Clays and Clay Minerals, Vol. 27, No. 4, pp. 293–296, 1979.

A MODIFIED FREEZE-DRYING PROCEDURE FOR THE ELECTRON MICROSCOPIC EXAMINATION OF HECTORITE

Key Words—Freeze-drying, Hectorite, Morphology, Sample Preparation, Transmission, Electron Microscopy.

INTRODUCTION

The use of freeze-drying techniques in the preparation of biological materials for electron microscopic investigation was introduced by Williams (1953) to minimize distortion of fragile specimens. The technique has since been used successfully to show the unique morphology of sodium montmorillonite for electron microscopy by Corbet and Wolffe (1956) and Bates (1958) but, as has been pointed out by Hofmann *et al.* (1962) and Mering and Oberlin (1971), the freeze-drying technique causes the formation of continuous films and massive aggregation and curling of smectite minerals. In recent studies of clay textures (McKee and Brown, 1977), the critical-point drying method was preferred over the freeze-drying method. Although critical-point drying has been shown to reduce aggregation and particle orientation in montmorillonites (Jernigan and McAtee, 1975) and thus furnish a more accurate picture of the morphology of aggregates, details of the relationships between individual particles may be partially obscured by the intense curling of single plates.

To reduce the amount of curling and aggregation of clay platelets, to show better the shapes of individual clay particles, and to preserve the delicate morphology of aggregates, a modified freeze-drying technique was developed employing a special holder which allowed only one side of a carbon-coated grid to be exposed to the suspension during the freezing and drying process. Electron microscopic examination of samples prepared by this method was successful in depicting the subtle morphological differences among a group of hectorite clays and showed a greater number of individual particles than those prepared by other techniques.

EXPERIMENTAL

Several hectorite samples representing different locations within the NL Industries mine at Hector, California, were examined by electron microscopy. Approximately 40 ml of 0.05% suspensions were mixed by submerging the microtip of a Sonifier cell disruptor (Model W185) into a small beaker with the suspension for 5 min at a constant energy setting for all samples. The experimental conditions such as concentration of solids and total amount of water and time, energy and temperature of sonification were held constant to minimize differences in specimen morphology due to sample preparation.

A special holder for carbon coated grids (400 mesh) held the grids stationary with only one side exposed to the suspension during the freezing process (Figure 1). The inch-long base possessed three equally spaced indentations, $^{9}/_{16}$″ wide and $^{4}/_{64}$″ deep, to accommodate three grids. When the grids were in place, the top section of the holder was secured by screws passing through both the top and bottom sections of the holder. The top section contained three holes exposing the interior of the grids while overlapping the edges to secure the grid in a place and to prevent the suspension from having access to both sides of the grids. The grid holder was then placed into a 125-ml VirTis freeze-drying flask. A small amount of the freshly prepared 0.05% clay dispersion drawn from the upper half of the suspension was applied by a disposable micropipet to each hole until the top meniscus was flush with the top of the holder. The flask was then placed in the precooled (−60°C) shell-freezing bath of a VirTis Bench Top Freeze Dryer (Model #10-030). The rotary motion of the shell-freezing bath was stopped just prior to the insertion of the flask into the bath. Visual observation showed that sample freezing was complete in less than a minute. When the sample was frozen, the flask was attached to the freeze dryer, and the suspension was allowed to dehydrate for 4 hr. The samples were then examined in an Hitachi HU-11A electron microscope at an accelerating voltage of 75 kV. Magnification was calibrated by means of polystyrene latex spheres obtained from Ladd Research Industries, Inc.

In addition to the modified freeze-drying technique described here, all samples were prepared for electron microscopic examination by fine spraying onto carbon-coated grids, bulk freeze-drying, and critical-point drying.

