Clays and Clay Minerals, Vol. 40, No. 5, 620–623, 1992.

NOTES

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

Key Words—Cryofixation, Freeze-fracture, Microstructure of clay suspensions, Smectite, Transmission electron microscopy.

Investigations of the structural state of colloidal inorganic and organic systems in conventional air- or freeze-dried samples face considerable problems as a result of artifacts produced by sample-preparation procedures. In an attempt to overcome these problems, Gu and Doner (1992) recently applied freeze-fracture electron microscopy (FFEM) to clay and humic-acid suspensions. The most critical aspect of this cryofixation technique is the cooling rate. If the cooling rate is too slow, the formation of ice-crystals and phase separation still disturb the original structure of the suspension. Gu and Doner (1992) quote maximum cooling rates achievable for FFEM on the order of 10^4 K s^{-1}, which would probably be sufficient to minimize artifacts due to sample preparation, although rates an order of magnitude higher (10^5) are preferred to exclude the possibility of artifacts (see below). However, we doubt that Gu and Doner (1992) have achieved sufficiently high rates in their experiments, for two reasons. First, cooling rates reported in the literature for the conventional freeze-fracture technique used by Gu and Doner (1992) are on the order of 10^2–10^3 K s^{-1}, insufficient for avoiding cryo-artifacts (Moor, 1964; Costello and Corless, 1978; for review see Plattner and Bachmann, 1982; Robards and Sleytr, 1985; Menco, 1986). Secondly, in our re-interpretation of their transmission electron microscopic (TEM) images, we identify the effects of cryo-artifacts. In order to elaborate on this point, we shall discuss their Figures 1–3 by comparing them with results obtained by us (1) with high-rate cryofixation-techniques (Bachmann and Schmitt, 1971; Moor *et al.*, 1976; Müller *et al.*, 1980; Pscheid *et al.*, 1981) and (2) with conventional freeze-etch techniques similar to the ones used by Gu and Doner (1992).

High pressure freezing (Riehle, 1968; Riehle and Hoechli, 1973) for cooling rates of 10^5 K s^{-1} are required to limit segregation below the 10 nm level. Cooling rates of this order can be achieved with spray-freezing (Bachmann and Schmitt, 1971) or sandwich-jet-freezing (Moor *et al.*, 1976; Müller *et al.*, 1980; Pscheid *et al.*, 1981), which are based on the principle of dividing the fluid to be frozen into small enough "parcels." These techniques have been applied successfully to clay-colloidal systems (Vali and Bachmann, 1988; Vali *et al.*, 1991).

A freeze-etch replica of a 3% dispersion of the <0.2 μm fraction of Na-smectite Nevada UCJ from northern Nevada (supplier: Südchemie AG, Munich) in deionized water showed individual free particles dispersed in water after jet-freezing (Figure 1a). The particle size varied between 0.05 to 0.5 μm. The individual particles are isolated from each other and randomly oriented. Formation of T-shaped or H-shaped aggregates as reported by Gu and Doner (1992) from their 1% montmorillonite dispersion (their Figure 1b) was not observed in the jet-frozen, dispersed smectite-suspension. The quality of cryofixation was checked by a 5% glycerol/water control solution which was jet-frozen under the same conditions as the smectite suspension (Figure 1b). Micro-segregation in the glycerol solution produced dimensions in the range of <0.05 μm, much smaller than the size of individual smectite particles. In contrast, freezing the same smectite dispersion by the conventional technique used by Gu and Doner (1992) resulted in the aggregation of free particles (Figure 1c). The structure shown in this replica is very similar to that in Figure 1b of Gu and Doner (1992). The low cooling rate permits the growth of relatively large ice crystals, which push the clay particles aside and lead to the formation of a three-dimensional clay network. In part, the clay particles may be rotated and their original distribution in dispersion destroyed. Obviously, such a structure represents a cryo-artifact and does not reflect the true microstructure of the colloidal system as suggested by Gu and Doner (1992) for their Figures 1b, 2a and 2b. Freeze-etching of the 5% glycerol/water control-solution under the same conditions clearly showed that, in contrast to jet freezing, the cooling rate of conventional cryofixation is insufficient to prevent the formation of larger ice crystals (Figure 1d). The dimensions of the crystals are much larger than those of the free smectite particles shown in Figure 1a. It is therefore not possible to distinguish between ef-

