THE MICROSTRUCTURE OF DILUTE CLAY AND HUMIC ACID SUSPENSIONS REVEALED BY FREEZE-FRACTURE ELECTRON MICROSCOPY

Direct observation of the structural organization of dilute clay and humic acid (HA) suspensions has not been reported, probably because of the difficulties and problems associated with the sample preparation procedures. To date, most electron micrographs of clay and HA samples are those of samples that had been air-dried or freeze-dried (Sides and Barden, 1971; Chen and Schnitzer, 1989; Stawinski *et al.,* 1990). No doubt, the structural features of clays and HA revealed by those studies differ significantly from the original structure in suspension, due to artifacts of the drying processes. Although the method of freeze-drying is less disruptive than air-drying, slow cooling rates and lack of a cold stage during freeze-drying often lead to unacceptable distortions of the suspension structure due to water crystallization.

A freeze-fracture electron microscopy (FFEM) technique can minimize the distortion. Attia *et al.* (1987) indicated that the FFEM method produced fewer artifacts than did freeze-drying, air-drying, or gel substitution of samples, and provided high-resolution imaging of structured colloidal systems. In essence, the FFEM technique is a means of preparing "wet" materials for examination in the electron microscope without the need for drying procedures. By this method, a sample is frozen extremely rapidly (about 10^4 K/s) to vitrify water (Zasadzinski and Bailey, 1989). This rapid phase change is accompanied by much smaller ice crystals than those produced by slower freezing, and therefore the possible damage to colloid structure is minimized. The FFEM technique has been widely used in the preparation of biological materials and polymer colloids for electron microscopy (Attia *et al.,* 1987; Zasadzinski *et al.,* 1987; Abeysekera *et al.,* 1990; Disanayaka *et al.,* 1990). However, it has received much less attention in the characterization of soil/clay dispersion-flocculation studies. This study was undertaken to explore the structural arrangements and dimensions of dilute clay and HA suspensions.

MATERIALS AND METHODS

The <2-µm fractions of Na-illite (IMt-1, Silver Hill, Montana) and of Na-montmorillonite (SWy-1, Crook County, Wyoming) were prepared as described by Gu and Doner (1990). The humic acid (HA) sample was isolated from a local soil (Gu and Doner, 1992). It was purified with a mixture of 0.1 M HCl and 0.1 M HF,

and with a previously-washed chelating resin (Chelex 100, Na-form). The purified HA contained 1.2% ash. One percent clay and HA suspensions were used for all preparations observed with electron microscopy.

The steps of freeze-fracturing and etching were as follows. About 4 µl of the dispersed suspension was pipetted onto a 3 mm disc. The specimen was then rapidly immersed in molten Freon-22 cooled to about −150°C with liquid N_2. After several seconds the specimen was transferred to liquid N_2 for storage. Four frozen samples were then placed on a cold stage (−150°C) of a Balzers BA360M freeze-etch device, fractured and etched for 1 minute with the knife placed normal to the specimen at −100°C under a vacuum of 2×10^{-6} Torr. The etching removes approximately 75 nm of frozen water by sublimation from the fractured surface (Zasadzinksi *et al.,* 1987). The low pressure was necessary to avoid condensation of water on the fresh surface. The replication of the surface was accomplished by depositing a platinum-carbon film at a shadow angle of 45° for 7.5 seconds, immediately followed with a carbon film at 90° for added strength. The Pt-C and C films were deposited using resistance-type evaporators. The clay specimens were dissolved in 12 M HF, whereas the HA dissolved in 0.1 M NaOH. The remaining replica was rinsed twice with distilled water and mounted on a 300-mesh copper grid for observation by TEM. A Zeiss 109 microscope operated at 80 kV was used for all observations. Resolution of the freeze-etched surfaces, limited by the platinum particle size on the replica, was about 4 nm (Harsh and Doner, 1985).

