

THE ELECTROCHROMATOGRAPHIC BEHAVIOR OF SOME
LAYER-SILICATE MINERALS

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1962

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Date thesis is presented October 26, 1961

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ACKNOWLEDGMENTS

The association with Dr. J Lowell Young, Dr. Moyle E. Harward and Dr. Wendell H. Slabaugh throughout the course of this study and in the preparation of the manuscript has been a rewarding experience. The helpful suggestions of Dr. David L. Carter during the initial stages of the project are also appreciated.

The cooperation of both the National Science Foundation and the Soil and Water Conservation Research Division in the Agricultural Research Service of the United States Department of Agriculture is gratefully acknowledged. Their aid in the form of financial assistance has largely made this study possible.

The only-mild protests offered by a patient wife to the frequent absences from home and the family necessitated by the research program and the preparation of the manuscript are truly appreciated.

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THE ELECTROCHROMATOGRAPHIC BEHAVIOR OF SOME LAYER-SILICATE MINERALS

INTRODUCTION

In the early 1940's, A.J.P. Martin and colleagues replaced the silica gel of earlier chromatographic workers with a filter paper supporting medium (9, p. 3). Since then, the field of paper chromatography has enjoyed a steadily-increasing popularity in such research areas as organic chemistry, biochemistry, plant physiology and medical research.

Filter paper was first used as a supporting medium for electrophoretic work by König in 1937 (9, p. 489), even prior to the advent of paper chromatography. The "rediscovery" of paper electrophoresis, however, was delayed until the early 1950's. By then, paper chromatography had become a fairly well-established technique. Soon after the development of one-dimensional or strip electrophoresis procedures by several laboratories (9, p. 489-490), an essentially new field of "electrochromatography" or "continuous electrophoresis" was born from a union of the paper chromatographic and paper electrophoretic techniques. Several of the pioneers in this area (17, 22, 34, 36) continually supported its development. They believed that the technique should allow the separation and identification of components from mixtures which had been impossible to study adequately through the use of paper chromatography or paper electrophoresis alone.

Electrochromatographic techniques have been applied to the study of various mixtures of organic and inorganic compounds. These same techniques have apparently never previously been applied to the study

of inorganic particles as large as the layer-silicate minerals, although the charge-to-mass ratios of such mineral species as kaolinite and montmorillonite appear sufficiently different to theoretically allow their separation by electrochromatography. The process, if applicable to the study of layer-silicates, should provide an excellent means for the purification of layer-silicate mixtures. It should also allow classification of mineral mixtures into those containing discrete mineral species and those containing a more or less continuous electrochromatographic spectrum of minerals. The technique should prove useful in the study and characterization of various artificial and natural complexes of layer-silicates with organic material. The present study was concerned with a preliminary evaluation of as wide a range of experimental conditions as possible in the time available. It was hoped to determine the feasibility of the electrochromatographic approach to layer-silicate studies and to outline the most profitable avenues for future development and refinement in this area.

The specific objectives of the study were:

1. To develop a suitable means for detecting minute quantities of layer-silicate minerals on the supporting medium (filter paper or similar modified material).
2. To find a suitable combination of electrochromatographic conditions which would permit movement of the minerals on a supporting medium.
3. To find a suitable combination of pH, buffer system, ionic strength, voltage and supporting medium which would allow differential

migration of different minerals and thereby permit separation of a layer-silicate mixture into its individual mineral components.

4. To apply these conditions to a few simple mineral mixtures, and to examine the type of patterns obtained from such studies.

REVIEW OF LITERATURE

Previous Work on the Electrophoretic Behavior of Layer-Silicates

The electrophoretic behavior of layer-silicate minerals has been studied by several workers. Bayer (4) and Anderson (3) investigated the electrophoretic mobility of layer-silicates as influenced by the amount and type of exchangeable cations. Tal (37) attempted to purify layer-silicates by electrophoresis in conjunction with electro-decantation. Reifenberg (30) examined the electrophoretic behavior of kaolin suspensions, and then projected this work to the study of soil colloids (31). Similar work was being done simultaneously by Kovda (24). Urbain (38) used electrophoresis to separate what he called the constituents of clays, although his work leaves one in doubt as to what actually had been separated upon completion of his study. Electrophoresis of layer-silicates was studied by Beavers and Larson (6), as well as by Bergna (7). The latter used the migration of minerals in a micro-electrophoresis cell as a clue to charge density and mineral structure. Separations of layer-silicate minerals by electrophoresis were also attempted by Beavers and Marshall (5), who selectively coagulated one mineral while measuring the mobility of another. Such separations are briefly discussed by Weiser (39, p. 241). Fridrikhsberg and Tsai (15) described macro-scale separations of layer-silicates from other minerals which, although not at the time economically feasible, were capable of preparing up to two tons of clay per day.

The above work has been confined to solution electrophoresis,

either in some type of Tiselius cell or in a modified cell employing much the same principle. Employment of such techniques for the separation of individual minerals from complex layer-silicate mixtures has always appeared theoretically promising, but the limitations of technique and the rather slight mobility differences between mineral species has never allowed sufficiently-complete mineral separations for preparative laboratory work. More than a little difficulty has also occurred in maintaining any separation sufficiently long for accurate sampling and complete separation of the components. The electrochromatographic approach, if feasible, was felt to offer much potential in the area of layer-silicate mineral studies.

General Electrochromatographic Principles

The principal objective of electrochromatography is to subject the charged sample particle to simultaneous chromatographic and electrophoretic forces, with the forces preferably acting at right angles to one another. The resultant effect is an angular movement of each particle across the supporting medium. The chances are small that two species in a given mixture will have exactly the same electrochromatographic behavior. Thus, introduction of the sample onto the supporting curtain and subsequent movement in response to electrochromatographic forces should yield a separate migration path for each of the different components of the mixture (Figure 1).

It is claimed that the superimposed electrophoretic and chromatographic effects are often much greater than would be predicted from the effect of each alone on a given particle (22, p. 1). In addition,

with the electrochromatographic procedure the slower components are not forced to move across a path previously saturated with the faster components, as is the case in horizontal or strip electrophoresis. Problems of component contamination, of differentially detecting the various sample components, and of unpredictable interactions between components should therefore be minimized through the use of such a technique.

Electrochromatography Apparatus

A few early workers attempted to use paper chromatography in one direction, followed by paper electrophoresis in another (8). This procedure did not prove entirely satisfactory and obviously did not actually yield electrochromatographic movement of the particles in the mixture. In the early 1950's, several workers developed electrochromatographic apparatus, each worker attempting to eliminate the apparent defects of existing apparatus. Continuous electrophoresis apparatus were designed and utilized by Dicastro (12), Durrum (14), Grassman (17), Karler (22), Sato (34), Strain (35) and Svensson (36), as well as by others. A few of these units will be briefly discussed in the following paragraphs.

One of the inherent difficulties of the apparatus developed by Durrum (14) was the non-uniformity of the imposed electrical field (Figure 1). In this apparatus a filter paper sheet was suspended from an electrolyte trough, with a tab dipping into an electrode vessel on each side of the hanging sheet. The non-uniformity of the field was obvious from the curved paths of the sample components

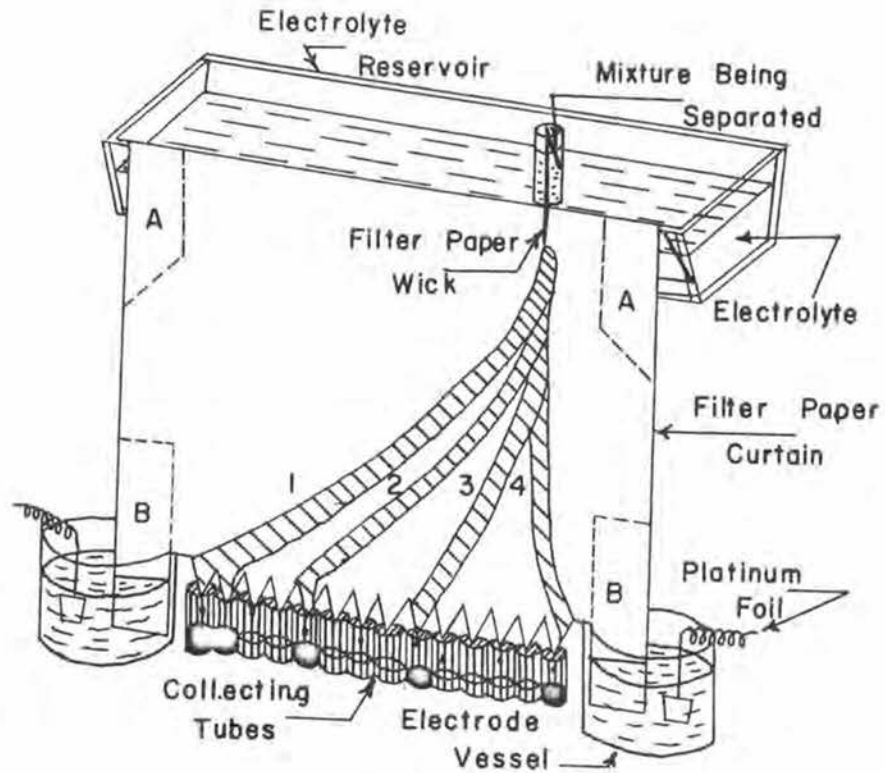


Figure 1. Continuous Electrophoresis Apparatus (Durrum).

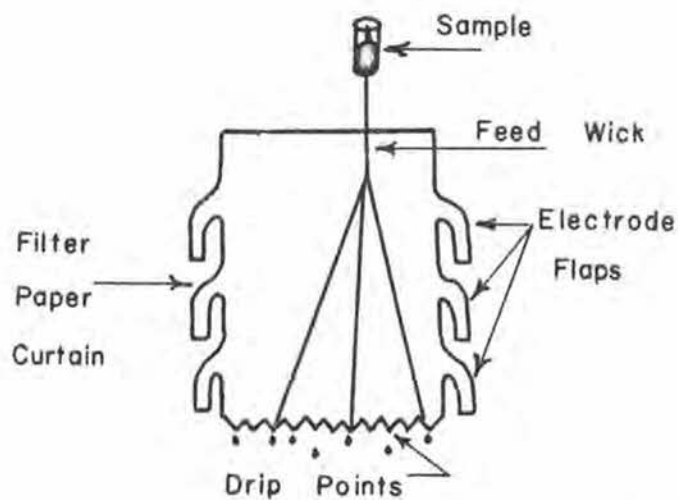


Figure 2. Flow Pattern on a Three-Paired Side-Tab Curtain (Dicastro).

(Figure 1); theoretically the paths should have been straight lines. Another inherent difficulty was the rather poor temperature control possible, due to the free-hanging nature of the sheet and to the temperature differences which developed at high voltage gradients.

The apparatus of Dicastro and San Marco (12) also employed a free-hanging paper curtain, but the curtain contained a number of paired tabs along the sides which dipped into the electrode vessels (Figure 2). This curtain design did give essentially linear flow of particles down the paper. The increased contact between paper and electrodes, however, presented considerably more possibility for contamination of the fractionation area with electrode products. The products, chiefly H^+ and OH^- ions in most aqueous systems (22, p. 55), frequently produced undesirable pH gradients across much of the paper curtain.

