

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

Key Words—Clay suspensions, Electron microscopy, Freeze fracture, Microstructure, Montmorillonite.

Vali and Hesse's comments (1993) on our studies on the microstructure of dilute clay and humic acid suspensions (Gu and Doner, 1992) again raised an important issue of possible artifacts associated with freeze-fracture electron microscopy (FFEM) technique. As we had pointed out (Gu and Doner, 1992), artifacts such as a slow cooling rate, freezing rate variation within a single sample, and damaged replicas are common when using this technique. Therefore, electron microscopy of replicas of dilute colloidal suspensions requires discrimination between artifacts and original structure and deserves further investigation.

Following our studies on dilute clay and humic acid suspensions with the conventional freeze-fracture technique, we re-examined the structural organization of the montmorillonite suspensions with a high-rate cryofixation technique, i.e., sandwich jet-freezing (Gilkey and Staehelin, 1986). As will be presented below, the results were in general agreement with those reported earlier in Gu and Doner (1992). The association of montmorillonite particles was believed to be between individual montmorillonite particles instead of face-to-face (FF) associated aggregates, as Vali and Hesse (1993) suggested. It also must be emphasized that the smectite sample (Nevada smectite) and particle size ($<0.2 \mu\text{m}$) used by Vali and Hesse (1993) are very different from ours (Wyoming montmorillonite, $<2 \mu\text{m}$). The Nevada smectite is more likely hectorite (containing 21% MgO and 1.5% Li_2O , Vali and Bachmann, 1988). Direct comparison of our results (Gu and Doner, 1992) with those obtained by Vali and Hesse (1993) is, consequently, doubtful. Thus, we strongly disagree with Vali and Hesse (1993) for their criticism of our interpretation of the observed montmorillonite particle arrangements based upon the comparison between our montmorillonite (Figure 2B) with illite (Figure 1A) (Gu and Doner, 1992) both for the above-cited reason and because the sample preparation procedures, especially suspension pH and washing procedures, could also greatly influence the particle arrangements of the colloidal dispersion.

We agree with Vali and Hesse (1993) that our cooling rate with the conventional plunging-freezing method could have been slow. Because we did not measure the actual cooling rate, we later re-examined the structural organization of montmorillonite suspensions (3.2%) with the sandwich propane jet-freezing technique

(Gilkey and Staehelin, 1986). Figure 1A shows the particle arrangements of the Na-montmorillonite suspension at pH 6.8 and $20 \text{ mol m}^{-3} \text{ NaClO}_4$. The suspension was observed as a gel. The micrograph shows a cubic network with many particles edge-to-face (EF) associated, results similar to those observed earlier by Gu and Doner (1992) by the conventional-freezing method. Since the thickness of the platelets shown in the micrograph depended on the replicating coating, angle of shadowing, etching depth, and angle of observation in EM, FF stacking of montmorillonite particles could not be determined by the EM appearance. In addition, the resolution of the replica is limited by the replicating Pt-C coating (approximately 4 nm, Gu and Doner, 1992). Vali and Hesse claimed that their 3% Nevada smectite suspension in $6 \text{ mol m}^{-3} \text{ NaCl}$ at pH 7.5 was aggregated FF (within several nm range, Figure 2, Vali and Hesse, 1993), leading them to conclude that montmorillonite particles shown in our Figure 2B (Gu and Doner, 1992) were not individual montmorillonite particles but composed of layers stacked FF upon each other. We disagree for reasons noted below:

- 1) As mentioned previously, the material they used (Nevada smectite, or hectorite) is different from ours (Wyoming montmorillonite). In appearance, the Wyoming montmorillonite was larger and thinner than the Nevada smectite and often bent, while Nevada smectite looked like platelets (Vali and Bachmann, 1988). Also, 1% Wyoming montmorillonite showed a single silicate layer, while the Nevada smectite showed a few FF-associated silicate layers with increasing clay concentration. In other words, the Nevada smectite was more likely to form tactoids or quasicrystals than the Wyoming montmorillonite. Therefore, the use of observations with Nevada smectite (Figure 2, Vali and Hesse, 1993) to extrapolate to our montmorillonite (Gu and Doner, 1992) is invalid.
- 2) Vali and Hesse (1993) showed a reduced number of smectite particles in their Figure 2A ($6 \text{ mol m}^{-3} \text{ NaCl}$) compared with Figure 1A (deionized water) and concluded that this effect was due to the predominant FF associations of individual smectite particles. Using the jet-freezing technique, however, we did not see an apparent reduction in the number

