

ELECTRON-MICROSCOPY OF MONTMORILLONITE DISPERSED AT VARIOUS pH

JAMES L. MCATEE, JR. AND WALTER HENSLEE, *Baylor
University, Waco, Texas 76703.*

ABSTRACT

Montmorillonite dispersions at pH 1 to 13 have been studied by electron microscopy utilizing the adsorption of the clay on cytochrome c protein film. This method is easy and quick and yields specimens that show the type of particle-particle interaction which is occurring in the clay dispersions.

INTRODUCTION

In suspensions of plate-like particles such as montmorillonites, three different modes of particle association can be considered: edge-to-edge, edge-to-face, and face-to-face. The type of association will be related to the electrical properties of the clay-water system and these are primarily determined by the electric double layers on the clay surfaces (Van Olphen, 1963).

The composition of the electrical double layer about both faces and edges of montmorillonite particles will be altered with variations in pH. Thus, the type of particle association might be expected to vary with pH.

In order to examine if this were true, a series of montmorillonite dispersions were prepared from pH 1 to 13. The montmorillonite was then adsorbed onto a monomolecular film of the protein cytochrome c, and examined by electron microscopy.

EXPERIMENTAL TECHNIQUES

Approximately 2 percent dispersions of sodium montmorillonite and sodium hectorite were prepared by ion exchange with Rohm and Haas IR120 exchange resin in the sodium form. The initial montmorillonite and hectorite¹ had been centrifuged and spray dried by the Baroid Division, National Lead Company for removal of the non-clay material. The cytochrome c was purchased from the Nutritional Biochemical Corporation.

The samples were prepared for electron microscopy in a manner reported by Lang (1967) for the study of DNA molecules. The procedure was as follows: a dilute clay dispersion (approximately 0.05% concentration) was poured into a teflon-coated dish that had a capacity of 35 ml. The surface of the clay dispersion was swept clean with a teflon-coated bar and then a small amount of dry, powdered cytochrome c was delivered to the surface of the dispersion by means of a needle. Talc particles previously sprinkled onto the surface of the bulk dispersion allowed the spreading of the protein film to be seen as it pushed the talc aside. Care was taken to keep the film area near the center of the solution to avoid the film from going over the meniscus of the dispersion.

After some given amount of time (usually five minutes), a carbon-coated microscope

¹The original montmorillonite was from the Newcastle formations of the Colony, Wyoming, area and the hectorite was from Hector, San Bernardino Company, California.

grid was touched to the cytochrome c surface, thereby picking up a small portion of the protein film with clay particles adsorbed to it. The grid was then placed on a filter paper to dry for a few minutes and then inserted into the electron microscope.

In order to make certain that the cytochrome c was indeed influencing the observations, several microscope grids were touched to the surface of the dispersion where no protein film had been placed. In all cases very little, if any, clay was found to be present on the grid. In contrast, almost any desired concentration of clay could be obtained on the grids touched to the protein film by variation of two parameters: 1) length of time after spreading the film before the grid was touched to its surface, and 2) concentration of the clay in the bulk solution. It was found by touching the grid to the film after various lengths of time that initial adsorption of the clay takes place quite rapidly.

A Hitachi Hu 11A with an accelerating voltage of 75 kV was used in these experiments.

RESULTS

Cytochrome c is a positively charged colloid and the adsorption is apparently simple electrostatic attraction between the negatively charged groups on the montmorillonite and the positively charged surface film. This was substantiated by the fact that kaolin, which does not have a negatively charged basal surface, did not adsorb to the cytochrome c film.

This method of specimen preparation was utilized to study the effect on clay dispersions by varying the pH. After adjusting the pH with either hydrochloric acid or sodium hydroxide, the cytochrome c film was placed on the clay dispersion and allowed to adsorb the clay for a five minute period. After this time the microscope grid was touched to the film and the specimen examined under the electron microscope.

