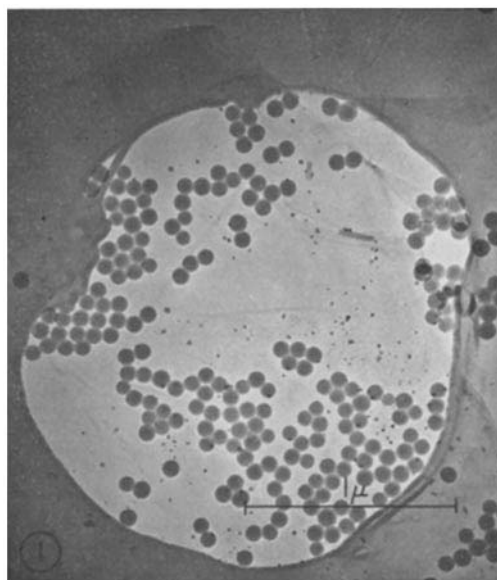


FIGURE 1 Graphite membrane spanning a hole in a carbon-backed, perforated collodion film. The graphite is torn on the upper right edge, permitting thickness measurements by comparing the opacity of the substrate and suspended 880 A spheres. (Photograph by D. S. O'Hara.)

⁴ Woods, G. 1966. Personal communication.

the thickness of the films was found to range between 50 and 150 Å; much thinner ones have occasionally been observed.

Visual comparisons indicate that the new technique, although simple and requiring minimal equipment, is capable of routinely producing grids with small areas covered by films frequently better than conventional amorphous carbon substrates.

These impressions were confirmed by preparing special grids with adjacent areas covered by the two substrates. Both films were subsequently photographed on the same plate, which was then qualitatively examined on a Joyce-Loebl microdensitometer (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England).

SUMMARY

A technique is reported for preparation of thin graphite specimen support films for electron microscopy. The procedure, involving preparation, chemical cleavage, and reduction of graphite oxide, is extremely simple and requires minimal equipment. With practice, 100 Å thick films are readily prepared and it is possible to prepare thinner ones.

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