NOTE

A METHOD OF SPECIMEN PREPARATION FOR ELECTRON MICROSCOPE OBSERVATION OF ALLOPHANE PARTICLES

The specimen usually used for electron microscope observation of allophane is a drop of suspension dried on a microgrid. The feature we can observe is, however, only an aggregate of hollow spherules (Kitagawa, 1971; Henmi & Wada, 1976). It may be desirable to observe allophane particles as they exist in suspension. In this note, a suspension of allophane and collodion is used to form a plastic film containing densely arranged minute hollows. The film surrounding the particles is very thin in the hollows. The formation of such hollows in a Formvar or collodion film was studied in the early days of microgrid preparation. A modification of Saka-ta’s (1958) technique is used here in an attempt to obtain a dispersion of the particles throughout the film.

Materials and methods

Allophane. The allophane used was separated from a weathered pumice from Moro, Kanuma, Japan. After the gel film was removed, the pumice was treated with 6% H₂O₂, and then dispersed at pH 4-4.5 with ultrasound (19.5 kHz). The <100 nm fraction was collected after centrifugation at 3000 g for 30 min and stored in a solution of 1 M NaCl and 1 mM HCl at a concentration of 0.1% (w/v) of the clay. The suspension had a pH of 4.3 when used for the following experiment.

Specimens for electron microscopy observation. One ml of the suspension was pipetted into a centrifuge tube, mixed with 4 ml of ethanol-0.2 M HCl, 199 : 1 (v/v), solution, and then centrifuged at 150 g for 20 min. After removal of the supernatant, the sediment was washed with 2 ml of the same solution in the same way. The washed clay was mixed with 4 ml of an 8% collodion solution in amyl acetate by hand-shaking. Absolute ethanol was not used in this washing operation because the clay washed with absolute ethanol and then centrifuged does not readily deflocculate when mixed with collodion by hand-shaking.

The apparatus for making plastic films (Fig. 1) was placed in a room where a temperature of 15–17°C and a relative humidity of 80-90% was maintained. The humidity was generated by the evaporation of mist from an ultrasound sprayer agitating the water with ultrasound. A box, the front of which had dimensions of 60 × 60 cm and the depth of which was 30 cm, contained a motor fan and a large petri dish of 15 cm diameter placed in a line along the front. The dish was filled with water having the same temperature as the air. The front of the box was then covered with a vinyl sheet with an opening 20 × 20 cm on the dish side. Beside the box, an insulated flask containing warm (45–50°C) humid air and warm water was placed on an electric heating plate. Two tubes were connected to the flask. One, having an inner diameter of 6 mm, extended from the bottom filled with the warm water and was connected to a compressed-air cylinder linked to a compressor via a tube. The other, having an inner diameter of 8 mm, extended from the top filled with the warm humid air and reached the point in the box 30 cm above the surface of the water. This tube was heated by an electric heating tape and covered with heat insulating material.

The atmosphere within the box was saturated with water vapour by using a hand sprayer. The
fan, rotated by the motor, created a flow of air over the surface of the water. The velocity of flow was just enough to slightly stir a hanging piece of tissue paper. A drop of the suspension of allophane and collodion was dripped on to the surface of the water. It spread and immediately became a thin layer. After 5 s, warm humid air was blown on to the surface of the collodion layer by passing air, at a pressure of 0.1–0.2 bar, through the warm water in the flask for 1–2 s. As the air over the surface was continuously being moved by the motor fan, the warm humid air was removed quickly after arriving at the surface. The same blowing operation was repeated at intervals of 2 s. The amyl acetate ceased to evaporate with the fourth operation, 12–15 s after the first operation was done. The resulting film was 9–10 cm in diameter and the surface had a blue interference colour. It was scooped up with a microscope slide on which copper grids had been placed, and then air-dried.

The above film contained densely arranged minute hollows, the formation of which can be explained in the following way. Water vapour from the warm humid air, combined with water vapour in the air, arriving at the surface of the collodion layer, forms droplets, which depress the collodion. These droplets evaporate quickly and a cycle of condensation and evaporation is induced by the repeated blowing operation. The water droplets on the point of evaporation cannot penetrate deep into the collodion and therefore cannot push the particles aside although they are able to form the hollows by depressing the collodion.

For comparison, Sakata's technique (Sakata, 1958) was examined in the following way. A large petri dish of 15 cm diameter was filled with cold (5°C) water containing pieces of ice which were then arranged along the round edge of the water. A drop of the suspension was spread on the surface of the cold water. After 7 s, it was breathed upon, and then allowed to stand for 1.5–2 min until the amyl acetate had all evaporated. The idea of the method is that water droplets continue to exist for the period of the evaporation of the solvent, probably penetrating deep into the collodion and pushing the particles aside.

Every film prepared was coated with carbon, and each was examined with a JEOL-JEM 2000 EX electron microscope. An accelerating voltage of 120 kV and a magnification of 60,000 was used to obtain an image of the specimen.

Results

Allophane particles in the film prepared by the technique described are shown in Fig. 2. The smallest particles are hollow spherules and the larger particles comprise similar spherules. The spherules observed correspond to the unit particles of allophane (Kitagawa, 1971; Henmi & Wada, 1976).

Figure 3 shows an aggregation of particles around the edges of the hollows in the film prepared by the technique of Sakata (1958).
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REFERENCES


