A new apparatus for paper electrophoresis

Robert John Hodges
Union College - Schenectady, NY

Follow this and additional works at: https://digitalworks.union.edu/theses

Part of the Chemistry Commons

Recommended Citation
https://digitalworks.union.edu/theses/1919
A NEW APPARATUS FOR PAPER ELECTROPHORESIS
A NEW APPARATUS FOR PAPER ELECTROPHORESIS

by

Robert John Hodges

A thesis presented to the Department of Chemistry of Union College in partial fulfillment of the requirements for the degree of Bachelor of Science with a major in Chemistry.

by Robert John Hodges

Approved by William B. Martin Jr.

Date May 26, 1956
ACKNOWLEDGEMENT

I would like to express my thanks to Dr. William B. Martin of the Chemistry Department of Union College for his inspiring help which he willingly gave me in carrying out this research project. I also want to thank Dr. Howard E. Sheffer and Dr. Galen W. Ewing for their help in making this project possible.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgement</td>
<td>1</td>
</tr>
<tr>
<td>Table of Plates</td>
<td>iii</td>
</tr>
<tr>
<td>Historical</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>Apparatus</td>
<td>10</td>
</tr>
<tr>
<td>Experimental Results</td>
<td>21</td>
</tr>
<tr>
<td>Calculations</td>
<td>35</td>
</tr>
<tr>
<td>Summary</td>
<td>37</td>
</tr>
<tr>
<td>Suggestions for Further Work</td>
<td>39</td>
</tr>
<tr>
<td>Bibliography</td>
<td>41</td>
</tr>
</tbody>
</table>
TABLE OF PLATES

Plate I
Closed Strip, Solid Support .......... 7

Plate II
Closed Strip, Non-Polar Liquid ...... 9

Plate III
Current Decrease With Time .......... 14

Plate IV
New Apparatus .......................... 18

Plate V
Glucose Migration ...................... 25

Plate VI
Migration Curves ....................... 29

Plate VII
Migration of DNP-DL-isoleucine ...... 32
HISTORICAL

Paper electrophoresis was first reported on in 1937 by König (1) in a paper which appeared in Portuguese. But electrophoresis in stabilized media can be traced back to 1886 when Lodge (2) determined ionic mobilities in a tube filled with an indicator-containing jelly. In 1939 König published another paper in collaboration with von Klobusitsky (3) in Brazil in which they described their work in separating a yellow snake venom pigment on filter paper. This was the first application of paper electrophoresis to the study of protein mixtures.

Application of electrophoresis in other stabilizing media such as agar (4)(5)(6), alumina (7), asbestos fiber (8), starch (9), ground glass wool (10), cotton gauze (11), silk (12), and cation exchange resins (13), has been very widespread.

There have been over three hundred papers published which deal with zone electrophoresis. Of these, ninety per cent have dealt with paper electrophoresis. At present, there are several books available which deal with the topic extensively, such as (14)(15)(16) and others.

Within the area of electrophoresis using filter paper as the stabilizing medium, many types and modifications of apparatus have been designed and are being used today.

The applications of paper electrophoresis are already staggering in number and undoubtedly many more applications
will be devised in the future for the separation of more and more complex mixtures and for the study of various electrokinetic properties of charged particles. Some of the mixtures which are being separated today are: inorganic ions and complexes, proteins, peptides, amino acids, carbohydrates, lipoproteins, enzymes, hormones, vitamins, alkaloids, carcinogenic materials, and radioactive materials, to mention a few. Some of the electrokinetic properties which are being determined are: ionic mobilities, isoelectric points, zeta potentials, and others.

Valuable information is being obtained everyday by means of paper electrophoresis which is aiding significantly in pushing back the boundary of the unknown in biological and chemical research.
INTRODUCTION

In paper electrophoresis, taking the idealized case, a charged particle, i.e. a particle which has an excess or deficiency of electrons, resulting in a net electrostatic charge, is placed on a strip of moistened filter paper. The moistening is usually done by a buffer solution which serves two purposes:

1. It provides a liquid phase within the structure of the paper through which the particle can travel.
2. It keeps a constant pH throughout the run.

Then an electrical potential applied across the opposite ends of the paper strip by connecting the ends to the electrodes from a battery or a constant voltage output AC rectifier. Thus there is a potential gradient along the paper strip which will force the charged particle to move in one direction or the other, according to the sign of its charge.

Furthermore, in the ideal case, there is no attraction between the particle and the paper. Thus, no adsorption occurs and there are no chromatographic effects to be considered.

The force acting on the particle due to its charge can be given as the product of the charge and the field strength acting on it:

\[ F = qX \]
And the retarding force on the particle due to the viscosity of the medium can be seen in Stokes’ law:

$$\frac{F'}{V} = 6\pi r \eta$$

where $V$ is the electrophoretic velocity, $r$ is the radius of the particle, and $\eta$ is the viscosity of the medium.