The apparatus used in this series of experiments (Figure 3) was that developed by Karler (22). Though it, too, had a slightly non-uniform electrical field on the fractionation area (Figure 5), it was commercially available, easily operated and supposedly had a good temperature control system when used with a cooled fractionation surface. Electrode reaction products were fairly effectively (22, p. 55) eliminated from the fractionation surface by the over-size electrode vessels and by the use of electrode baffles (Figure 4). This was not the hanging curtain type of apparatus as designed by Durrum and by Dicastro, but was instead a "horizontal curtain" type of apparatus (22, preface) in which there was still a chromatographic component in the vertical direction. The curtain was supported on a

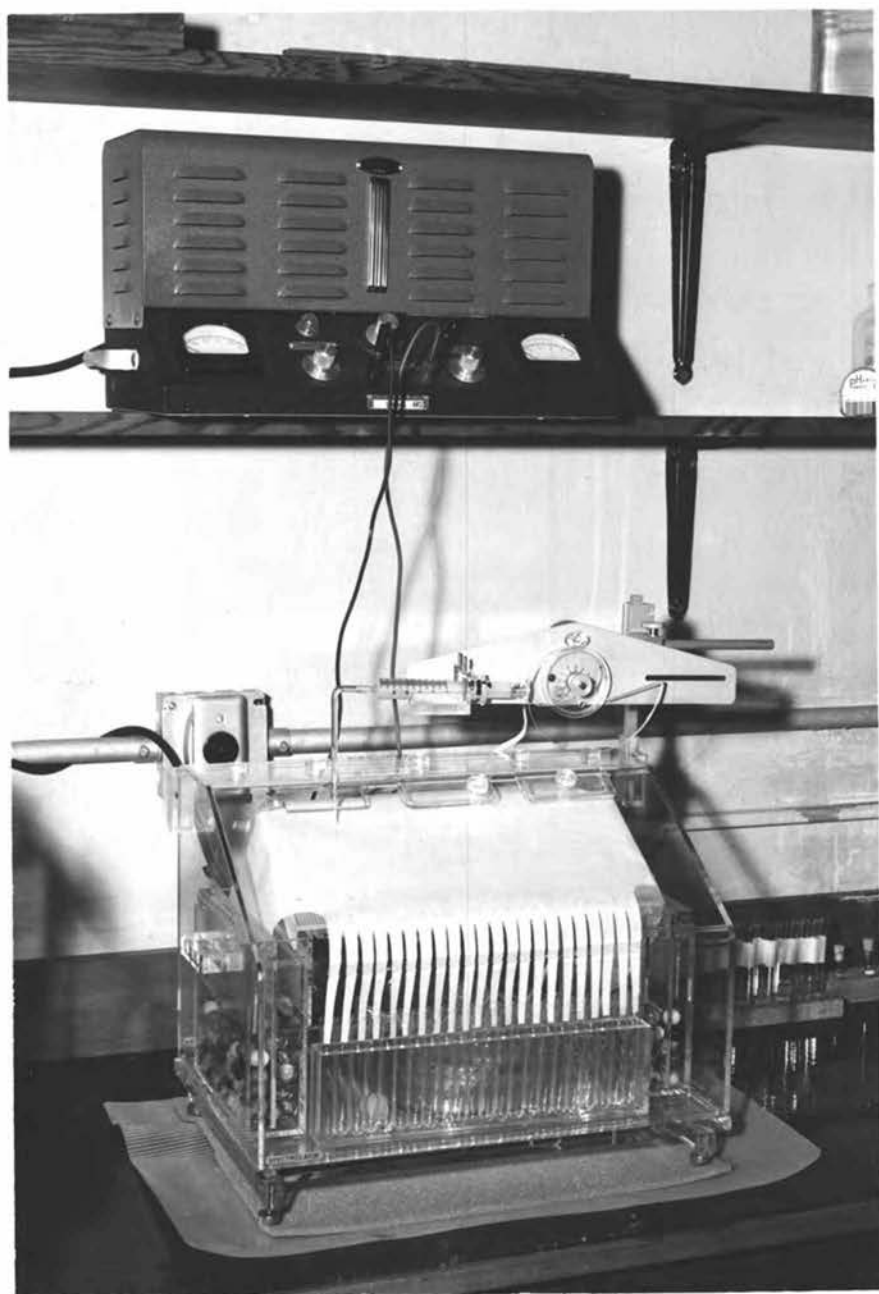


Figure 3. The Karler-Misco Electrochromatography Apparatus

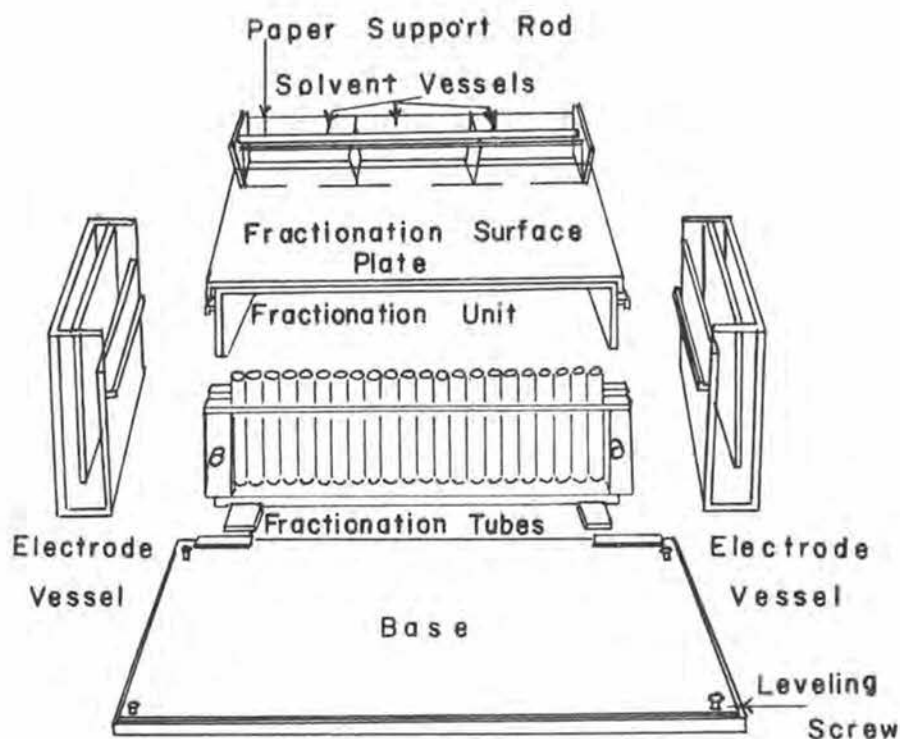


Figure 4. Working Components of the Karler-Misco Electrochromatography Apparatus.

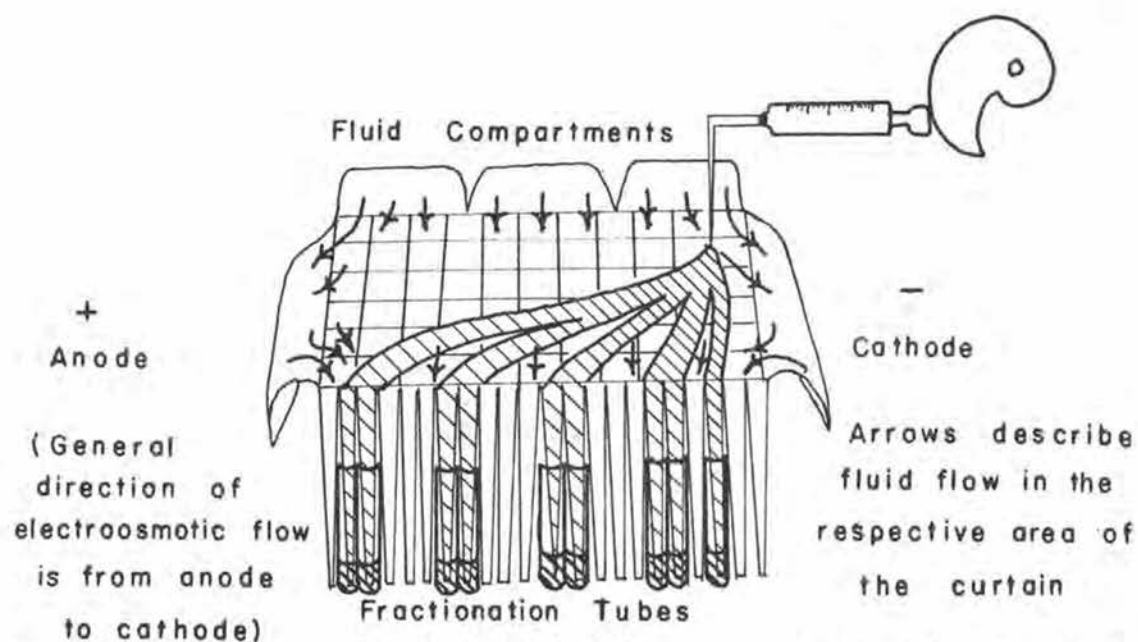


Figure 5. General Directional Pattern of Fluid Flow in the Karler-Misco Electrochromatography Unit (Karler, p.25).

pyramidal-plastic fractionation surface (Figure 4) instead of hanging vertically from the solvent vessels.

Use of Electrochromatography in Layer-Silicate Mineral Studies

Earlier work in chromatography, electrophoresis and electrochromatography was confined primarily to smaller particles such as amino acids (8, 14, 17), dye components (14), peptides (23) and small inorganic ions (1; 9, p. 410-442; 26; 34). In recent years techniques have been extended to proteins and similar large particles (14; 22, p. 79-83). Karler speculated on the use of electrochromatography for the processing of large particles, viruses and eventually even whole cells (22, p. 87-89), but Block, Durrum and Zweigg (9, p. 529) and other authors such as Grassman (16) felt that the procedure would have either none or at best only limited applicability for the larger particles. Their skepticism was attributed to such factors as drying of the paper at the high voltage gradients required and to the strong adsorption of larger particles on the paper.

The observation of Svensson that the pores of the paper are large enough to allow the passage of entire cells (36) only added fuel to the fire of hope that electrochromatography could be developed into a suitable technique for use by the soil chemist, clay mineralogist and colloid chemist in their studies of layer-silicate minerals. Thus, in spite of the admonition of Wunderly that :

"Migration in paper depends on . . . the properties of the ion, the magnitude and sign of its net charge, its colloiddally dispersed . . . or molecularly dispersed . . . nature, its spherical or threadlike form . . . the electrolyte concentration, ionic strength, hydrogen

ion concentration, temperature, viscosity . . . current
density, field intensity . . . and filter paper texture"
(41, p. 182)

the research to find a suitable set of conditions for the electro-
chromatographic examination of layer-silicates was begun.

MATERIALS

Characterization of Minerals

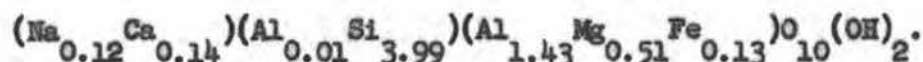
The layer-silicates used in this study, with the exception of the vermiculite and bentonite samples, were obtained from Ward's Natural Science Establishment, Inc., Rochester, New York. Characterization data were taken from the American Petroleum Institute (A.P.I.) report on reference clay minerals (2). The characterization data on the minerals are as follows:

Montmorillonite 11. This mineral (from Santa Rita, New Mexico) has the chemical formula



The sample contains 3% non-clay impurities, primarily basic plagioclase. The mineral has an exchange capacity of 96 me/100 g¹.