RESULTS AND DISCUSSION

Illite and montmorillonite suspensions

Figure 1 illustrates Na-illite and Na-montmorillonite dispersed in distilled water. The illite suspension showed a random arrangement of fine clay particles in the suspension (Figure 1A), whereas the montmorillonite particles tended to form T-shaped "duplets" or H-shaped "triplets" (Figure 1B). Both clay suspensions were very stable and no apparent flocculation was observed. It should be noted that the shadow areas (dark areas in the photos) indicate those protruded clay particles. Figure 1B shows montmorillonite sheets perpendicular to the fracture surface with a part of them
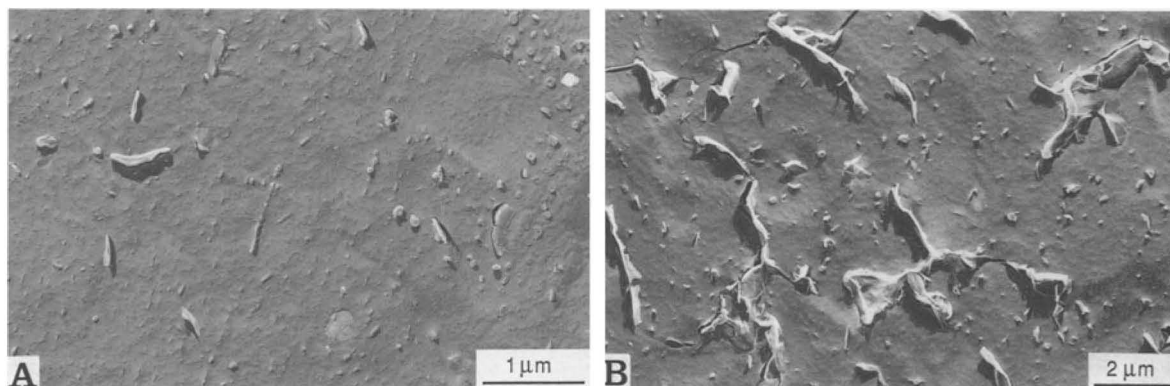
Figure 1.   Electron micrographs of 1% (A) Na-illite and (B) Na-montmorillonite dispersed in distilled water.

embedded in the ice. No particles parallel to the fracture face were observed. This is probably due to the fact that montmorillonite sheets parallel to the fracture surface will have the least resistance to being pulled out of the ice matrix, or they may be too thin to be observable at the resolution of the replicas. It should also be noted that the rough background of the replicated sample was due to sublimation of water vapor from the fractured surface, which produced etch pits and aspirates. Staehelin and Bertaud (1971) found that the size of aspirates depended on the amount of water vapor recondensation.

Figure 2 shows the illite and montmorillonite suspensions in the presence of 0.01 M $NaClO_4$. The illite suspension (Figure 2A) was coagulated since its critical coagulation concentration value was measured to be 0.0082 mole/liter in $NaClO_4$ (Gu, 1991), in agreement with that obtained by Arora and Coleman (1979) and Rengasamy (1983). Compared with Figure 1A, coagulating illite particles show two major types of fabrics: edge-to-edge association of individual platelets and stepped clusters of edge-to-face and face-to-face ori-

ented flakes. This orientation suggests that under these experimental conditions the double layer of each clay particle was compressed resulting in an increase in the van der Waals forces of attraction. However, most illite particles showed edge-to-edge associations, and were separated by a relatively large distance. It is possible that the process of transferring a small drop of the sample onto the disc disturbed the original structure of the illite suspension (coagulated) and the suspension was somewhat restabilized due to electrostatic repulsion between illite particles before it was frozen. This idea is supported by the fact that there is practically no difference in the negative electrophoretic mobility of illite particles in the presence or absence of 0.01 M $NaClO_4$ (Gu, 1991). Therefore, although the sample was allowed to set on the disc for several minutes, the original coagulated state of the particles was not completely regained before the specimen was frozen in Freon.

For the montmorillonite suspension (Figure 2B), most clay particles tended to form edge-to-face associations, but some formed edge-to-edge associations. An inter-
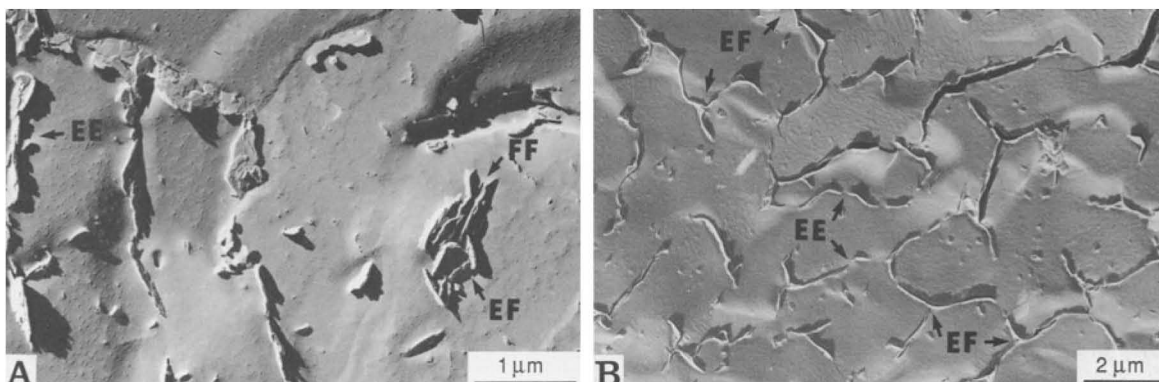