Then it can be seen that particles with different charges can be easily separated because of the different forces acting on them and thereby different mobilities of the particles. On the other hand, particles with the same charge but different particle size or shape (Stokes’ law holds only for spherical particles) can be separated because of the different retarding forces acting on them, again resulting in different mobilities.

In some cases where only separation is desired, some adsorption on the paper is desirable because then chromatography aids in the separation. Thus a wide variety of mixtures can be separated by paper electrophoresis even though they may be very similar chemically.

Mobility has been defined as the migration distance traveled by the particle per unit field strength:

$$u = \frac{V}{X}$$

The factors governing the migration of any ion in
solution and in an electrical field may be summed up in the following three classes:

"1. These characteristics related to the ion itself, namely, its charge (sign and magnitude), size and shape, tendency to dissociate, and amphoteric behavior, if any.

"2. These factors related to the environment in which the ion is being studied, such as the electrolyte concentration, ionic strength, dielectric properties, chemical properties, pH, temperature, viscosity, and the presence of non-polar molecules which may influence viscosity or dielectric properties of the electrolyte, or which may interact to form charged complexes (for example, carbohydrates in the presence of borate buffers).

"3. The character of the applied field, its intensity, purity (presence of alternating-current components), and distribution along the migration path. It is obvious that secondary interactions between factors 1 and 2, either electrostatic or by van der Waals' forces, may further influence the experimental situation." (17).

The types of apparatus for paper electrophoresis may be divided into three general classes:

I. Closed strip (evaporation prevented).
   A. Solid support.
   B. Non-polar liquid.
II. Semi-closed (evaporation permitted).

III. Open strip (evaporation permitted), minimal area of support.
   A. Horizontal types
   B. Hanging strip types (18).

An example of the solid support classification would be the case where the paper strip is pressed between two plates of glass or plastic (Plate I). Thus, evaporation of the buffer solvent along the paper strip is prevented by the plates.

In the non-polar liquid type of closed strip apparatus the paper strip is immersed in a non-conducting, non-polar liquid which likewise prevents evaporation (unless the buffer is appreciably soluble in the non-polar liquid). This type of apparatus (Plate II) was first described by Consden and Stanier in 1952 (19).

In semi-closed apparatus, evaporation is permitted or regulated. For example, one might support the paper strip on one side with a plate of glass and nothing on the other side or a perforated plate on the other. In this way, evaporation can be allowed to proceed at a known rate and thereby regulated.

In the open strip type of apparatus, nothing is touching the paper except air (or other gas) or a vacuum, and the support rods. Here the evaporation is either permitted, or else retarded by increasing the pressure on the surrounding gas.
PLATE I

CLOSED STRIP
SOLID SUPPORT

PAPER
ELECTRODE
GLASS
BUFFER
After experimenting with several types of apparatus, the author decided that a new type of apparatus was needed and could be designed which would eliminate many of the difficulties which were inherent in other types of apparatus.

Therefore, the purpose of this research was to design, build, and test a new apparatus which would satisfy this need. Some experimental results were also to be obtained.
PLATE II

CLOSED STRIP
NON-POLAR LIQUID

BUFFER

PAPER

ELECTRODE

NON-POLAR LIQUID
APPARATUS

It has been shown (20) that when an apparatus is to be used for other than empirical separations of mixtures of compounds, e.g. for mobility determinations, the apparatus must possess such a geometry that results can be obtained which satisfy two necessary requirements:

1. The movement of the migrant must be linear with respect to time. At constant potential gradient, if a particle moves 2 cm. in 1 hr. it should move 4 cm. in 2 hrs.

2. The movement of the migrant must be linear with potential gradient. For example, if a particle moves 2 cm. in 1 hr. at 3 volts/cm., then it should move 4 cm. in 1 hr. at 6 volts/cm., and also 6 cm. in 1 hr. at 9 volts/cm.

"When the two criteria discussed above are fully met, the particular design or geometry of the apparatus fades out of the picture as far as having any influence on electromigration rate is concerned, and it becomes legitimate to measure electromigration rates in terms of mobilities..." (20).

Mobilities are usually given in cm./sec./volt/cm. or in cm.$^2$/volt-sec.

The disadvantages encountered in the various types of apparatus experimented with were difficulty of preparation and/or operation, and failure to achieve good results.
With the closed strip, solid support type of apparatus, although good results have been reported in the literature (21), the following difficulties were met with:

1. It was necessary to keep an even pressure on the two plates with the paper between, so that a constant buffer-to-paper ratio could be kept along the paper. In one region of the paper, the pressure should be greater than in another, the buffer might not fill all the crevices and pores in the paper; thus the migrant particles would not move as fast here as they would in a region of lower plate pressure, and the criteria of linearity with time and potential would not be satisfied.