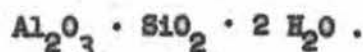
Montmorillonite 24. This montmorillonite (from Otay, California) has the chemical formula



Of the 4% impurities, 1% is quartz, with 0.5 to 1% orthoclase and 1 to 2% sericite. There are also traces of limonite and ferromagnesium minerals. The exchange capacity of the sample is 111 me/100 g.

¹Exchange capacity determinations made by Wallace J. Wipper, Department of Soils, Oregon State University. Procedure involved saturation of the exchange complex with sodium, followed by determination with a flame photometer of the sodium displaced by a neutral ammonium acetate solution. (Exchange capacity determinations were made on less than 40 mesh material.)

Kaolinite 5. The source of this mineral is the Lamar Pit, near Bath, South Carolina. The chemical formula of the kaolinite is



It contains 4.8% impurities, primarily sericite, quartz and leucosene, with traces of muscovite, orthoclase, limonite, ferromagnesium minerals and carbonaceous material. The exchange capacity is 4 me/100 g.

Utah Bentonite. This mineral is from Box Elder county in northern Utah.² It is a montmorillonitic mineral, with a chemical formula thus somewhat similar to those given for the montmorillonitic minerals above. Impurities were not determined, but bentonites normally contain a high percentage of iron, with varying amounts of feldspar, biotite, quartz, pyroxenes, zircon and other volcanic minerals (2). The cation-exchange capacity of the mineral is 99 me/100 g.

Vermiculite. This mineral was used as a packing material for glassware received at this laboratory. The source is unknown. Its use was prompted by a very strong $14.7 \overset{\text{O}}{\text{\AA}}$ peak upon X-ray analysis, indicating perhaps a greater proportion of vermiculite in relation to impurities than in many other vermiculite sources. The types and amounts of impurities are unknown. The sample has a cation-exchange capacity of 93 me/100 g.

²Obtained by Dr. M. E. Harvard from Dr. H. B. Peterson, Head, Department of Agronomy, Utah State University, Logan.

Preparation of Mineral Suspensions

Suspensions of the layer-silicates were obtained using Jackson's (21, p. 73) boiling Na_2CO_3 procedure. No attempt was made to remove organic matter or free iron oxides from the samples. According to Bayer (4) and Beavers and Larson (6), the size of particles of a given layer-silicate does not noticeably affect migration velocity in an electrical field, so elaborate size fractionations were not felt necessary for the study. After fractionation by centrifugation to yield the $<0.2\mu$ and $2-0.2\mu$ size fractions, a stock supply of each mineral was maintained in 0.01% Na_2CO_3 . Mineral suspensions were prepared from the stock supply as needed throughout the course of the experiment. (At the end of the experiment the minerals had been in contact with 0.01% Na_2CO_3 for approximately six months). Layer-silicate concentrations in the samples varied from 1.0 to 0.001%. By comparison, Beavers and Marshall (5) used a 0.04% mineral concentration in their electrophoresis studies, while Beavers and Larson (6) used 1.4 to 2.1% suspensions. However, these latter high concentrations were necessary for detection of the minerals by the moving boundary procedure which they used.

Though much work with layer-silicate minerals in electrophoresis cells has included peptization with water glass (14; 39, p. 241) and ammonia (15), such treatments were not considered necessary in this series of preliminary experiments. The suspensions prepared in the above manner were deemed sufficiently stable to yield little flocculation during the course of a given electrochromatographic run.

Description of Apparatus

As was mentioned previously (page 8), the apparatus used for this series of experiments was the Karler-Misco electrochromatography unit distributed by Microchemical Specialties Company, Berkeley, California. It is pictured in Figures 3-5. The unit was equipped with a 0-1000 volts d.c. power source, with a maximum current capacity of 50 ma. The pyramidal fractionation plate allowed drainage of excess buffer from puddled zones on the paper, so that a more uniform electrochromatographic field would result. It also allowed temperature control through the use of a water or ethylene glycol-water mixture which was circulated from a water bath through the plate at a rate of approximately 600 ml per minute. Feed rate and flow pattern of the buffer were adjusted by varying individually or in combination: the height of liquid in the buffer compartments; the angle of the fractionation plate (from 0 to 25°); the type of paper; or the viscosity of the buffer system.

Samples were introduced onto the paper for analysis from a motor-driven syringe (available from Microchemical Specialties Company) which delivered a full syringe of sample per day to the paper. Feed rates were varied by changing the size of the sample syringe, the concentration of the sample suspension, or both.

SELECTION OF SUITABLE EXPERIMENTAL CONDITIONS

Development of Staining Technique

In order to determine the position of minute amounts of layer-silicates on the filter paper curtain, a suitable detection technique had to be developed. It was felt that ultraviolet scanning, used successfully by some workers for the detection of organic compounds on paper (9, p. 66-67; 22, p. 58-60), would not prove effective with layer-silicates, due to the normal lack of fluorescent groups in such mixtures and thus to the low absorption of the sample as compared to the "background" absorption of most papers. According to Block, Durrum and Zweigg (9, p. 67), infra-red spectrophotometry is not satisfactory for use directly on filter paper, since milligram quantities of most substances are necessary for successful sample detection. After consideration of these and other detection methods, it was felt that the use of a selective stain for locating the mineral might offer the most promise as a detection technique.

Much work on the staining of layer-silicates has been reported in the literature, although most of it has been in connection with petrographic studies. Mielenz and King (28) used safranin "Y", malachite green and benzidine in such research. The use of benzidine for the petrographic differentiation of minerals was extensively studied by Hendricks and Alexander (19), while both malachite green and safranin were studied by Dodd (13). Color reactions between layer-silicates and amines were examined by Hauser and Leggett (18).

Based on the information in the above reports, malachite green, safranin and benzidine were selected as the most promising of the possible stains. Benzidine was prepared in an aqueous medium. Safranin and malachite green were prepared in nitrobenzene. (The nitrobenzene was used because of its low surface tension and because of stain solubility properties. It was considered much less likely to enter into oxidation-reduction reactions with the stains than were other possible carriers such as aniline (13).)

To determine the most desirable detection reagent, the stains were added dropwise to approximately 0.25 g of mineral (<40 mesh) in a spot plate until a paste was obtained. The colors of the pastes were then examined while wet and also upon drying. Definite color differences between layer-silicates were observed under these conditions. Unfortunately, such differences were not evident when the stains were applied to minerals previously spotted on filter paper strips. Thus, hope of mineral differentiation on paper by the staining techniques had to be abandoned for the present, although the stains showed sufficient color under such conditions to outline the location of the minerals on the paper.

Differing concentrations of minerals were added to filter paper strips, dried, and then dipped through stains of varying concentrations. Half of the papers were also dipped in 0.01% Na_2CO_3 prior to staining to ascertain the effect of Na_2CO_3 on mineral detection properties of the stains. The stains were tested for interference by glycerated systems to determine if the presence of residual glycerol (used in some electrochromatographic techniques) would seriously

hamper efforts to detect layer-silicates on the paper.

Benzidine was initially selected as the most suitable detection agent. In contact with minerals, it produced a blue-tinged spot on the white background of the paper. Unfortunately, the blue color, which has been attributed to oxidation of the stain by the ferric iron in minerals (19), was not evident on samples subjected to a potential gradient of 20 v/cm for several hours. This may have been due to reduction of the iron under these conditions, with a resultant loss in detection properties of the stain.

After rejection of the benzidine stain, attention was turned to the safranin and malachite green systems. Both stains were found to detect as little as 10^{-6} g of the layer-silicate minerals studied. Malachite green was more effective than safranin on all but the kaolinite and vermiculite samples at low stain concentrations. Since it gave the lower background at higher stain concentrations and a generally better detection at both lower stain and mineral concentrations, malachite green (0.002%) was eventually selected as the most satisfactory stain for use in subsequent experiments. (A 0.004% concentration was usually better on the thicker curtains.) Dodd (13) reported that malachite green and safranin were not satisfactory stains for dilute suspensions of montmorillonite, but this did not appear to be the case for dilute suspensions of montmorillonite which had been applied to filter paper. The main condition under which staining was not found to be a satisfactory method for detection was when the last interfering traces of glycerol were not removed from the paper prior to the application of the

malachite green. In these cases the pattern either failed to stain or stained weakly and faded rapidly with time. When this limitation was kept in mind, however, the staining technique presented a rapid and sensitive means of locating layer-silicates on paper curtains.

Once the stained patterns had dried, the mineral spot was obvious as a dark blue or light blue area on the very light blue or white background of the paper. Malachite green, an acid-base stain, has been used as the basis for a petrographic separation of layer-silicates, through its color reactions with minerals of varying acidic nature. Its effectiveness in locating mineral spots on the filter paper medium was probably more the result of a concentration of dye in the region of the minerals, however, than of a differential pH reaction. This conclusion was supported by the following observations: The entire paper was a greenish-blue color when wet, and the same color was retained by the mineral spot or band while the background faded out as the paper dried; When the curtain was hung up to dry after staining, white "tails" appeared below the mineral bands as the excess stain moved down through the paper, indicating that the stain had been retained by the mineral while the nitrobenzene was continuing to move down through the drying curtain.

Development of Suitable Conditions for Layer-Silicate Mineral Studies

Flow rates and capacities of selected filter papers

Karler (22, p. 27) claims that fluid flow rate is the most critical electrochromatographic variable. Lack of sufficient

information on flow rates of the various filter papers under the conditions present in the Karler-Misco apparatus prompted a study of relative fluid-flow capacities for an array of filter papers.

Strips of paper, 40 cm long and 1.25 cm wide were placed in the Karler-Misco apparatus and allowed to wet by capillarity from a 0.01% Na_2CO_3 solution at 8 °C. Initial wetting rates, capacities and other pertinent data for the papers examined are listed in Table 2 of the Appendix.

According to Karler (22, p. 28), initial wetting rate is at best only a poor indicator of fluid flow capacity for porous supporting media such as filter paper, since this wetting rate is a function of both the liquid gradient and the capillary forces of the paper fibers. Total capacity, as defined by the average number of mls of fluid coming off a drip point per unit time, is a more desirable means of expressing the electrochromatographic potential of a given paper under a given set of experimental conditions.

The papers may be roughly classified into five groups according to capacity (Table 2): hydrophobic, 0 ml/hr; extremely low, <0.01 ml/hr; low, 0.01-0.04 ml/hr; intermediate, 0.07-0.19 ml/hr; fast and extra-fast, 0.51-1.20 ml/hr. Representative papers from the last four groups selected for more detailed study were Whatman 1, Whatman 3MM, Schleicher and Schnell 598 and Schleicher and Schnell 470A. The S & S 470A paper was felt to be especially promising, since the rate of fluid flow and hence its capacity could be varied by a factor of nearly 20-fold (22, p. 27). Selection of a paper was dictated by the above-mentioned capacities of the various papers, as well as by

any special properties attributed to the papers by the manufacturer.

Often several days were involved in assessing the effect of varying conditions on the electrochromatographic pattern. Limited time available for the study restricted the number of different supporting media that could be examined. For a given degree of resolution the fractionation time under a given set of experimental conditions increases as the capacity of the paper used decreases. Papers of low capacity were thus not considered feasible for eventual use in preparative electrochromatography. Such papers were still used throughout much of the study to assess the effect of varying experimental conditions on mineral patterns. Since a great deal of pioneering work was necessary for evaluation of mineral behavior on the standard papers, the various "specialty" papers, such as ion-exchange papers, acetylated papers, glass "papers" and cellulose acetate foils were reserved for future research.