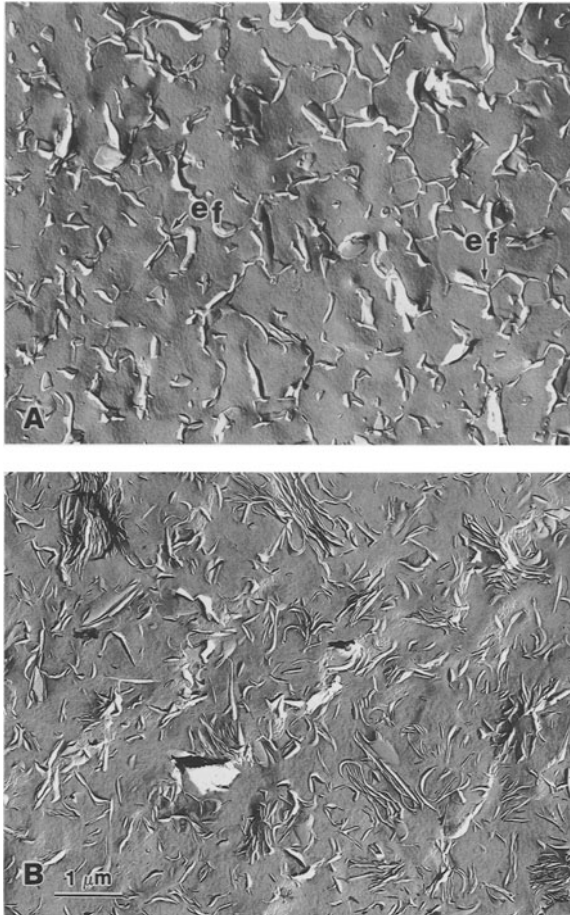


Figure 1. TEM images of jet-frozen freeze-fracture replicas of 3.2% Na-montmorillonite suspensions at A) pH 6.8 and $20 \text{ mol m}^{-3} \text{ NaClO}_4$, and B) pH 3.6 and $2 \text{ mol m}^{-3} \text{ NaClO}_4$ (coagulated). EF represents "edge-to-face" associations of montmorillonite particles.

of montmorillonite particles (Figure 1A) compared with those dispersed in deionized water or even in $80 \text{ mol m}^{-3} \text{ NaClO}_4$. Results presented in Figure 2A by Vali and Hesse (1993) may only represent part of the suspension structure since Figure 7B (Vali and Bachmann, 1988) for the same material and conditions showed a greater number of particles in general than that of Figure 2A (Vali and Hesse, 1993), although it is noted that the magnification in Figure 7B is about two times lower than that of Figure 2A.

- 3) A number of other studies using such techniques as X-ray scattering, viscosity, light scattering, chloride exclusion, and electron microscopy showed an absence of quasicrystals in dilute Na-montmorillonite suspensions (1.2 ± 0.2 unit layers, Sposito, 1984).

It also appears that Vali and Hesse (1993) have misinterpreted our Figure 2B (Gu and Doner, 1992) as coagulation caused by cryoartifacts. We considered it to be gel-formation when the montmorillonite particles formed an EF- and EE-associated cubic network, and the montmorillonite suspension was visualized as a stable gel. The actual coagulation of the Na-montmorillonite and Nevada smectite suspensions can be illustrated in Figure 1B at a reduced pH (3.6) and in Figure 7C of Vali and Bachmann (1988) at $10 \text{ mol m}^{-3} \text{ CaCl}_2$. A number of large aggregates were observed, which were composed of FF-associated montmorillonite particles (quasicrystals?). Figure 1B also shows that suspension pH has a pronounced effect on the association of the particles. A low pH may be related to the appearance of Al (or hydroxy-Al) on the surfaces of montmorillonite released from the clay structure. Both H^+ and Al (or hydroxy-Al) can form "inner-sphere" surface complexes with montmorillonite and effectively screen the surface negative charge (Sposito,

