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A PREPARATIVE TECHNIQUE FOR ELECTRON MICROSCOPIC EXAMINATION OF COLLOID PARTICLES

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Abstract—Adsorption of clay particles from very dilute aqueous suspensions onto positively charged films (Cytochrome C) on carbon coated support grids has been used to obtain well dispersed samples of the particles suitable for detailed electron microscopic examination. The natural negative charge of the carbon coated grid was used for ferric polycations and precipitates.

INTRODUCTION

In many studies of colloidal material by electron microscopy, it is very difficult to obtain good, representative dispersions of the colloidal material on the carbon coated grids due to artefacts arising in the process of transferring material from an aqueous phase to a dried state. The usual method of drying a drop of dilute suspension of the material on a carbon coated grid has proved of limited application for suspensions of ferric–hydroxy polycations and clay particles (in particular, sodium–montmorillonite and sodium–illite).

As it dries, the drop passes through a state of increasing concentration; clumping of the particles results. However, these problems are eliminated if the colloidal material is directly adsorbed from suspension onto an oppositely charged film coating a copper grid. The natural negative charge of a carbon coating or the positive charge of an appropriate protein film may be used.

The procedure is a modification of that developed by Lang *et al.* (1964) and later improved by them (1967) for studying DNA molecules. Their procedure involved the direct adsorption of the DNA onto an oppositely charged film spread onto a water surface. The film was then transferred to a microscope grid.

Using the improved technique of Lang *et al.* (1967), whereby the Cytochrome C was added to the surface in solid form dried on a needle, poor dispersions on the grids were obtained with electrolyte-free illite suspensions. Instead of obtaining good dispersions of separate illite crystals, the illite was found to be present in the form of strands in which individual crystals were loosely linked one to another.

Lang *et al.* (1967) mention that they also had difficulty in obtaining good dispersions of DNA molecules on the grids, when the ionic strength of the DNA soln was less than 0·05. These effects were found to disappear, and separate DNA molecules could be found, if the Cytochrome C film is pre-formed and stabilized on a salt solution, before being transferred to a salt-free DNA solution. McAtee and Henslee (1969) used the improved technique of Lang *et al.* (1967) to obtain dispersions of montmorillonites on electron-microscope grids.

Using the modifications described in this paper, excellent dispersions on the grid of ferric–hydroxy polycations and precipitates, and of a range of clay minerals were obtained, without the necessity to preform and stabilize the Cytochrome C film.

EXPERIMENTAL

Iron species adsorbed on carbon films

The ferric–hydroxy polycations and precipitates were adsorbed directly from solution on a carbon film deposited on a copper microscope grid. Silicon monoxide films were also suitable but, due to the experimental difficulties involved in preparing grids, carbon films were preferred. The samples were diluted with distilled water to give 0·01 per cent w/v solution and a final pH value ~3·5. Immediately after dilution, a drop of solution was placed on a clean Teflon surface and a carbon coated grid was floated on the surface of the drop, carbon film down.

After an appropriate time, the grid was lifted off the surface, excess solution removed by dipping in ethanol and then the grid was dried on filter paper, carbon film uppermost. The amount of material adsorbed onto the carbon film could be varied either by changing the adsorption time or the solution concentration. For an 0·01 per cent solution, 1–2 min was usually adequate.

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Clay minerals adsorbed on protein films

Clay minerals were adsorbed on an immobile protein (Cytochrome C) monolayer of opposite charge to the clay, deposited on carbon coated grids.

The first stage of this process involved layering a close-packed monolayer of Cytochrome C onto a clean water surface and then transferring a portion of the monolayer onto a carbon coated grid so as to form a stable and coherent film over the carbon.

A small Teflon trough was filled with deionized water so that the meniscus was above the top of the trough and the surface cleaned by sweeping across the meniscus with Teflon bars. The surface was then lightly sprinkled with talc and an immobile monolayer of Cytochrome C applied to the surface by touching a few small crystals, held on the end of a fine glass needle, onto the surface. If the surface was thoroughly clean, the cytochrome rapidly spread out from the point of application, pushing aside the talc, to form a monolayer across the surface, the boundaries of which were defined by the talc.

A copper grid which had previously been coated with a thin carbon film was then lightly touched, carbon film down, onto the layer of Cytochrome C, so that the grid just broke the meniscus of the surface, and held in this position for a few seconds. The excess water was removed by dipping the grid into ethanol and dried on a filter paper, Cytochrome film uppermost.

The grid was then touched for a few seconds onto the surface of a drop of approx 0·01 per cent clay suspension, which had been placed on a clean Teflon surface. Excess water was removed by dipping into ethanol and the grid was then dried, carbon film uppermost on filter paper. If the particles are large, sedimentation away from the surface will occur with a resulting low particle density on the grid. This may be overcome by using a hanging drop.