The use of a fluid level of 3 cm in the solvent vessels, coupled with drip points shortened from 13 cm to 5 cm was found to provide optimum flow rates at 8 °C with S & S 470A paper and a 0.01% Na_2CO_3 -20% glycerol fluid mixture. Since the shortened drip points were only rarely submerged, a relatively constant liquid gradient was thus also provided during the rapid filling of the fraction tubes. With the low capacity papers, drip points of 13 cm length and 5 1/2 cm of buffer in the solvent vessels provided maximum flow rates when used with a 0.01% Na_2CO_3 , non-glycerated system.

Assessment of fluid flow pattern

To determine the uniformity of the electrochromatographic field at various points on the curtain, amino acid solutions were spotted across the paper. The paper was then rapidly wetted and the desired potential applied. Movement of DL-Aspartic acid (0.0714 M in 10% isopropanol, 1000 v, 2 ma for 20 min) was as would be predicted from the fluid flow lines shown in Figure 5. Similar experiments with Utah bentonite (0.01% Na_2CO_3 , 1000 v, 2 ma for 4 hr) rather than aspartic acid at first yielded anomalous results, with the minerals apparently moving toward first one and then another section of the fractionation area. Greater care in effecting complete contact of the paper curtain and the fractionation plate to insure uniform temperature across the curtain resulted in apparently normal flow patterns of the mineral on the paper.

It will be noted from the arrows in Figure 5 that the middle 1/2 or 2/3 of the curtain has linear chromatographic flow (22, p. 25). Near the edges of the curtain the buffer appears to flow out onto the electrode tabs and then back onto the main fractionation surface once again. Since the paper is negatively charged at most pH values (9, p. 501), there is also a net flow of electrolyte toward the cathode due to electroosmosis (9, p. 501; 22, p. 26). This electroosmotic flow is particularly pronounced at low ionic strengths and high pH values, as were used in this series of experiments (9, p. 501). The electroosmotic flow may have pronounced effects on particle movement, and is even used to counterbalance electrophoretic flow in

the method spoken of as "electrorheophoresis" (36; 41, p. 188). It tends to offset flow of the cathode reaction products onto the paper, but also tends to exaggerate flow of anodic products onto the paper.

Choice of buffer system

According to Beavers and Larson (6) and Dayhuff and Hoagland (11), varying the pH between 5 and 10 has little effect on the electrophoretic mobilities of clay particles. Since one of the primary objectives of using different buffer systems in electrophoretic and electrochromatographic studies is to vary the pH of the system, the initial feeling was that the actual chemical buffer systems used should not be too critical. In the present study, the buffer was considered as primarily an electrolyte and a dispersion medium. Variation of such physical properties as viscosity and conductivity was thus first evaluated to determine the effect of these variables on the electrochromatographic behavior of layer silicates. Examination of various organic buffer systems was not deemed advisable, under existing conditions. Organic buffers are generally employed where the sample is so much more soluble in either the water or the organic phase that considerable variation in the chromatographic behavior of the particles can be effected through variation of the ratio of water to organic material in the mixture. Furthermore, the solubility properties of the plexiglas apparatus prohibited conduction of experiments involving a rather wide range of organic buffer systems. The organic systems were held in reserve as a possibility for use in the case of the hydrophobic papers, perhaps in conjunction

with organophilic complexes of layer-silicates with organic materials.

Most of the electrochromatographic work was carried out in a 0.01% Na_2CO_3 medium, since the mineral suspensions had been previously prepared in a Na_2CO_3 system. This was not a buffer system in the strictly-chemical sense of the word, but it was considered a satisfactory electrolyte by virtue of the non-dependence of layer-silicate migration on pH within a rather wide pH range (6, 11) and because of the relative constancy of pH of the system maintained by frequent changing of the electrolyte in the electrode and solvent vessels. The Na_2CO_3 system will be referred to as the "buffer" throughout the remainder of this manuscript, though such terminology is more dependent upon tradition established by other electrochromatographic workers than upon the chemical nature of the system. The effects of varying buffer concentration were also evaluated by employing either 0.05%, 0.01% or 0.005% Na_2CO_3 "buffers".

Water-glycerol systems of varying proportions were effective in retarding somewhat the chromatographic flow rates of the high-capacity papers. For the S & S 470A paper, a 0.01% Na_2CO_3 buffer containing glycerol, used with a potential gradient of 20 v/cm, permitted the greatest horizontal displacement of the bentonite components during electrochromatographic determinations. The glycerol was also felt to be of possible value at low temperatures in minimizing diffusion of mineral particles on the paper, thus leading to narrower band widths than might be expected in water systems at higher temperatures.

pH and specific conductance values for the various Na_2CO_3 and

Na_2CO_3 -20% glycerol systems employed are recorded in Table 3 of the Appendix.

Choice of voltage

With the high-capacity papers such as S & S 470A, it was found expedient to operate at maximum capacity of the power source, which was 1000 volts (20 v/cm). Though such high potential gradients were not actually necessary on the low and medium capacity papers such as Whatman 41H, Whatman 3MM and S & S 598, the combination of low buffer feed rate and low potential gradient would have necessitated a week or longer to obtain sufficient electrochromatographic displacement or separation of mineral particles. Therefore, experiments with the low capacity papers were also run at the maximum potential gradient of the unit, i. e. 20 v/cm.

Choice of temperature

The temperature-viscosity relationships of the buffer systems studied and the supposed effect of temperature on limitation or accentuation of the diffuse nature of the particle migration bands led to the use of low temperatures for the electrochromatographic studies of layer-silicates. Temperature control was effected through the use of a cooled fractionation plate, as described in the REVIEW OF LITERATURE section. High temperatures usually result from the use of the higher potential gradients unless some means of refrigeration is supplied. This effect is even more pronounced in glycerated systems where the cooling effects of evaporation are greatly decreased.

A temperature of 8 °C was selected with the above considerations in mind, as well as the limitations imposed by the available water bath.

Choice of sample delivery rate

Sample delivery rates actually turned out to be a critical property of the electrochromatography system. Excessive delivery rates resulted in mineral accumulation around the feeding origin. Slow delivery rates necessitated extremely long periods before satisfactory movement of minerals could be achieved, as well as in some cases a resultant mineral concentration on the paper so weak as to be marginal for detection. Thus the maximum delivery rate which still yielded essentially a "point source" of minerals at the origin was continuously sought. As previously stated, sample delivery rates were varied by using syringes of various sizes, by varying the concentration of the mineral samples, or both. The sample was applied in approximately the same buffer system as used for the chromatographic flow, though this was not always necessary (22, p. 47).

Procedure Used for a Typical Electrochromatographic Run

The exact electrochromatographic conditions will vary from one run to the next, but certain considerations are necessary for each determination. The general sequence of runs during the course of the study was as follows:

1. The experimental conditions to be used and evaluated were selected.
2. The necessary materials for the run were prepared, including:

- a. Preparation of suitable and sufficient buffer solution;
 - b. Preparation of a sample of the desired mineral(s), in the buffer to be used for the run, if possible;
 - c. Preparation of the paper sheet by cutting the necessary drip points and tabs.
3. The temperature of the water bath was adjusted to the temperature desired for the run.
4. The electrode vessels were filled with buffer to within an inch of the top. The solvent vessels were filled to a level providing the desired hydraulic head.
5. The sheet was positioned in the apparatus and allowed to wet by capillarity (with S & S 470A) or by addition of buffer to the fractionation area from a syringe (for the slower papers).
6. The desired voltage was applied to the paper approximately 1/2 hour after the paper had become entirely wetted. (This 1/2 hour equilibration period was lengthened to overnight by Karler whenever exact analytical patterns were desired (22, p. 16).
7. Sample feeding was commenced after attainment of current-voltage equilibrium, as indicated by stabilization of the current flow.
8. The syringe was refilled during the run whenever necessary, and the buffer changed in the electrode and solvent vessels every 24 hours for runs greater than a day in length.
9. The voltage was turned off and the sample case removed at the end of the run.
10. The electrode tabs, solvent tabs and drip points were

severed from the fractionation area. This area was then transferred to a copper screen and dried in a forced-air oven at 80 °C. (Due to adsorption of the layer-silicates on the paper curtains, there appeared little danger of the pattern "running" during the drying process, as had been observed with patterns of highly-mobile constituents such as the amino acids.)

11. The curtain was dried overnight (this step was necessary to remove the last interfering traces of glycerol from the curtain and thus prevent interference with the staining procedure). It was then dipped through a trough containing either 0.002% or 0.004% malachite green dissolved in nitrobenzene. (Unless wetting at this stage was done rapidly and smoothly, splotchy staining resulted, with some areas of the curtain heavily stained and other areas stained only weakly or not at all.)

12. The curtain was hung in the hood overnight to dry after it had been stained. By the following morning the dark green background of the wet curtain had faded to the light blue or even white background of the dry curtain, with the blue color of the mineral pattern showing up clearly.

ELECTROCHROMATOGRAPHIC BEHAVIOR OF LAYER-SILICATE MINERALS

Adsorption of Layer-Silicates on Filter Paper

Paper electrophoresis or paper electrochromatography of layer-silicates is characterized by a strong adsorption of the minerals on the paper, similar to that observed by Karler in his electrochromatographic studies of some protein mixtures (22). When an amino acid solution is fed onto the electrochromatography curtain for a period of time and then feeding halted while electrochromatography continues, the amino acids move down their individual paths and are eventually "electrochromatographically eluted" from the paper. Under a similar set of conditions, a layer-silicate system leaves a streak on the paper, the movement of particles in the streak ceasing soon after sample feeding has been discontinued. This adsorption should make horizontal electrophoresis of minerals an extremely difficult phenomenon to study. The slower-moving components would be forced to travel over a path previously saturated with the faster-moving components, and would be rather intimately mixed with the faster components when detection of the minerals on the paper was attempted.

If the limitations imposed by this adsorption of components are realized prior to determinations, electrochromatography can be profitably used in the study of such a system. In electrochromatography, as contrasted to conventional paper electrophoresis, each component is moving over its own individual path, and thus is less subject to interference from the other components of the system.