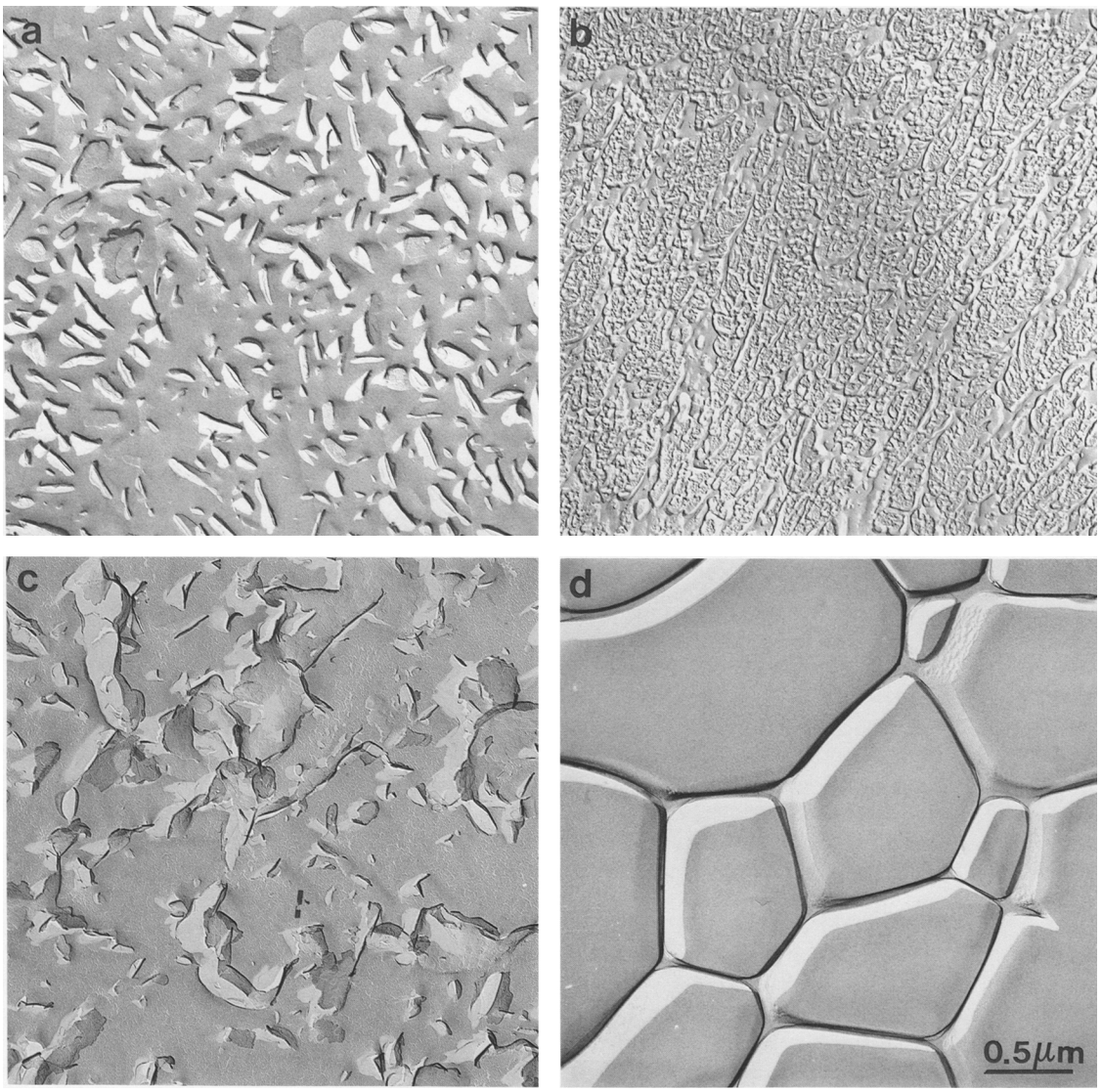

Figure 1. TEM images of freeze-etch replicas; a) 3% smectite dispersion in deionized water jet-frozen, showing randomly oriented, dispersed free smectite-particles; b) jet-frozen 5% glycerol/water solution confirming the quality of freezing; c) the same dispersion as in (a) conventionally frozen, exhibiting particle aggregation resulting from ice crystallization; d) the same glycerol/water solution as in (b) showing network structure resulting from a freezing artifact.

fects of coagulation caused by dispersing the suspension in an electrolyte solution, and those caused by cryo-artifacts, if cooling rates are too low. The fabric seen in Gu and Doner's (1992) Figure 2b is not a reliable indicator of coagulation.

The occurrence of coagulation, which is not a freezing artifact, can be demonstrated in high cooling-rate experiments. The microstructure of the 3% smectite suspension dispersed in a 0.006 M NaCl solution (pH = 7.5) and viewed in a non-sheared sandwich-jet-frozen specimen (Figure 2a) exhibits a section through the three-dimensional honeycomb-like network composed of extended sheet-like, face-to-face (FF) aggregates. The high-resolution image of the freeze-etch replica (Figure 2b) revealed predominant FF associations of individual smectite particles. The surface of these extended large aggregates is covered with steps indicating that they are composed of units (free smectite particles?) which are stacked upon each other. Triple points representing EF contacts are small in number compared to the many hidden EE and FF contacts between individual smectite particles. Aggregates must be present because they are required to form the network of Figure 2a from the small free particles shown in Figure 1a. One of the triple points (arrow in Figure 2b) suggests splitting of an FF aggregate at the end and bending of one of the two branches at termination.