RESULTS AND DISCUSSION

Iron containing species

Plate 1 shows the polycations present in a ferric perchlorate solution, Plate 2 those present in a ferric–chloride solution. The reasons for the different appearance of the polycations in the two solutions will be the subject of separate papers (Murphy, Posner and Quirk, to be published). The method provides an excellent dispersion of the polymeric species present in solution. These dispersions were superior to those prepared by the usual technique of drying diluted samples of the solutions.

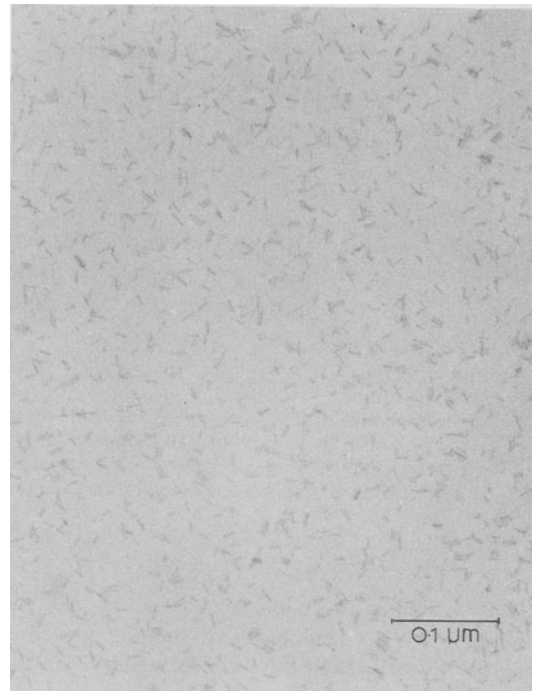


Fig. 1. Polycations in a solution of 0·1 M $FeCl_3$ with OH/Fe = 2·0 aged for 28 days at 20°C. Close examination shows the rods to be made up of dot-like material similar to that shown in Fig. 2.

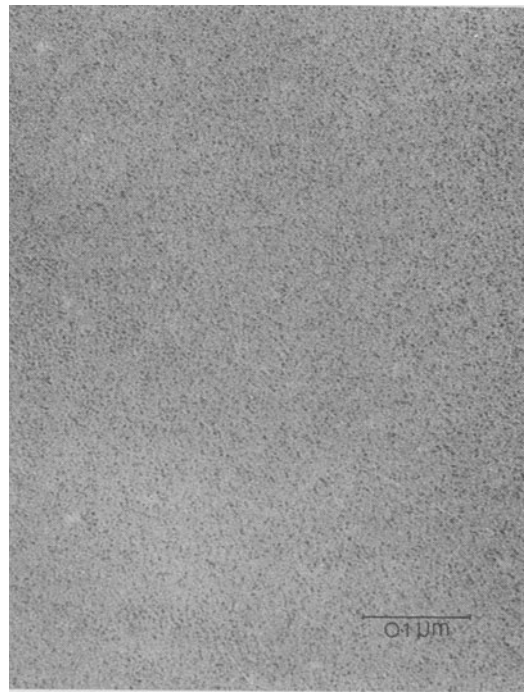


Fig. 2. Polycations in a solution of 0·02 M $Fe(ClO_4)_3$ with OH/Fe = 2·0 aged for 2 hr at 20°C.

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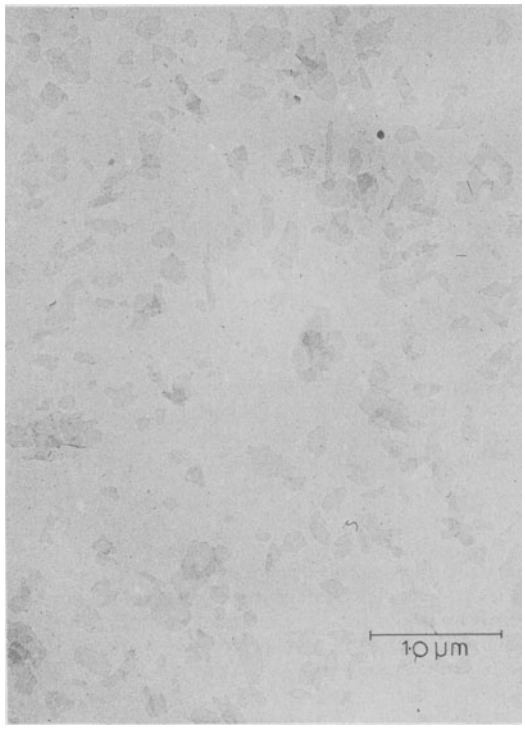


Fig. 3. Fine fraction of Na–montmorillonite (Wyoming) dispersed onto a Cytochrome C film from an upright drop.