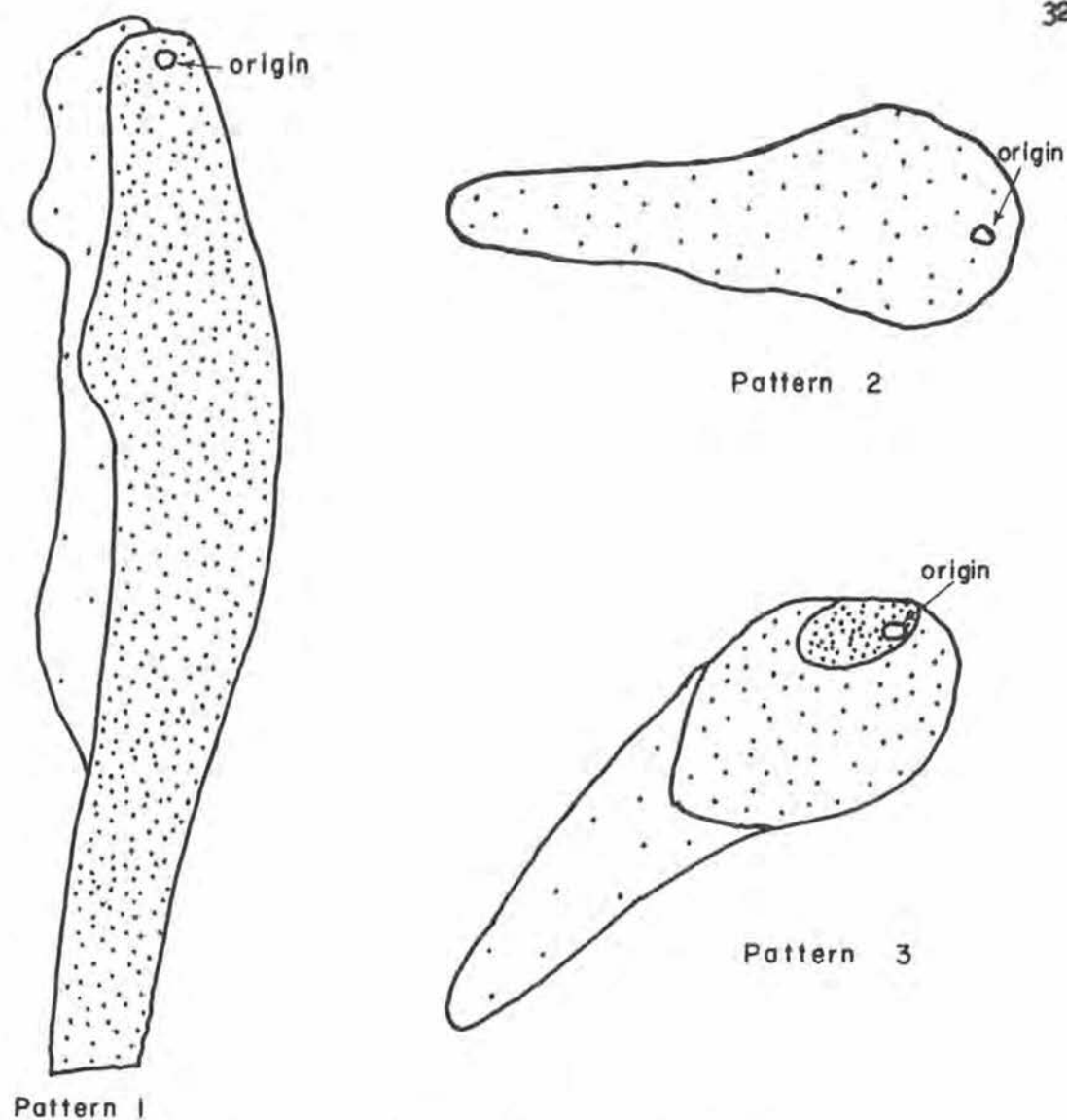
Movement under conditions of strong adsorption requires a path previously saturated with the strongly-adsorbed component over which subsequent particles can then move. The very mechanism of such a process would seem to necessitate rather long periods for saturation of the paper before the various fractions of the mixture could be taken from the drip points of the curtain. That there are ways to overcome the problem of adsorption of components on the paper will be indicated in the section on preparative electrochromatography and in the GENERAL DISCUSSION.

Effect of Varying Buffer Flow Rate

Figure 6 provides a clear indication of the effect of varying buffer flow rates on the electrochromatographic movement of layer-silicate minerals.³ Although variables other than buffer flow rates must be considered, the present comparison (see Figure 6 and Table 4 of the Appendix) was concerned more with the direction of mineral movement than with the shape of the mineral pattern. The essential difference between patterns 1 and 2 was in the flow capacities of the papers represented, while patterns 1 and 3 represent the same paper but buffer systems of widely differing viscosities.

It will be noted that buffer flow on pattern 1 was so rapid that practically all movement of the Utah bentonite was down the paper in response to chromatographic buffer flow. A diffuse blue area had

³All electrochromatographic patterns presented in this paper are full size, with the relative density of dots indicative of relative mineral concentrations as revealed by the intensity of the blue color of the malachite green-clay complex.



Electrochromatographic Variables					
Pattern No.	Paper	Buffer	Current (ma)	Feed Method	Time (hr)
1	S & S 470A	0.01% Na_2CO_3	—	10 ml syringe	24
2	Whatman 3MM	0.01% Na_2CO_3	2	1 ml syringe	27
3	S & S 470A	0.01% Na_2CO_3 plus 20 % Glycerine	3	5 ml syringe	24

All Patterns Run With 0.1% Utah Bentonite, at 8°C and 1000 v.

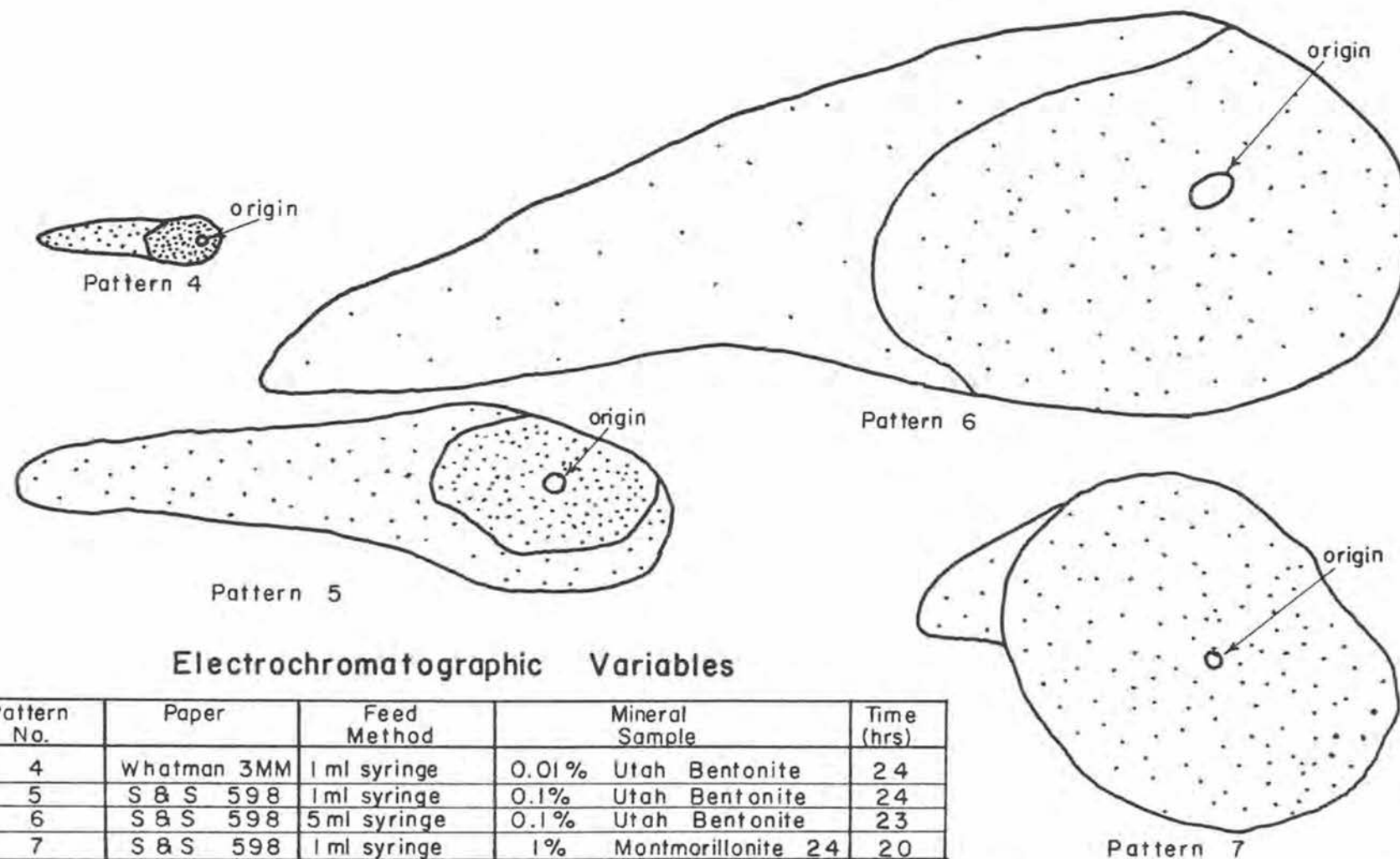
Figure 6 - Effect of Varying Buffer Flow Rates on Electrochromatographic Behavior of Layer-silicate Minerals.

begun to develop on the anode side of the pattern, however, as some mineral gradually moved toward the anode in response to electrophoretic forces. The narrow band with pattern 1 was probably due to a rapid chromatographic removal of layer-silicates from the origin as soon as they were applied to the paper, thus preventing accumulation at the origin as present in patterns 2 and 3. In pattern 2, the electrophoretic effect was by far the dominant one, with very little chromatographic movement down the paper. The bentonite particles in pattern 3 demonstrated the more desirable type of electrochromatographic movement in that electrophoretic and chromatographic forces on the particles were nearly equal, with a resultant electrochromatographic displacement of approximately 45° from the vertical. The accumulation of layer-silicates around the origin in patterns 2 and 3 (discussed in the following section) illustrates the inter-relation existing between buffer flow rate and sample delivery rate, for each of these patterns represents a layer-silicate delivery rate less than that represented by pattern 1. Slow electrochromatographic removal of sample from the origin resulted in mineral accumulation in the case of these last two patterns.

Effect of Varying Sample Delivery Rate

The four patterns in Figure 7 demonstrate the effect of varying sample delivery rate on electrochromatographic movement of layer-silicate minerals. The size and shape of these patterns should be noted.

Pattern 4 represents a sample delivery rate of $4.2 \mu\text{g/hr}$, with



All Patterns Run in 0.01% Na_2CO_3 , at 8°C , 1000v and 2 ma.

Figure 7. Effect of Varying Sample Feed Rate on Electrochromatographic Movement of Layer-Silicate Minerals.

a total of 0.10 mg of mineral added to the paper. The pattern approximates the desirable narrow band of uniform or slightly increasing width which was observed for less-strongly adsorbed materials such as amino acids. However, even at this low delivery rate there was a definite tendency for bentonite to accumulate around the feeding origin. Total migration of the layer-silicates was also quite small. At this delivery rate, periods of days or even weeks might be required to achieve a given degree of separation of a mineral mixture under conditions of more rapid chromatographic flow. The requirement for a paper path saturated with adsorbed layer-silicates before subsequent particles could move further across the paper was no doubt one factor contributing to slow migration in this case.

The mineral delivery rate in pattern 5 was $62 \mu\text{g/hr}$ for a total addition to the paper of 1.5 mg of bentonite. The accumulation around the origin was larger than in pattern 4, and the zone of mineral movement was appreciably wider. The zone was widest near the origin, indicating that the mass of bentonite at the origin was continually increasing with time, with the bentonite moving from an ever-widening zone of accumulation. The mineral moved much further across the curtain than in pattern 4, though the diffuse pattern was less desirable.

Pattern 6 represents a total of 9.2 mg of bentonite delivered at a rate of $400 \mu\text{g/hr}$. The zone of accumulation at the origin was extremely large, with the band of mineral movement much broader and

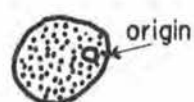
more diffuse than in the two previous patterns. The band definitely tapers to a point, supporting the hypothesis that the zone of accumulation at the sample delivery origin gradually increased during the course of the run. However, the mineral moved a total of 15 cm from the origin, or well over half-way across the reference grid of the fractionation area, during the determination.