Figure 2.   Electron micrographs of (A) Na-illite and (B) Na-montmorillonite suspensions in 0.01 M $NaClO_4$. The illite suspension was coagulated and therefore clay concentration shown in graph (A) was >1%. EE, EF and FF represent edge-to-edge, edge-to-face and face-to-face associations of clay particles, respectively.
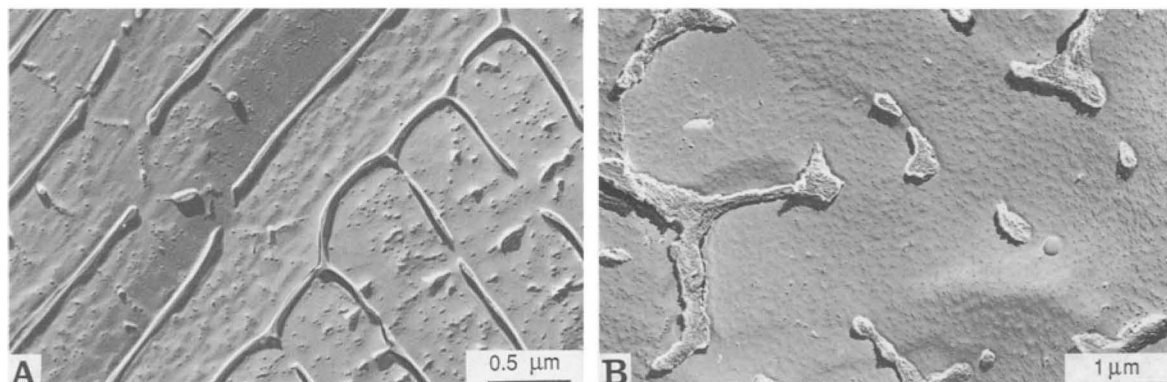
Figure 3. Electron micrograph of 1% humic acid at (A) pH 7 and (B) pH 2.

linked solid-particle network was seen clearly. Such particle association was also observed by the formation of a gel-like suspension. An idealized hypothetical model for particle association in montmorillonite suspensions has been proposed by van Olphen (1977, 1989). In this model, three different modes of particle association are considered: edge-to-face, edge-to-edge, and face-to-face. These different associations are governed by different sets of double-layer and van der Waals interaction forces. He has indicated that because of the presence of opposite charges on edge and face surfaces edge-to-face association must always occur, unless the edge charge has been reversed by an increase of pH or the adsorption of additives. He has also considered that in dilute, salt-free montmorillonite suspensions, edge-to-face association may only be limited to the formation of light-weight, T-shaped duplets or H-shaped triplets, which would not readily settle as flocs. With addition of salt, the double layers on both edge and face surfaces are compressed so that in addition to edge-to-face association, edge-to-edge association occurs. The results presented here (Figures 1B, 2B) are apparently in excellent agreement with van Olphen's hypothesis.

Humic acid suspensions

A 1% HA suspension at pH 7 was examined and is shown in Figure 3A. The HA macromolecules showed an interesting, well-oriented, parallel structure in the suspension. Each HA macromolecule is an elongated sheet or fiber, about one-half to several micrometers long and about 30 to 40 nm in thickness (or diameter). The parallel orientation of the HA macromolecules is believed to be caused by water crystallization during freezing, and also probably an electrostatic repulsion between elongated sheets or fibers of HA. Such a long sheet or fiber is possibly a bundle of hydrogen-bonded HA molecules, as indicated by Chen and Schnitzer (1989). At pH 2, the HA was precipitated or aggregated, and appeared as a massive and short-rod structure (Figure 3B). Such a massive HA unit was probably formed

by conformational change of HA molecules to randomly coiled units at low pH, followed by aggregation through hydrogen bonding.