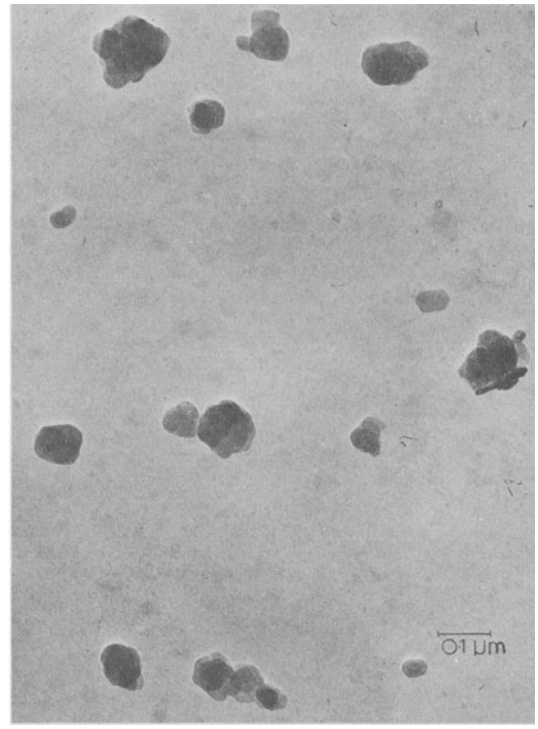


Fig. 5. Sample of sodium illite (Muloorina) dispersed onto a Cytochrome C film from an upright drop.

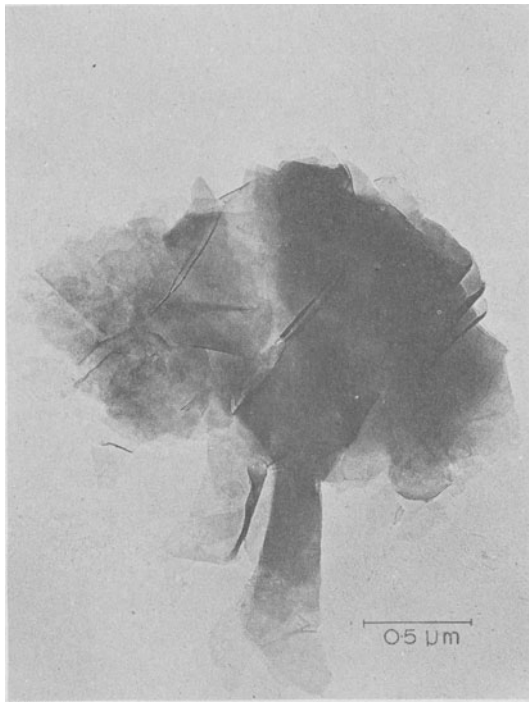


Fig. 4. Coarse fraction of Na–montmorillonite (Wyoming) dispersed onto a Cytochrome C film from a hanging drop.

Clay minerals

Though good dispersions on the grids have been obtained for a range of clay minerals, our technique has mainly been applied to the examination of sodium montmorillonite (Wyoming bentonite API 25b from the John C. Lane Tract, Upton, Wyoming) (Figs. 3 and 4) and sodium illite (Muloorina) (Fig. 5). Using conventional techniques, good dispersions of these two clays on the grid, especially of the finer fractions, had been difficult to achieve.

Since there was not a great range in the particle size distribution of the illite material, an upright drop was used in all cases.

The hanging drop was used for the large sized fraction of Na–montmorillonite (Fig. 4).

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Résumé—L'adsorption de particules d'argile en suspension aqueuse très diluée sur des films chargés positivement (cytochrome C) déposés sur des grilles support recouvertes de carbone a été utilisée pour obtenir des préparations de particules bien dispersées adaptées à une étude détaillée au microscope électronique. La charge négative naturelle des grilles recouvertes de carbone a été mise à profit dans le cas des polycations et des précipités ferriques.

Kurzreferat—Die Adsorption von Tonteilchen aus sehr verdünnten wässrigen Suspensionen an positiv geladenen Filmen (Cytochrom c) auf kohlenstoffbeschichteten Trägergittern wurde benutzt, um gut dispergierte Proben der Teilchen für eine eingehende elektronenmikroskopische Untersuchung zu erhalten. Die natürliche negative Ladung der kohlenstoffbeschichteten Gitter wurde für Eisen-III-Polykationen und Niederschläge benutzt.

Резюме — Адсорбция частиц глины из очень разбавленных водных суспензий на положительно заряженные пленки (цитохром С) на опорных решетках с угольным покровом для получения образцов с хорошо диспергированными частицами использовалась для детального исследования под электронным микроскопом. Естественный отрицательный заряд решетки с угольным покрытием использовался для адсорбции железистых поликатионов и для осаждений.

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