Very significant morphological changes are noted when the pH of the bulk clay solution is varied. This is demonstrated by the series of pictures in Figure 1. At pH 1 (Fig. 1A) there is a great degree of stacking and aggregation of the platelets, and adsorption to the cytochrome c film is small. At a pH of about 4 (Fig. 1B) a fairly large degree of stacking and aggregation still occurs but adsorption to the film was greater than was observed at pH 1. A significant change was observed at about a pH of 6 or 7 where the individual clay particles become clearly visible. This is shown in Figure 1C. Figure 1D shows the individual, very thin montmorillonite platelets adsorbed from a dispersion adjusted to a pH of 11. At pH 13 (Fig. 1E) the platelets have largely become aggregated. Since cytochrome c has an isoelectric pH value of 10.6 (West *et al.* (1966)), the only clay-cytochrome c adsorption specimen that might be adversely effected would be those prepared at a pH value greater than 11. However, the clay sample prepared at pH 13 appeared to adsorb in a quantity that suggested that the cytochrome c monolayer still retained its positive charge although a partial change in the cytochrome c charge may contribute to the clay curling that was observed.

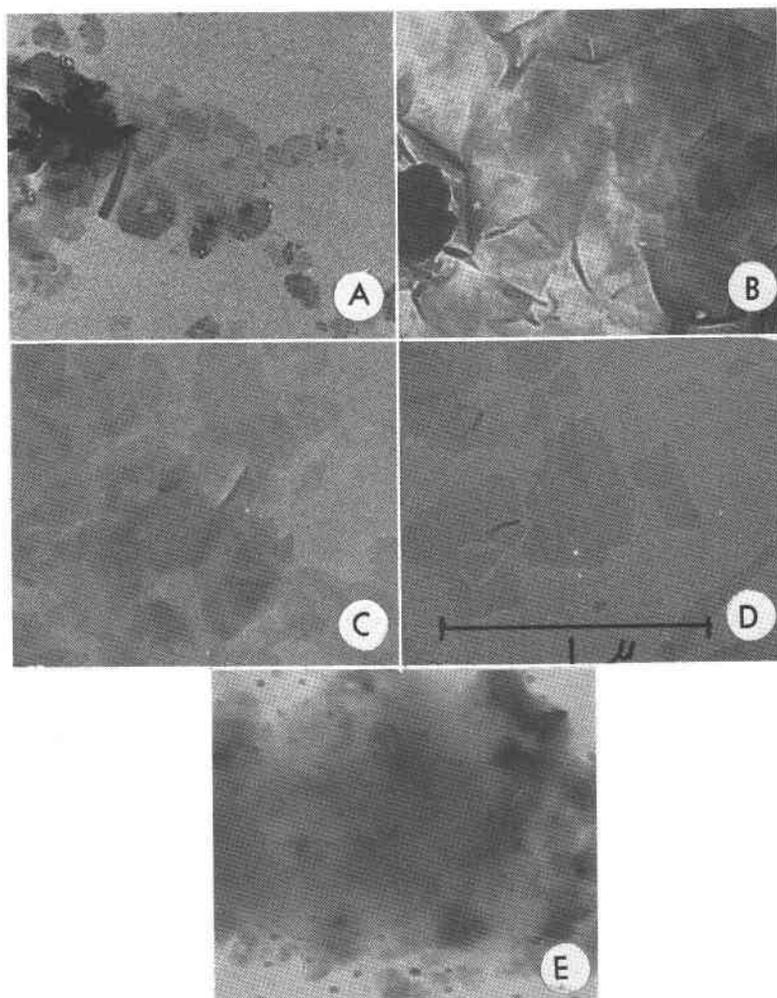


FIG. 1. Electron micrographs of sodium montmorillonite at (A) pH 1, (B) pH 4, (C) pH 6, (D) pH 11 and (E) pH 13. Adsorption time 5 minutes.