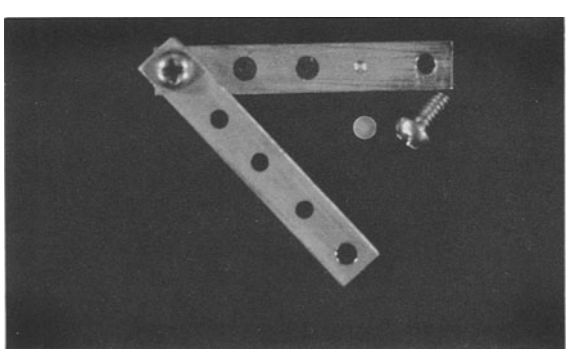

Figure 1. Photograph of holder for carbon-coated grids for freeze drying. (Base plate = 1 inch in length.)

RESULTS AND DISCUSSION

All conventional preparation techniques produced mainly aggregates of hectorite or continuous films. Differences in the morphology of individual laths in the several specimens examined could be seen only when the specimens were prepared using the special grid-holding device described above.

In Figure 2, different freeze-dried hectorites display variations in particle length, particle width, amount of aggregation, amount of curling, and amount of fine-grained anhedral material in the background. Figure 2a illustrates a relatively pure centrifuged hectorite that is more highly aggregated than the specimen shown in Figure 2b. The clay particles in Figure 2b are smaller than those in Figure 2a, and the specimen contains more background material which may be non-clay but whose source is believed to be from the sample since such material was observed on a blank grid. The laths in Figures 2c and 2d are about one third larger than those in 2a and 2b, while the clay particles in Figures 2e and 2f are two to five times larger than those in Figures 2a and 2b. The sample in Figures 2e and