Pattern 7 represents a condition of such rapid sample delivery that almost the entire mineral fraction remained in the mass around the origin. A total of 9.4 mg of montmorillonite was added in this instance at a rate of 460 $\mu\text{g/hr}$. By the end of the run, the mineral was just starting to move from the mass into its fractionation pattern. Though the total mineral concentration and rate of delivery were only slightly greater than that of pattern 6, the change to pure montmorillonite in place of the more impure and partially cemented montmorillonite of the Utah bentonite sample has apparently resulted in a feed rate of the magnitude necessary for "auto-catalytic accumulation" of montmorillonite at the origin. This accumulation is probably similar to that mentioned by Karler (22, p. 88) in connection with the separation of large particles by means of electrochromatography. This excessive accumulation at too-rapid sample delivery rates may also be the reason that Block, Durrum and Zweigg (9, p. 544) warned against attempting to separate mixtures which were too concentrated by means of electrochromatography. In any case, the pattern in question demonstrates a possible result of excessive sample delivery rates in mineral studies.

Effect of Varying Buffer Concentration

The three patterns shown in Figure 8 demonstrate the effect of varying buffer concentration on the electrochromatographic movement of layer-silicate minerals. The movement in pattern 8 resulted from a buffer of 0.05% Na_2CO_3 and an equilibrium current of 7.5 ma. The spot was globular, with no apparent tendency for electrochromatographic movement of the mineral exhibited. Pattern 9 (also discussed as pattern 4 on page 33) shows the movement attainable using a buffer concentration of 0.01%, with an equilibrium current of 1.5 ma. The electrochromatographic migration path was sharper, and the mass of bentonite at the origin was smaller than in the previous case. Pattern 10 demonstrates the type of movement obtained in a buffer concentration of only 0.005%. The total migration distance was greater than with either pattern 8 or 9. The wider band than in pattern 9 is attributed to increased chromatographic flow in this case (Table 5, Appendix).

The sharper and longer electrochromatographic migration path in buffers of lower ionic strength is probably the result of an increased amount of current being carried by the mineral particles themselves in such buffers. In the buffers of higher ionic strength more of the current would be carried by the buffer ions and less by the minerals. This would result in less electrophoretic displacement of particles (22, p. 39). This view is also supported by Overbeek and Lijklema (29, p. 13) who stated that electrophoresis will be slower, the higher the ionic strength of the buffer used.



Pattern 8



Pattern 9



Pattern 10

Electrochromatographic Variables

Pattern No.	Buffer	Current (ma)
8	0.05 % Na_2CO_3	7
9	0.01 % Na_2CO_3	2
10	0.005 % Na_2CO_3	0.5

All Patterns 0.01% Utah Bentonite Fed from a
1ml syringe onto Whatman 3MM Paper for 24
Hours at 8° C

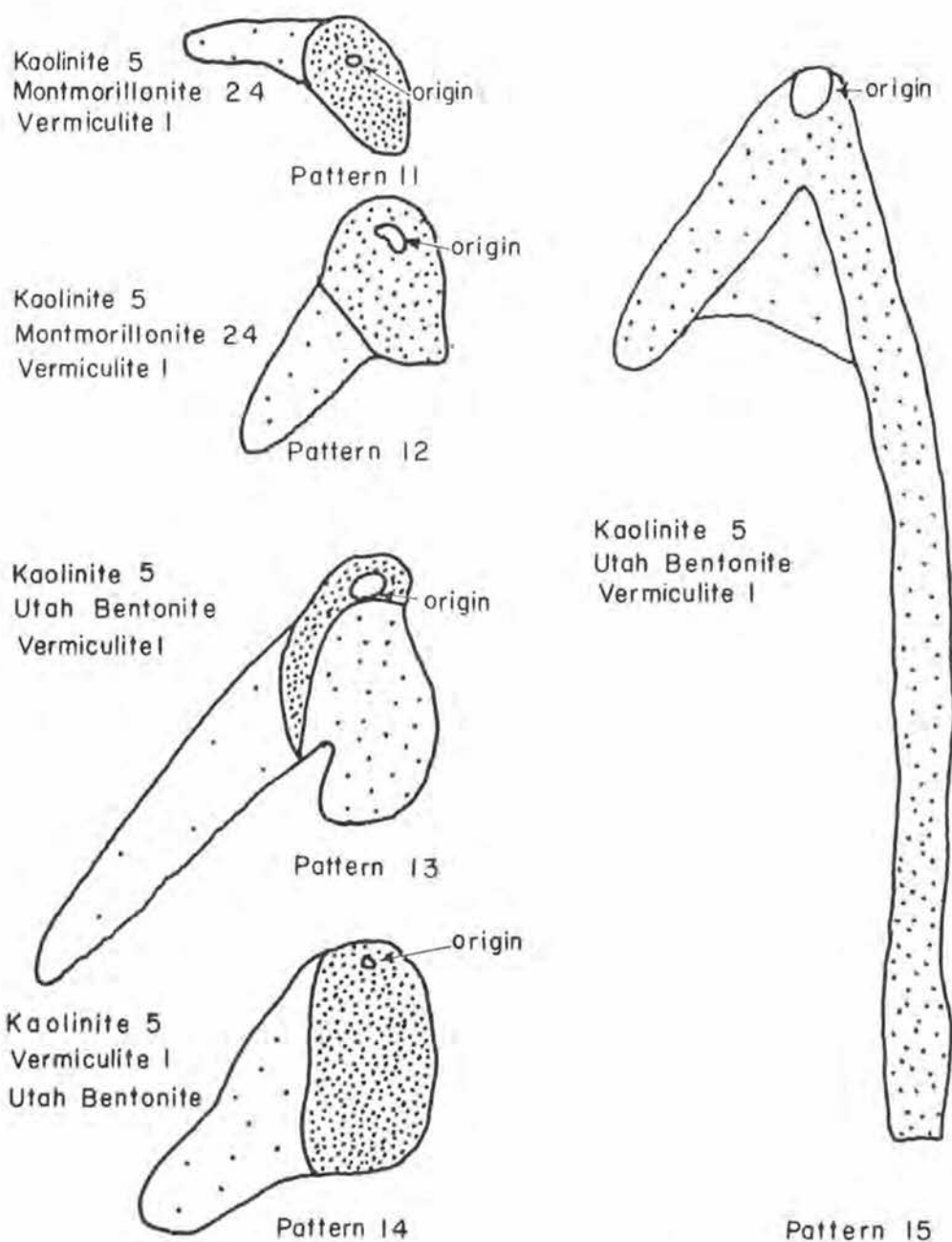
Figure 8. Effect of Varying Buffer Concentration on Electro-Chromatographic Movement of Layer-Silicate Minerals.

Electrochromatographic Movement of Mineral Mixtures

Though conditions to produce layer-silicate mineral migration in clear-cut flow lines still needed to be better defined, it was felt desirable in the limited time available to see if some separation of minerals could be effected. Patterns showing the electrochromatographic movement of layer-silicate mineral mixtures are shown in Figure 9.

The first sample mixture on which separation was attempted was a mixture of Kaolinite 5, Montmorillonite 24 and vermiculite. Separation was attempted on S & S 598 paper. The mixture was introduced at a rate of $4.3 \mu\text{g}$ of each mineral per hour, with a total application of 0.291 mg of each mineral. As can be seen from pattern 11, Figure 9, some separation into two components was underway at the end of the run, although a third mineral band was not readily discernible. The band extending down the paper and to the right was by far the darker of the two, indicating that perhaps it contained the larger concentration of layer-silicate minerals.

Incorporation of 20% glycerol in the sample delivery system frequently led to problems such as siphoning of the sample from the syringe and ejection of sample solution from the rear of the syringe barrel. An attempt was made to introduce a sample mixture suspended in a Na_2CO_3 -water system to a glycerated buffer system. Results are presented in pattern 12, Figure 9. The mineral mixture was the same as that used in pattern 11, with S & S 470A paper used in this case. Sample delivery rate was $23 \mu\text{g}$ of each mineral per hour. The pattern



Electrochromatographic Variables

Pattern No.	Paper	Buffer	Current (mg)	Feed Method	Time (hr)
I	S & S 598	0.01% Na_2CO_3	2	1 ml syringe	20
12	S & S 470A	0.01% Na_2CO_3 All	2	5 ml syringe	44
13	S & S 470A	0.01% " in 20% Gly-	1.5	5 ml syringe	48
14	S & S 470A	0.005% " cerol	0.5	5 ml syringe	53
15	S & S 470A	0.01% "	2	5 ml syringe	48

All Patterns Run at 1000 V and 8°C .

Figure 9. Electrochromatographic Movement of Layer-silicate Mineral Mixtures.

was rather inconclusive, with movement of one component definite and of any other components speculative. That the water in the sample markedly affected the viscosity of the system and thus the chromatographic fluid flow pattern is evident from the data of Table 5 of the Appendix, where the marked increase in the total number of ml. collected from the drip points beneath the sample delivery origin can be observed. Experiments employing non-glycerated samples for introduction to glycerated buffers were repeated with single-mineral systems, with the decision finally made to abandon this approach and to use sample solutions of essentially the same viscosity and density as that of the buffer when working with glycerated systems. As long as the syringe was maintained in a horizontal position, little trouble with siphoning was encountered. Maintenance of the tip of the syringe needle at approximately $1/2$ mm off the curtain, with the syringe checked occasionally to correct any tendency of the plunger to bind in the barrel, eliminated to a large extent the problem of sample ejection from the rear of the syringe barrel.

Pattern 13 represents a mixture of Kaolinite 5, Utah bentonite and vermiculite run in a glycerated system on S & S 470A paper. The sample was delivered at the rate of $25 \mu\text{g}$ of each mineral per hour. Separation into two definite mineral components was observed, with the right-hand band once more by far the darker of the two. It was observed during the run that mineral floccules were developing in the syringe barrel, indicating a lack of stability of at least one mineral in the mixture under these experimental conditions. The effect of this flocculation on mineral movement will be

mentioned again in connection with the discussion of pattern 15.

Pattern 14 shows the effect of reducing the buffer concentration to half that used in the fractionation illustrated in pattern 13. All other experimental conditions remained the same. Any separation of the band into more than one component is once again largely speculative rather than definite. It was concluded that in the presence of 20% glycerol the 0.01% Na_2CO_3 buffer was better for the separation of layer-silicate minerals than was 0.005% Na_2CO_3 .

Pattern 15 represents the same conditions used in pattern 13, with a 0.01% Na_2CO_3 -20% glycerol buffer and S & S 470A paper. The mixture of Utah bentonite, vermiculite and Kaolinite 5, introduced at a rate of 25 μg of each mineral per hour, produced the most promising pattern obtained. Two rather well-defined flow lines appeared. One band, which one would expect to be kaolinite from charge-to-mass ratio considerations, was carried with the flow path of buffer down the paper. Another component moved at an angle of 45° from the vertical, which was the same direction that the Utah bentonite had migrated in previous experiments under these conditions. The much better migration of the apparently-kaolinitic component in this pattern would tend to indicate that it was actually this right hand (kaolinitic) component which had started to flocculate during the run represented by pattern 13. There appears to be no ready explanation for the lack of stability of the component in one case and its stability in the other case under apparently the same experimental conditions. It is realized that the reasonably-good separation and well-defined flow lines of

pattern 15 represent only a crude beginning. Nevertheless, there should be little doubt that separation of colloidal silicate minerals by electrochromatography is not only theoretically but is also experimentally possible.

Preparative Electrochromatography

All of the preceeding work could be classified as analytical electrochromatography, in that the main concern of the methods was to observe the mineral patterns under varying experimental conditions. Attention was next briefly turned to the possible use of paper electrochromatography in preparative work, through the collection and analysis of the fractions leaving the different drip points of the curtain.