By relating their results to van Olphen's (1977, 1989)

https://doi.org/10.1346/CCMN.1992.0400517 Published online by Cambridge University Press

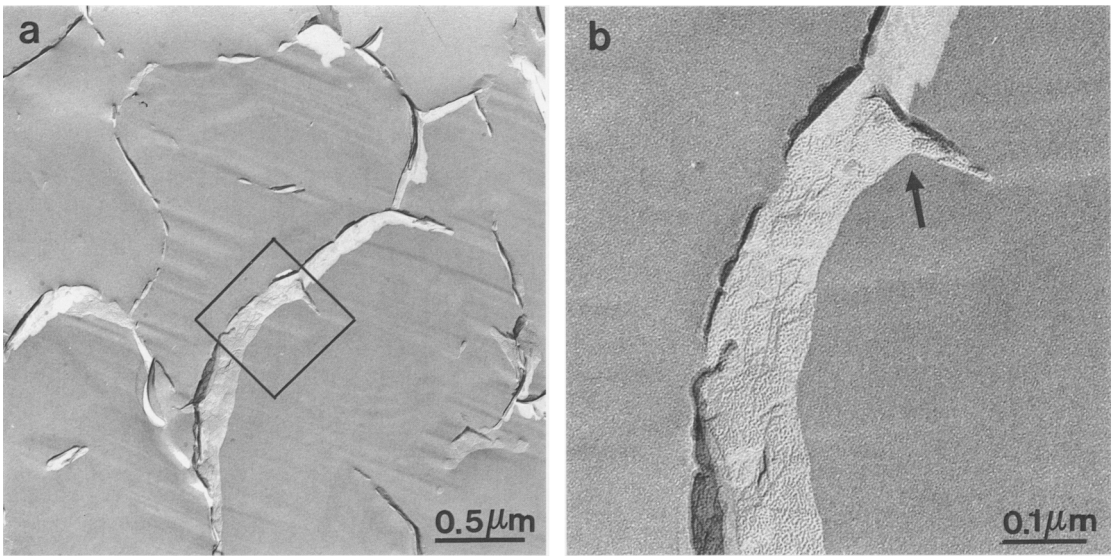

Figure 2. TEM images of freeze-etched replicas of the 3% smectite suspension dispersed in 0.006 M NaCl and jet-frozen, showing gel structure. a) Honeycomb-like network composed of predominant face-to-face (FF) aggregates that may include numerous hidden EE contacts originating from the small free particles in Figure 1a. b) Higher magnification of (a) reveals numerous steps on the surface of elongated aggregate as well as bending of feathered termination of one particle (arrow).

hypothesis concerning particle associations, Gu and Doner (1992) state an excellent agreement between their TEM observations and the proposed edge-to-edge (EE) and face-to-edge (FE) particle-fabrics in Na-montmorillonite suspensions. It is important to note, however, that van Olphen's model of particle associations is based on the interaction between the individual particles, which occur as free particles in a colloidal dispersion (Figure 1a). Employing the same terms used to describe these interaction models for the FFEM images (Figure 2b) by Gu and Doner (1992) is misleading. If there were indeed EF or EE interactions between *individual* smectite particles, the dimensions of cells in the network of Figure 2a or Figure 2b of Gu and Doner (1992) should be in the same size range as those of the free particles in the dispersion of Figure 1a.

Gu and Doner (1992) also used FFEM to study structural features of humic acid (HA) suspensions. Based on their freeze-etch replicas (their Figure 3a), the HA macromolecules are described as elongated sheets or fibres. Again, the quality of the replicas does not warrant such an interpretation. Since the morphological features of isolated molecules are not visible, it is most likely that the observed structure is a cryo-artifact, as already suspected by the authors. The fact that this structure is very similar to the feature seen in the 5% glycerol/water solution after conventional freezing (Figure 1d) supports this suggestion. The true structure of isolated macromolecules can be examined in TEM using spray- or jet-freezing (for review see Bachmann, 1987).

The objective of this discussion has been to demonstrate the capability of fast cryofixation techniques in preserving the original structure of colloidal dispersions. It is important to emphasize that inadequate techniques of cryofixation do not permit the original distribution of the particles in dilute dispersions to be observed. The quality of freeze-etch replicas should be checked by using dilute solutions of glycerol or other compounds of low molecular weight as a test for the actual experiment. The cryotechniques discussed have important applications in colloid chemistry, soil mechanics, soil chemistry, clay mineralogy, and other fields dealing with the interaction between colloidal particles and organic matter.

ACKNOWLEDGMENTS

The cryofixation and freeze-etching work was performed in L. Bachmann's EM-Laboratory at the Institut für Technische Chemie, Technical University of Munich, West Germany. Helpful discussions with L. Bachmann are gratefully acknowledged. This research was supported by grants from the Deutsche Forschungsgemeinschaft (DFG), Germany, the Natural Sciences and Engineering Research Council (NSERC) of Canada, and the Petroleum Research Fund administered by the American Chemical Society (ACS).

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(Received 26 June 1992; accepted 21 September 1992; Ms. 2238)