When specimens are obtained at times other than five minutes the effect of pH on the clay dispersions is further illustrated. These results are shown in Figure 2 for sodium montmorillonite dispersions at a pH of 4 and a pH of 9. As the time of adsorption is increased from five minutes to twenty minutes at a pH of 4 (A-C), it is found that the aggregates grow larger and larger and more numerous and the density or thickness of the aggregates increases. At no time is an even distribution of the clay particles formed on the microscope grid. That is, in all cases large areas

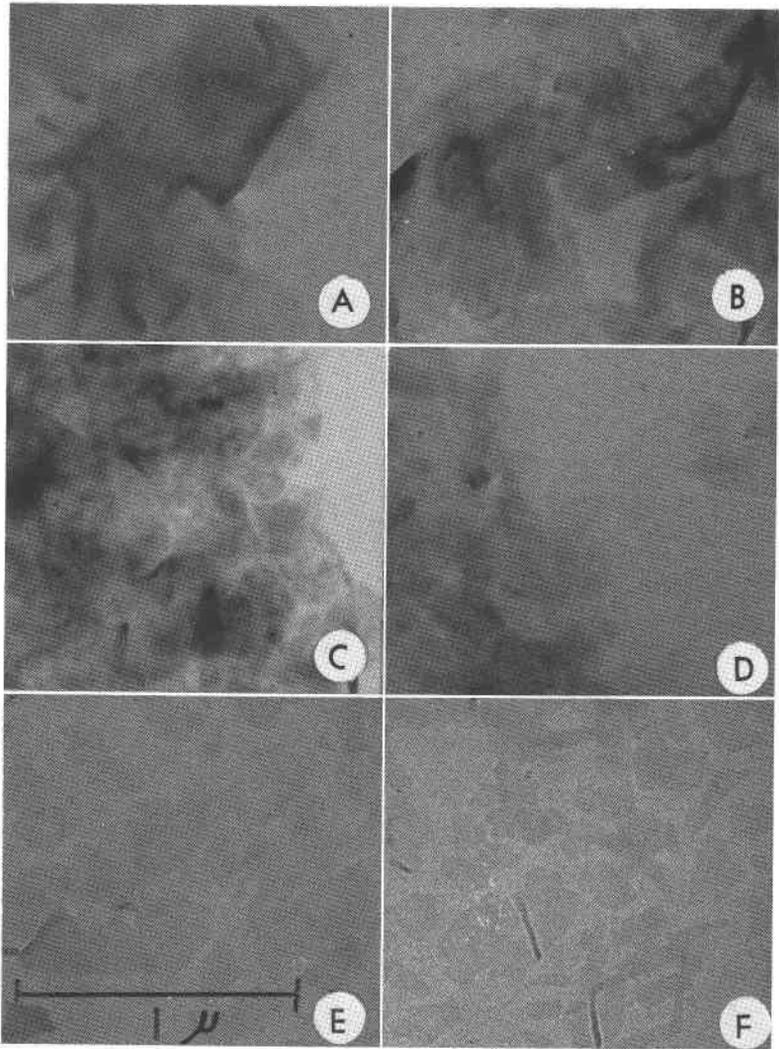


FIG. 2. Electron micrographs of sodium montmorillonite at various adsorption times, pH 4: (A) 5 minutes, (B) 10 minutes, (C) 20 minutes; pH 9, (D) 5 minutes, (E) 10 minutes, (F) 20 minutes.

of film do not adsorb clay and other areas show thicker and thicker aggregates of the clay on the surface of the cytochrome c film.

This is in direct contrast to what is observed at pH 9 (Fig. 2D, E, and F). The five-minute adsorption at pH 9 (Fig. 2D) resembles the five minute adsorption at pH 6 (Fig. 1C), while the 20 minute adsorption at pH

9 (Fig. 2F) resembles the five minute adsorption at pH 11 (Fig. 1D). The platelets appear to distribute themselves over the entire grid as time passes, and new platelets adsorb preferentially to the empty spaces on the cytochrome c, rather than forming clusters or stacking.

The changes in morphology, as well as the behavior of sodium montmorillonite in the time-dependence study, might be explained by changes in the edge-to-edge and plate-to-plate interaction of the clay particles. At low pH perhaps hydrogen bonding acts as a bridge for the silicon-oxygen tetrahedra at the surfaces of the clay particles. This then leads to the stacking and aggregation observed in Figure 2A through C. In contrast, at high pH, one might visualize a negatively-charged ion sheath of hydroxyl groups about each clay particle, thus giving repulsion of the individual clay particles. This would explain the pH effect as well as the time dependence, since more stacking and aggregation takes place as this "hydrogen-bond bridge building" occurs at the protein film. The film acts as a substrate for the process.