In spite of possible effects on sample movement, a curtain was soaked for 4 hours in 0.4% $<0.2\mu$ Montmorillonite 11 in an attempt to presaturate the adsorption sites of the paper with layer-silicate particles. It was hoped to thus speed up the movement of the sample particles down the paper. After drying, the curtain was placed in the electrochromatography chamber and excess montmorillonite removed by "electrochromatographic elution" for 48 hours with a 0.01% Na_2CO_3 -20% glycerol buffer system under an applied potential of 1000 volts. Sample feeding of a Kaolinite 5, Utah bentonite and vermiculite mixture at the rate of $25\mu\text{g}$ of each mineral per hour was commenced. After feeding for 24 hours, effluent from the various drip points was collected for 72 hours.

The effluent was concentrated approximately ten times through

evacuation at 90 °C in a vacuum oven to remove excess water and glycerol. Visible floccules in the tubes were formed by this treatment, the total amount of flocs varying from tube to tube. The flocs, apparently consisting of a mixture of filter paper residues and layer-silicate minerals, were removed from the tubes with an eye dropper. They were placed on glass slides and dried in the vacuum oven at 90 °C. (Slides were also prepared from the concentrated effluent containing no visible flocs, but insufficient layer-silicates were present to yield definite X-ray diffraction patterns.) X-ray analysis of the dried flocs was made on a Morelco X-ray diffractometer, equipped with a Geiger counter and a Brown recorder. The radiation was Cu K α . A tube potential and current of 40 kilovolts and 20 milliamperes, respectively, were used. Divergence and scatter slits of 1/4° and a 0.006 inch receiving slit equipped with a Ni filter were used for all patterns. For the recorder, the rate meter settings of 2 x 0.6 corresponding to 60 counts per second full scale with a time constant of 8 were used. Several of the dried and analyzed slides were also treated with a drop of 0.5 N KCl, allowed to air dry at room temperature, and once more scanned with the X-ray unit. Criteria used for identifying the minerals present in the flocs from a given tube were established by X-ray analysis of the original sample and its components under similar conditions of glycerol saturation and glycerol-potassium saturation. These criteria are given below:

Montmorillonite (including bentonite) - 18 Å peak on the glycerated slide, collapsing to 14-15 Å upon K⁺ treatment and drying.

Vermiculite - 14-15 Å peak on the glycerated slide, tending to collapse to 11-12 Å upon K^+ treatment and drying.

Kaolinite - 7.2 Å peak on the glycerated slide, and also on the unheated K^+ -treated slide.

(All "dried" slides thus apparently retained appreciable glycerol.)

The results of the X-ray analysis are presented in Table 1.

Insufficient residue for adequate analysis was found in tubes 3, 5, 15 and 17, even upon reheating at 90 °C for several hours. Residue was found in tubes 6, 7, 9, 10, 11, 12 and 14, but no peaks were evident in the X-ray patterns, even after the slides had been repositioned and rescanned several times. The residue in these cases might be filter-paper fibers eluted from the paper during the course of the run or amorphous decomposition products resulting from electrochromatographic treatment of the sample minerals.

The results of the X-ray analysis verify that at least a portion of the minerals in the sample mixture were able to survive the exposure to electrochromatographic conditions without complete disruption or degradation of their crystal lattices. This fact, coupled with the general portion of the curtain from which the minerals were eluted, strongly indicates that the movement obvious in the analytical electrochromatography phase of the research was that of the minerals themselves, rather than of possible mineral decomposition products resulting from the treatment.

The kaolinitic component was observed in tube 20, almost directly below the point of sample origin, and in no other tube. Vermiculite, although only weakly evident in tubes 18 and 19, was not detected in

Table 1. X-ray Diffraction Properties of Drip Point Effluents Following Preparative Electrochromatography of a Layer-Silicate Mineral Mixture.

Drip Point	Layer-Silicate Minerals Present ¹		
	Vermiculite	Montmorillonite	Kaolinite
1		**	
2		**	
3			
4		***	
5			
6			
7			
8		*	
9			
10			
11			
12			
13		*	
14			
15			
16		***	
17			
18	*	****	
19	*	****	
20		*****	***
21		**	
22		**	
Original Sample Mixture	***	***	****

¹Number of asterisks denotes the relative intensity of the 1st order, 001 peak characteristic of the mineral.

any other tubes. It apparently moved with the kaolinitic mineral during the early stages of the analytical electrochromatography patterns represented by patterns 11-15 and then separated from the kaolinite in the lower portion of the curtain, being pulled toward the anode into tubes 18 and 19. This movement with kaolinite would not be predicted from charge-to-mass ratio considerations. Montmorillonitic minerals appear concentrated in two regions, tubes 1-4 and tubes 16-22. These results at first seemed rather discouraging, but it may be possible to consider tubes 1-4 as representing the actual Utah bentonite of the sample mixture, with the montmorillonite in tubes 16-22 attributable to another effect as described in the following paragraph. Thus the mineral mixture may have been at least roughly separated by means of preparative electrochromatography into its three components, with the bentonite coming off in the region of tubes 1-4, the vermiculite coming off into tubes 18-19, and the kaolinite coming off into tube 20. Further refinement of the technique will be necessary to verify such a separation and to improve on the separation if the minerals are actually being only partially separated.

The X-ray analysis of minerals in the fractionation tubes may aid in more clearly elucidating the process of adsorption of layer-silicates on the filter paper curtain during electrochromatography. The montmorillonite in tubes 16-22 could conceivably result from a poor separation of the sample mixture, with some Utah bentonite actually moving almost vertically down the curtain to emerge from drip

points 16 to 22. It could also be excess Montmorillonite 11 not removed from the curtain by the 48-hour period of electrochromatographic elution of the paper. (This is not a likely explanation, however, for it fails to explain the virtual absence of montmorillonitic components in tubes 5-15, which would also be expected to contain Montmorillonite 11 "contaminant".) In light of the apparent separation of mineral mixtures observed under nearly identical conditions during the analytical electrochromatography phase of the research, it appears that the montmorillonitic components in tubes 16-22 may actually represent Montmorillonite 11 displaced from the adsorption sites of the paper by kaolinite and vermiculite particles as they moved down the paper. Such a process seems a plausible one, and would explain this observed presence of montmorillonite particles in a portion of the curtain where theoretical considerations and previous experimental work would not predict them to be. The continuing nature of the process would explain the large number of tubes on the right side of the curtain containing montmorillonitic minerals. Such a process would also lead to a prediction that relatively more vermiculite and kaolinite and less montmorillonite would appear with increasing time in tubes 16-22, as larger percentages of the kaolinite and vermiculite paths became saturated with their respective minerals. If the occurrence of such a process can be verified or disproved by future laboratory work, it may shed light on the

mechanism of the adsorption process of minerals on filter paper, and may eventually lead to the discovery of a medium on which such adsorption is considerably lessened or even absent. In any event, there lies ahead considerable potential research in the area of preparative electrochromatography of layer-silicate minerals.

Consideration of Anomalous Results

Several types of "anomalous" electrochromatographic patterns added confusion and frustration to the challenge presented to the inexperienced electrochromatography worker. Some of these are briefly discussed in the following paragraphs.

Flooding patterns may occur when the sample is delivered to the paper so rapidly that the ability of the paper to assimilate the sample solution is exceeded. This type of pattern is highly irregular, and is easily recognized by the "arms" of sample extending down the paper in a nearly random manner in response to gravitational flow of the sample over the surface of the paper. The electrochromatographic pattern will develop in time from the flooded pattern, but it will naturally be wide and diffuse due to the large "origin" from which the electrochromatographic separation proceeds. Flooding was particularly pronounced on Whatman 41H paper to which the sample was introduced at a rate of 60 μg per hour. The experiment was repeated with 0.001% Utah bentonite fed at a rate of 0.42 μg per hour, but the resultant mineral concentration was so dilute as to be undetectable against the background of the paper.

In cases where there is an appreciable difference in viscosity between the buffer and the sample, the sample may apparently flow a short ways down the curtain on the surface of the paper before it is finally assimilated by the buffer for subsequent movement through the curtain in the normal electrochromatographic manner. Flow on top of the curtain appears to correspond to the chromatographic flow of buffer in that region of the curtain. In these instances rather diffuse electrochromatographic movement of the minerals eventually takes place from this fairly wide zone established by initial mineral movement down the paper.

There is also a danger of placing the sample delivery origin in an area of the curtain where buffer flow differs drastically from normal vertical flow. Such positioning may yield anomalous patterns as the mineral particles move more in response to the actual buffer flow in that portion of the curtain than to the theoretical electrophoretic and chromatographic forces.

Finally, anomalous mineral movement in unpredictable directions may result during low-temperature operation if there is not intimate contact between the curtain and the coolant plate. Temperature differences thus established may result in mineral movement with the buffer toward areas of excessive evaporation from the curtain.

GENERAL DISCUSSION

It was somewhat discouraging to find that the electrochromatographic behavior of layer-silicate minerals was not characterized by narrow, clear-cut bands such as were obtained in separations of smaller organic particles. It is felt, however, that the experimental results have proven promising enough to warrant further research in this area. The present work should aid future workers to more rigidly define experimental conditions so that the electrochromatography of layer-silicates may become a useful experimental technique.

The Karler-Misco apparatus has been satisfactory for the preliminary examination of electrochromatographic conditions to be used in layer-silicate studies. A major limitation of the apparatus has been the total potential possible from the power source. If the electrochromatography of layer-silicate minerals is to be made sufficiently rapid for practical laboratory usage, the fractionation time must be appreciably decreased. A considerably larger power source probably could be used to advantage in enhancing the lateral movement of the mineral particles, thereby allowing an increase in chromatographic flow with a subsequent decrease in fractionation time. This decreased fractionation time should also lessen the diffuse nature of the electrochromatographic bands for the various layer-silicates (9, p. 531). The maximum permissible potential gradient would be that just short of causing decomposition of the mineral lattice. Kickhofen and Westphal (23) used gradients of up

to 120 v/cm for peptide separations, as compared to the only 20 v/cm used in this study.

Once its limitations in the presence of glycerol are realized, the malachite green stain becomes a useful means for detecting mineral bands on the paper curtain. If it becomes desirable to use methods allowing not only detection but also differentiation of the layer-silicate minerals while on the curtain, perhaps work such as that of Ishibashi (20), in which several staining reagents were used to differentiate layer-silicates, might be used. Karler has implied (22, p. 82) that iodine vapor is a good and nearly "universal" reagent to be used for the detection of bands on the paper, so its substitution for malachite green in cases where traces of glycerol may be present during staining might well be considered.

The same need for a relatively rapid procedure mentioned earlier in this section makes it almost imperative that S & S 470A or similar rapid papers be used for electrochromatographic work with layer-silicates. The high capacities of such papers allow a great deal of variation in buffer flow rates. The use of water-glycerol systems of varying proportions offers an effective means for changing the buffer flow rate of such papers to provide suitable electrochromatographic movement of particles. Methods for simple, rapid removal of the glycerol and recovery of the eluted minerals need to be devised yet, however.

Care must be exercised in work with layer-silicates to avoid excessive sample delivery rates. Such rates may lead to localized

flooding of the paper or to autocatalytic accumulation of minerals at the origin. For analytical work the lowest delivery rates which still allow detection of the minerals on the paper are most desirable, while for preparative work the maximum feed rate not giving flooding or accumulation at the feeding origin should be sought.