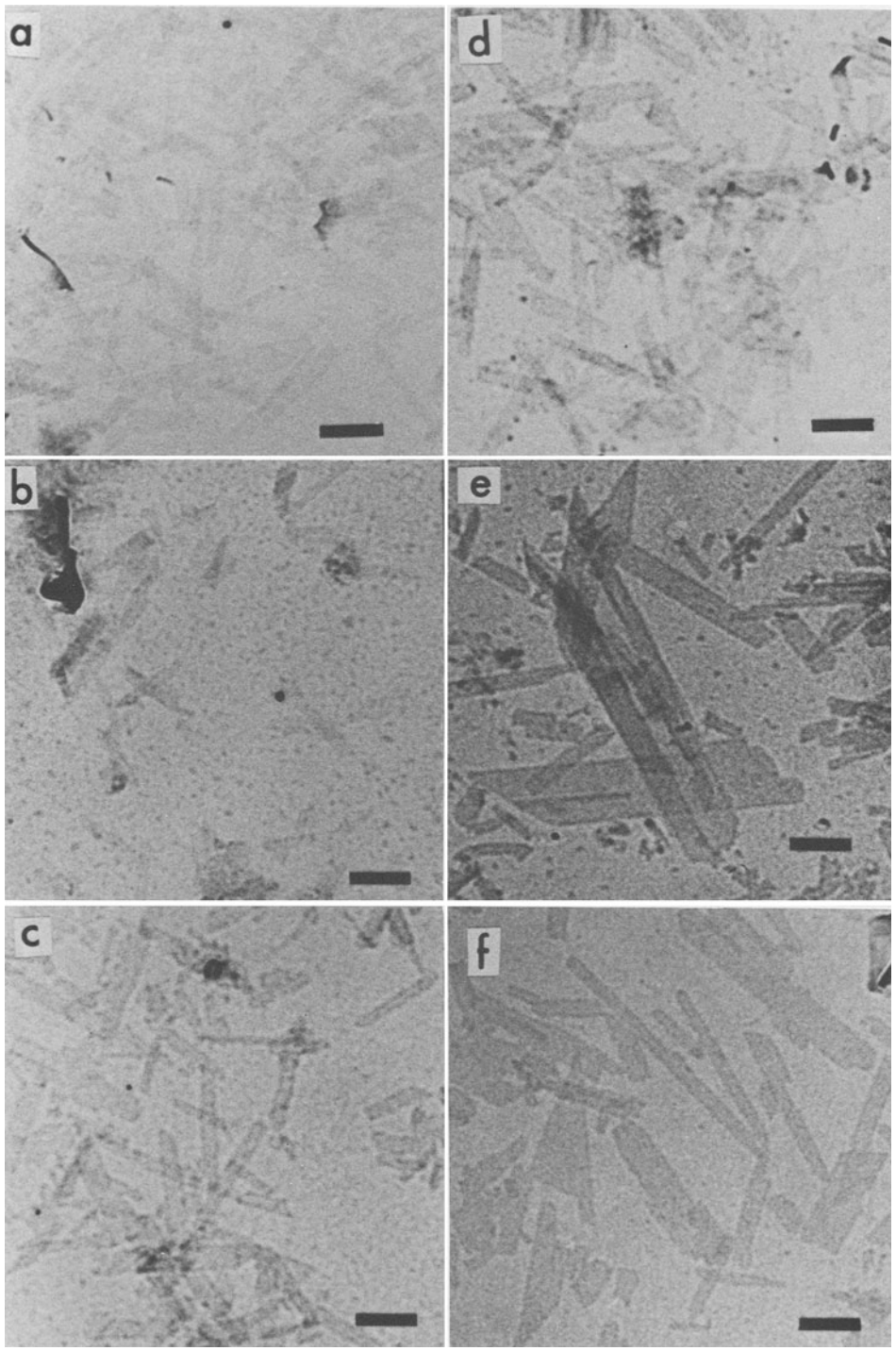

Figure 2. Electron photomicrographs of various hectorite specimens from same clay locality. Bar unit = 0.25 μm.

https://doi.org/10.1346/CCMN.1979.0270409 Published online by Cambridge University Press