This explanation is further supported by the fact that if the calculated amount of hydrochloric acid necessary to adjust the pH to a value of about 3 is added, the measured pH is actually about 5. This disappearance of many hydronium ions could be caused by the phenomenon mentioned above.

Studies were also conducted on the long-term effects on exposure of sodium montmorillonite to pH 1. Initially aggregation occurs, but little difference was noted in micrographs after one hour of pH 1 and those after 127 hours. A typical large aggregate is pictured in Figure 3A. After 127 hours standardized sodium hydroxide was added in an amount which

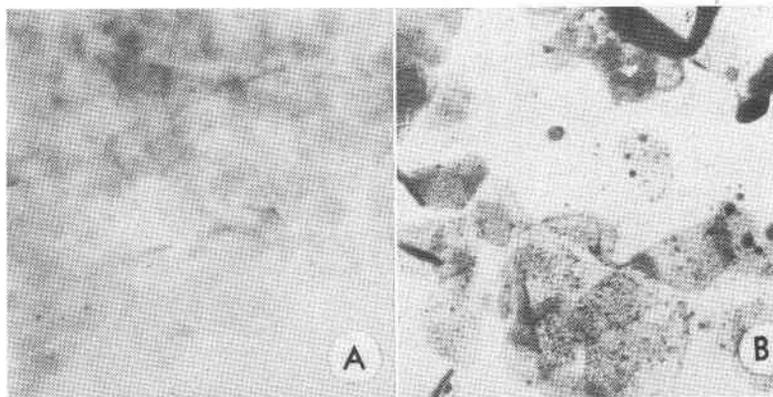


FIG. 3. Electron micrographs of sodium montmorillonite at pH 1 (A) exposure time 25 hours, (B) 72 hours after "neutralization." Adsorption time 5 minutes.

should have yielded a pH of 7. However, the pH was found to be approximately 2 instead of 7. It is reasoned that hydronium ions from the acid solution have replaced aluminum ions in the clay structure. The aluminum ions, in turn, have replaced sodium ions as the exchangeable cation on the clay. When NaOH was added, the aluminum acted as a Lewis acid forming hydrous aluminum oxide with the hydroxide ion. These conclusions are supported by electron micrographs taken following the NaOH addition to the acid clay dispersion. A micrograph shown in Figure 3B shows hydrous aluminum oxide crystals growing in the clay, and they closely resemble micrographs by McAtee and Wells (1967) of the adsorption of hydrous aluminum oxide on clays. As time increases, appreciable curling of the clay occurs as well as heavy aggregation of these curled particles. 166 hours after treatment of the dispersion with NaOH, micrographs show many individual hydrous aluminum oxide crystals in a fairly even distribution of curled particles with little aggregation. In all cases, the adsorption time on the cytochrome c film was five minutes.

This method of specimen preparation for electron microscopy was also successful on samples of hectorite. At neutral pH a large number of individual laths are observed. At pH 4 hectorite forms aggregates, as does sodium montmorillonite, while at pH 11 curling of the laths occurs with little adsorption by the protein film.

ACKNOWLEDGEMENTS

The authors wish to acknowledge financial aid from the Robert A. Welch Foundation which made this research possible.

REFERENCES

- LANG, D., H. BEYARD, B. WOLFF, AND D. RUSSELL (1967) *J. Mol. Biol.* **23**, 163-181.
- MCATEE, J. L., JR., AND LINDA M. WELLS (1967) Mutual adsorption of clay minerals and colloidal hydrous aluminum oxide—an electron microscopy investigation. *J. Coll. Interface Sci.* **24**, 203-210.
- VAN OLPHEN, H. (1963) *An Introduction to Clay Colloid Chemistry*. Interscience Pub., New York.
- WEST, E. S., W. R. TODD, H. S. MASON, AND J. T. VAN BRUGGEN (1966) *Textbook of Biochemistry*. Macmillan Co., New York.

Manuscript received, August 12, 1968; accepted for publication, December 30, 1968