Adsorption is definitely a problem in layer-silicate studies by the means of paper chromatography or paper electrophoresis. The problem is mainly one of time, rather than of experimental accuracy, for Grassman (17) has indicated that adsorption modifies only the speed, and not the direction, of electrochromatographic movement. One approach to the problem would be attempting to locate a supporting medium on which there was little or no adsorption of the minerals studied. Instead of eliminating this adsorption, however, it may be more feasible or even necessary to continue the use of high-voltage electrochromatography to circumvent the problem. Electrochromatographic technique allows each mineral to travel over its own migration path on previously adsorbed particles of its own mineral species. Suitable individual paths down the curtain are thus possible if diffusion and overfeeding can be either minimized or eliminated. The technique is thus applicable in the presence of adsorption of particles on the paper curtain, whereas strip electrophoresis has at best only limited applicability under such conditions.

In general, less-sharp resolution must be expected on the thicker papers such as S & S 470A, since convection currents are set up in the increased buffer volume of the papers (9, p. 529). Other

authors have also obtained rather diffuse bands in using the electrochromatographic technique for the study of humic acids at high potential gradients (10).

It is not claimed that the conditions presented in this manuscript are the only ones allowing satisfactory study of layer-silicate minerals by electrochromatography. They merely appeared to be most satisfactory under the physical limitations which were imposed on this series of experiments, such as size and shape of apparatus, power source and available curtain material. It has been proposed by Kunkel and Trautman (25, p. 238-240) that zone electrophoresis on granular starch might prove desirable for large particles. They state that adsorption is often considerably lessened on such a medium. Block, Durrum and Zweigg (9, p. 52) also note that ion-exchange papers have been used for the separation of small inorganic anions such as SeO_3^{-2} and TeO_3^{-2} , so perhaps such papers could be used to advantage in layer-silicate mineral studies.

It might prove rather interesting to employ this type of procedure, once it has had the necessary modifications, for the study of particles such as the soil clay-organic matter complex. This was attempted in a Tiselius-type apparatus by Reinosa (32). A basic motive of the current research was to learn about the electrochromatographic behavior of layer-silicate minerals so that this knowledge, coupled with existing knowledge of the electrochromatographic behavior of soil organic matter constituents, would eventually allow the electrochromatographic study of soil clay-organic matter complexes.

With appropriate (and, at the present, rather numerous) modifications, it appears that colloidal mineral-organic particles of differing character may be separable by electrochromatography for subsequent study.

SUMMARY

Selected layer-silicate minerals were examined under varying electrochromatographic conditions in a Karler-Misco electrochromatography unit in an attempt to determine if the technique could be used to advantage for the study of such minerals and for their separation from mineral mixtures.

Malachite green (0.002% in nitrobenzene) was found to serve as a satisfactory detection reagent for the location of minute amounts of layer-silicates on paper curtains. Effectiveness of the stain was reduced in the presence of glycerol.

Initial wetting rates and capacities of various filter papers were evaluated. S & S 470A was selected as the most satisfactory paper for electrochromatographic studies of layer-silicates. A 0.01% Na_2CO_3 -20% glycerol buffer medium was used in conjunction with this paper for most studies.

Sample delivery rates were found to be quite critical in work with layer-silicate minerals. Too-slow delivery rates gave insufficient sample for adequate detection, while too-rapid rates caused flooding or accumulation of minerals at the sample delivery origin. Strong adsorption of the minerals on the paper was exhibited with all minerals studied.

Patterns are presented for individual minerals and mixtures of minerals under varying electrochromatographic conditions. Separation of a mixture of montmorillonite, kaolinite and vermiculite into its individual mineral components has been at least partially achieved.

Considerable refinement of experimental conditions remains before entirely satisfactory patterns are obtained and before layer-silicate separations are possible in a practical length of time. Nevertheless, it is concluded that, with refinement, electrochromatography should be a useful technique for the separation and study of layer-silicate minerals.

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APPENDIX

Table 2. Capacities and Characteristics of Selected Filter Papers.

Paper ¹	Special Characteristics ²	Initial Wetting Rate (cm/hr)	Capacity (ml/hr)	Thickness (in) ²
S & S 1001	100% Polyvinyl Chloride	0 ³	0 ³	0.009
S & S 2495	Partially Acetylated	0 ³	0 ³	0.0075
S & S 2497	Fully Acetylated	0 ³	0 ³	0.006
S & S 2499	5% Dowex DC 1107	0 ³	0 ³	0.0075
W 1	Standard	3.0	<0.01	0.005
W 2	Standard	4.0	<0.01	0.006
W 20	Standard	3.0	<0.01	0.005
W 40	Ashless	4.0	<0.01	0.006
W 42	Ashless	3.0	<0.01	0.007
W 52	Hardened	3.5	<0.01	0.006
S & S 26	Glass Fiber "Paper"	5.0	<0.01	0.0085
S & S 287	Impregnated with infusorial earth	3.5	<0.01	0.01
S & S 576	Very Smooth, Hardened	2.5	<0.01	0.004
S & S 589BR	Ashless, Acid Washed	3.0	<0.01	0.0065
S & S 589RR	Ashless, Acid Washed	3.0	<0.01	0.007
S & S 2040A	Standard, Natural	5.5	<0.01	0.0065
S & S 2043A	Standard, Natural	2.5	<0.01	0.0065
S & S 2043B	Standard, Smooth	4.0	<0.01	0.0075
S & S 2045A	Standard, Very Smooth	1.5	<0.01	0.0065
S & S 2045B	Standard, Very Smooth	2.0	<0.01	0.0075
W 3MM	Standard	5.0	0.02	0.013
W 4	Standard	6.0	0.02	0.007
W 41H	Hardened	5.0	0.04	0.005
W 54	Hardened	4.5	0.02	0.006
S & S 589WR	Ashless, Acid Washed	4.0	0.01	0.007
S & S 589OR	Ashless, Acid Washed	4.5	0.02	0.0095
S & S 2041	Standard	4.5	0.01	0.006
S & S 2043B	Standard	4.0	0.01	0.0075
S & S 507	Ashless, Hardened	2.0	0.07	0.004
S & S 589GR	Ashless, Acid Washed	6.5	0.12	0.0125
S & S 589BKR	Ashless, Acid Washed	6.5	0.09	0.008
S & S 598	Standard	7.0	0.19	0.012
S & S 2040B	Standard, Smooth	8.5	0.09	0.0075
S & S 2316	Standard	5.0	0.09	0.012
W 31DT	Ashless	11.0	0.51	0.017
S & S 470	Standard	13.0	1.20	0.035
S & S 470A	Smooth	12.0	0.99	0.022

¹W - Whatman S & S - Schleicher and Schuell²Data taken from manufacturer's literature³hydrophobic paper

Table 3. pH and Specific Conductance of Various Sodium Carbonate Systems.

System	pH	Specific Conductance (mhos)
0.05% Na_2CO_3	10.6	8.0×10^{-4}
0.05% Na_2CO_3 -20% glycerol	10.2	4.44×10^{-4}
0.01% Na_2CO_3	10.2	1.67×10^{-4}
0.01% Na_2CO_3 -20% glycerol	9.6	0.89×10^{-4}
0.005% Na_2CO_3	9.9	0.95×10^{-4}
0.005% Na_2CO_3 -20% glycerol	8.9	0.56×10^{-4}

Figure 4. Experimental Conditions for the Electrochromatographic Runs Illustrated in the Manuscript.

Pattern No.	Paper	Buffer	Current ¹ (ma)	Size of Feed Syringe	Sample	Total mls of sample applied	Time (hrs)
1	S & S 470A	0.01% Na ₂ CO ₃	—	10 ml	0.1% Utah bent.	—	24
2	Whatman 3MM	0.01% Na ₂ CO ₃	2	1 ml	0.1% Utah bent.	1.0	27
3	S & S 470A	0.01% Na ₂ CO ₃ plus 20% glycerol	3	5 ml	0.1% Utah bent.	6.2	24
4	Whatman 3MM	0.01% Na ₂ CO ₃	2	1 ml	0.01% Utah bent.	1.0	24
5	S & S 598	0.01% Na ₂ CO ₃	2	1 ml	0.1% Utah bent.	1.1	24
6	S & S 598	0.01% Na ₂ CO ₃	2	5 ml	0.1% Utah bent.	6.6	23
7	S & S 598	0.01% Na ₂ CO ₃	2	1 ml	1.0% Mont. 24	1.0	20
8	Whatman 3MM	0.05% Na ₂ CO ₃	7	1 ml	0.01% Utah bent.	1.0	24
9	Whatman 3MM	0.01% Na ₂ CO ₃	2	1 ml	0.01% Utah bent.	1.0	24
10	Whatman 3MM	0.005% Na ₂ CO ₃	0.5	1 ml	0.01% Utah bent.	1.0	24
11	S & S 598	0.01% Na ₂ CO ₃	2	1 ml	0.01% Kaol. 5 0.01% Mont. 24 0.01% verm.	2.9	68
12	S & S 470A	0.01% Na ₂ CO ₃ plus 20% glycerol	2	5 ml	0.01% Kaol. 5 0.01% Mont. 24 0.01% verm.	10.2	44
13	S & S 470A	0.01% Na ₂ CO ₃ plus 20% glycerol	1.5	5 ml	0.01% Kaol. 5 0.01% Utah bent. 0.01% verm.	10.9	48
14	S & S 470A	0.005% Na ₂ CO ₃ plus 20% glycerol	0.5	5 ml	0.01% Kaol. 5 0.01% Utah bent. 0.01% verm.	13.5	53
15	S & S 470A	0.01% Na ₂ CO ₃ plus 20% glycerol	2	5 ml	0.01% Kaol. 5 0.01% Utah bent. 0.01% verm.	9.5	48

¹All runs made at 1000 volts potential (20 volts/cm) and 8 °C.

Table 5. Milliliters of Buffer Collected from the Drip Points During the Electrochromatographic Runs Illustrated in the Manuscript.

Pattern No.	Drip Point Number ¹																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1																						
2	.5	.5	.3	.5	.5	.3	.3	.3	NOT	RECORDED	.3	.3	.3	.5	.5	.5	.3	.3	.3	.3	.3	.3
3	10	10	8	8	8	8	10	9	7	7	7	9	8	7	8	7	7	8	8	9	10	9
4	1	1	.8	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	1	1	1	1
5	4	4	3	4	3	3	3	3	3	3	2	3	3	3	3	3	3	4	4	4	4	4
6	3	4	3	3	3	3	3	2	3	2	2	2	3	3	3	4	3	3	4	4	3	4
7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3
8	.8	.8	.8	.8	.8	.8	.5	.5	.5	.8	.8	1	1	2	2	2	3	3	3	3	3	3
9	1	1	1	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.8	1	1	1	1
10	5	5	6	5	4	4	3	4	4	3	3	3	3	4	4	4	4	4	3	4	3	3
11	11	11	12	11	12	9	8	9	8	9	9	8	8	9	9	8	9	9	11	10	9	9
12	14	15	12	12	12	12	14	15	13	14	14	13	14	16	15	15	15	27	34	33	20	22
13	21	21	17	18	18	19	20	17	20	19	18	18	21	21	19	19	22	20	25	24	23	41
14	26	28	27	27	26	23	23	22	23	23	21	26	22	22	23	21	23	24	23	29	24	37
15	22	26	20	21	19	19	19	18	19	22	20	23	23	20	20	19	21	22	21	23	25	27
Preparative Electrochrom- atography	46	33	35	42	33	38	28	28	30	42	31	28	34	30	33	37	39	49	53	64	60	66

¹Drip points numbered consecutively from the left-hand side of the curtain, with points 18-20 generally directly below the sample delivery origin.