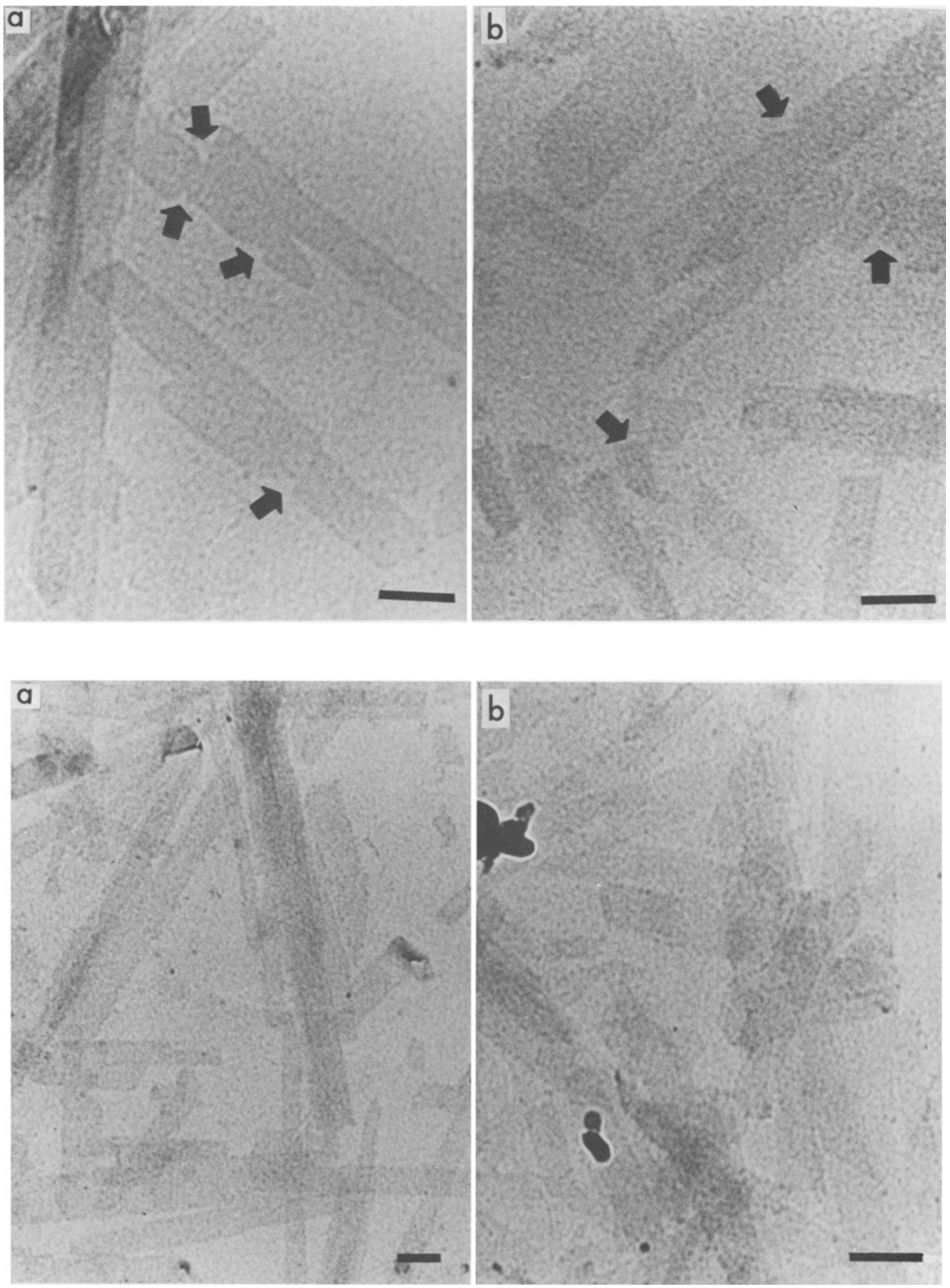

Figure 4. Electron photomicrographs illustrating longer laths in hectorite specimens exhibiting more angular stacking. Bar unit ≈ 0.1 μm.

https://doi.org/10.1346/CCMN.1979.0270409 Published online by Cambridge University Press

2f is characterized by more edge-to-edge associations. A few triangular-shaped particles can be seen in Figure 2f.

At higher magnification, photomicrographs of areas where particles remained flat during freeze drying suggest that some particle shapes might be due to edge-to-edge (or less likely end-to-end) association, followed by degradation along fissures in the laths as seen in Figure 3. In specimens with more angular stacking of laths, as in Figure 4, the longer laths are dominant.

ACKNOWLEDGMENTS

NL Industries and the Robert A. Welch Foundation are recognized for financial support of this research project.

Chemistry Department
Baylor University
Waco, Texas 76703

JAMES L. MCATEE, JR.
GWEN LAMKIN

REFERENCES

Bates, T. F. (1958) *Selected Electron Micrographs of Clays: 37–44. Circular No. 51:* College of Mineral Industries, Pennsylvania State Univ., 61 pp.

Corbet, H. C. and Wolffe, J. (1956) A freeze-drying technique for investigation of sodium montmorillonite: Proc. Stockholm Conf. Electron Microscopy, Almquist and Wiksell, Stockholm, 334–335.

Hofmann, U., Scharrer, E., Czerch, W., Fruhauf, K., and Burck, W. (1962) Grundlagen der trockengefreszten Massen: *Ber. Dtsch. Keram. Ges.* **39,** 125–130.

Jernigan, D. L. and McAtee, J. L. (1975) Critical point drying of electron microscope samples of clay minerals. *Clays & Clay Minerals* **23,** 161, 162.

McKee, T. R. and Brown, J. L. (1977) Preparation of specimens for electron microscopic examination: In *Minerals in Soil Environments,* J. B. Dixon and S. B. Weed, eds., Soil Science Soc. of America, Madison, Wisconsin, p. 209.

Mering, J. and Oberlin, A. (1971) The smectites: In *The Electron-Optical Investigation of Clays,* J. A. Gard, ed., Mineralogical Society, London, p. 195.

Williams, R. C. (1953) A method of freeze-drying for electron microscopy: *Exp. Cell Res.* **4,** 188.

(Received 10 January 1979; accepted 16 April 1979)