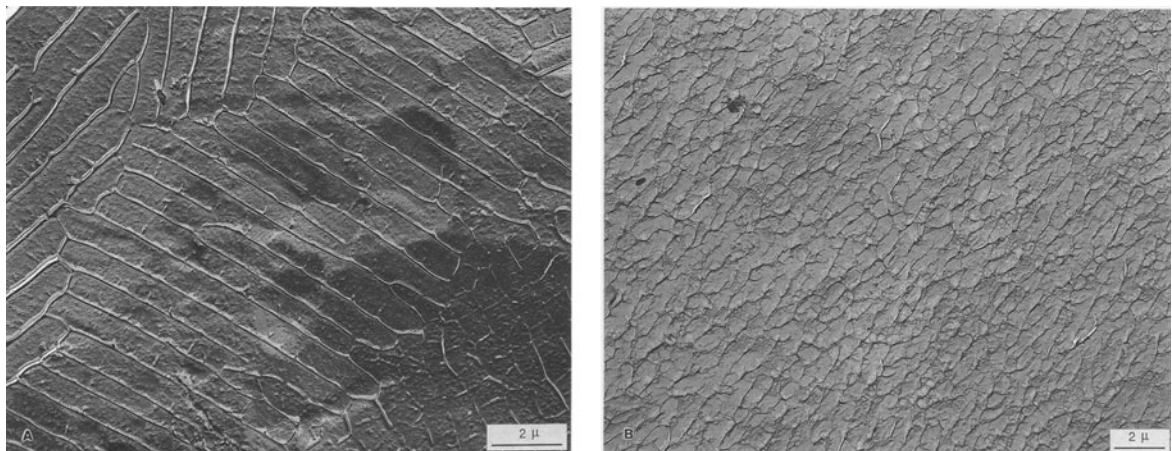


Figure 2. TEM images of conventionally frozen freeze-fracture replicas of 1% humic acid: A) an anionic polysaccharide; B) at pH about 7.

1984). A close FF interaction of the montmorillonite particles can thus be expected.

Regarding the structural features of the 1% humic acid (HA) suspension reported by Gu and Doner (1992), Vali and Hesse considered it to be a cryoartifact since the morphological features of isolated HA macromolecules (or units) were not visible. We, therefore, include herewith the same TEM image of HA at a reduced magnification (Figure 2A) along with the image of an anionic polysaccharide ($0.2 \times 10^6 < MW < 2 \times 10^6$, Aly and Letey, 1988) for comparison. Both the HA and polysaccharide replicas were prepared with the conventional-freezing method. It was unfortunate that we did not further examine their morphological features with the jet-freezing technique once we finished studies by the conventional-freezing technique. Although we did not check the replicas by using dilute solutions of glycerol (5%), as suggested by Vali and Hesse (1993), we did compare our replicas with that of glycerol (Staehelin and Bertaud, 1971; Menold *et al.*, 1976; or Figure 1D by Vali and Hesse, 1993). Ice nucleation is a random event in the absence of a structured solute and tends to form hexagonal ice crystals of various shapes and sizes (Echlin, 1992) (e.g., Figure 1D by Vali and Hesse, 1993).

We, therefore, believe that the morphological features and the pattern of HA shown in Figure 2A are correct although their spacing and orientation may be changed by a faster freezing method, as we pointed out previously (Gu and Doner, 1992). The elongated sheet or fibrous structure of HA at about neutral pH was also observed by a number of other investigators using freeze-dried samples (for review see Chen and Schnitzer, 1989). By comparing the image of HA (Figure 2A) with that of polysaccharide (Figure 2B), there are distinct differences in their morphological features although the concentration and sample preparation of the two samples were exactly the same: The polysaccharide showed a cross-linked polymer network. It is also interesting to note that the quality of our polysaccharide replica (conventional freezing) is comparable with a polysaccharide image (Figure 1B, jet-freezing) reported by Zasadzinski *et al.* (1987).

Finally, we would like to point out again that FFEM is a potentially useful tool for direct visualization of the structural organization, morphology, size, and size distribution of particles or units that make up the dispersion in their natural state; however, electron microscopy of replicas requires discrimination between artifacts and original structures. It is also important to realize that the high-rate cryofixation techniques such as spray freezing and jet freezing are not artifact free.

A concrete proof of perfect freezing is difficult to establish for any new sample (Zasadzinski and Bailey, 1989); therefore, structural studies of dilute colloidal suspensions by FFEM deserve further investigation.

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