Previous workers (Orlov *et al.,* 1975; Chen *et al.,* 1978; Ghosh and Schnitzer, 1982; Stevenson and Schnitzer, 1982, 1984; Tan, 1985) studied the structural features of HA which had been air-dried or freeze-dried. As can be expected, the structural features revealed by those studies appeared difficult to observe, especially for those samples that had been air-dried. For example, Orlov *et al.* (1975) distinguished air-dried HA at pH 6–7 as only "minute" particles with diameters about 3 nm. At higher concentrations, HA appeared as featureless strata or sheets of condensed material. Similar results were obtained by Visser (1963) and Flaig *et al.* (1975). Much improvement was made by employing the technique of rapid freezing and freeze-drying. For example, Tan (1985) observed that HA at pH 7.0 had the appearance of shredded sheets. Chen and Schnitzer (1976) studied the effect of pH on the shape and particle arrangement of HA. At pH 6, the particles were found to consist of bundles of fibers. At pH 8, the fibers tended to form a sheet-like structure, and at pH 10 the particles aligned themselves longitudinally to form thin sheets. A number of other publications using the same freeze-drying technique (Chen *et al.,* 1978; Ghosh and Schnitzer, 1982; Stevenson and Schnitzer, 1982, 1984) have confirmed that the most common structure found in humic substances prepared by drying solutions at neutral to slightly acidic pH values is that of fibers or bundles of fibers. Chen and Schnitzer (1989) indicated that the fibers or bundles of fibers were formed by hydrogen-bonded humic molecules. Several tens (possibly 50–100) of associated molecules can form a fiber 50 to 100 nm thick and several micrometers long.

Practical applications

The FFEM technique is a useful tool for studying the behavior of colloidal suspensions. First, it provides direct visualization of the structural organization of a

colloid suspension. The flow behavior, colloidal stability and viscosity of a suspension are largely influenced by such a structural organization, as in the case of montmorillonite (van Olphen, 1989; Low, 1989). Second, it gives valuable information on the shape, morphology, size and size distribution of the particles or units that make up the dispersion in their natural state, which is often unattainable by drying the sample, especially for organic polymers. The surface adsorption of organic polymers on clay surfaces and the structure of adsorbed polymers can be studied by such a technique. For example, with the FFEM technique Barker *et al.* (1982) visualized the ultrastructure of organoclay complexes in their natural state. Harsh and Doner (1985) reported the use of the FFEM technique to explore details of interlayer surfaces of a hydroxy-aluminum montmorillonite. Gu (1991) viewed an anionic polysaccharide by FFEM and found that polysaccharide chains formed a cross-linked network that largely explained its effectiveness in the formation of stable soil aggregates as compared with HA.

However, it must be pointed out that electron microscopy of replicas requires discrimination between artifacts and original structures. The replicas can often be damaged by ruptures of the carbon-platinum film and artifacts. In the present study, the question remains as to what extent the structural changes seen are due to water crystallization by this fast freezing method. The parallel HA macromolecules in Figure 3A were observed on replicas prepared at different times, and are thought to be caused by water crystallization and possible electrostatic repulsion between HA units. It is still unclear why this did not happen to the clay samples. Obviously, further study is needed using an even faster freezing method such as propane-jet freezing.

SUMMARY AND CONCLUSIONS

The FFEM technique made it possible to see the structure of particles or units that made up clay and HA dispersions, and revealed their orientation and distribution with high resolution. The Na-illite clay showed randomly distributed clay particles when dispersed in distilled water. With the addition of salt (0.01 M $NaClO_4$), illite particles tended to coagulate and form a "card-house" structure. The particle arrangement of montmorillonite in suspension was in excellent agreement with van Olphen's hypothetical model. At very low electrolyte concentration montmorillonite particles tended to form light-weight duplets or triplets, whereas the majority of the particles were edge-to-face associated and formed an interlinked solid particle network at 0.01 M $NaClO_4$. HA in suspension at pH 7 appeared as elongated sheets or fibers, whereas at pH 2 it showed massive short-rod structure, probably formed by aggregation of HA molecules through hydrogen bonding.

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