To keep the pressure uniform, much time and effort were spent, and yet poor results were obtained.

2. The particles on the uppermost surface of the paper came in direct contact with the glass plate. Therefore, difficulty was anticipated in achieving results which could be reproduced with other apparatus because slight adsorption would add to the retarding force acting on the particles, resulting in low mobilities. Also, the particles deeper in the paper which would not come in contact with the glass would show slightly higher mobilities than the particles on the surface. And the spot which, for example, might have been round, would, after migrating, be elliptical. This would give rise to difficulty in measuring the movements.
3. When the glass plates were removed so that the spots could be developed on the paper, e.g. by staining or by charring, a fair per cent of the spot adhered to the glass. Then, upon development, only very faint or poorly-defined spots appeared.

4. It was time consuming to set up the apparatus and dismantle it for each run.

Since these disadvantages were general for all apparatus of the closed strip, solid support type, it was decided to abandon this type of apparatus and work with another type.

In working with a few modifications of the open strip type, again many disadvantages were encountered. They were principally the following:

1. In types of apparatus where the paper strip is not exactly horizontal, gravity becomes an added force on the migrant particles. If the strip is stretched exactly straight and is either vertical or at a certain angle, gravity is a constant force, and linearity could probably be achieved. However, if the strip has any curvature to it at all, such as by catenary sagging, gravity will be a variable force and linearity cannot be achieved. In horizontal types it is difficult to prevent catenary sagging without subjecting the strip to tensions near its limit of wet strength, or using a large number of supports (thereby approaching the
closed strip type. In vertical types, although good for empirical separations (the development of a continuous electrophoresis apparatus \(22\)(23)(24)(25) has proven very successful for separations), difficulty is encountered due to the continuous flow of buffer solution down through the paper.

2. Evaporation of the buffer solvent from the paper is impossible to prevent without constructing an airtight enclosure to be able to increase the pressure of the surrounding gas (usually helium). Then, recovery of the gas becomes time consuming and difficult. Then, also, temperature control becomes very important in order to keep a constant pressure.

In the laboratory, in experiments where the strip was enclosed in a box to prevent temperature changes and drafts, but kept at room pressure, great difficulty was experienced with regard to water evaporation from the buffer. When evaporation occurred there were pH changes on the paper, and there was crystallization of buffer acid and salt which increased the resistance through the paper.

In one trial where a lactic acid buffer on Watman no. 1 paper was used and run at \(25^\circ\mathrm{C}\). and 8 volts/cm., the current decreased from 1,000 Amps to zero in less than a half-hour (Plate III).

3) Contamination by absorption of impurities from
the surrounding gas on the paper is an obvious source of difficulty.

Again, since these difficulties are general for all apparatus of this type, it was decided to avoid them.

The apparatus of the non-polar liquid type have some pronounced advantages, such as:

1. The evaporation from the strip is prevented (assuming the buffer is not appreciably soluble in the liquid, and this is a reasonable assumption, since the liquid must be non-miscible with the buffer solution.)

2. Uniform pressure on the strip is automatically assured as long as the paper is kept horizontal and at the same depth in the liquid.

3. The strip can be easily and quickly placed in the apparatus and removed after the run.

4. As long as the liquid is kept free from impurities, contamination of the paper from the surroundings cannot occur.

Some disadvantages which go along with this type of apparatus are in general:

1. The possibility that the migrant particles might be affected by the non-polar liquid, e.g. by denaturation or by chemical reaction, must not be ignored. It is possible that some combinations of migrant and non-polar liquid cannot be used together.
2. As in the case of the glass plate apparatus, but to a lesser degree, the contact of the liquid with the migrant particles will retard their motion.

However, it in general seemed that the advantages outweighed the disadvantages enough, and the type showed enough promise over the other types, that it warranted the channeling of effort on this type.

Before constructing and using the apparatus of Consden and Stanier (19), (Plate II), it was obvious that certain disadvantages of the particular design would prevent fast and efficient usage and/or good results. They were principally as follows:

1. The paper strip must be exposed to the air where it passes out of the buffer vessels and into the non-polar liquid vessel. Obviously, this is a place where contamination and buffer evaporation can occur.

2. There seems to be no provision for keeping tension in the strip. Without proper tension, catenary depression of the center will occur. The only provision to prevent sagging is the use of a non-polar liquid which has approximately the same density as paper. However, using different buffers in the paper, temperature differences and even non-uniformities in the paper will give cause for sagging or rising of the paper in the liquid.

3. Electrode reaction products can get onto the paper
after forming at the electrodes. This is a source of contamination which may be manifested in streaking, pH changes, etc.

4. There is no provision for keeping the level of the buffer in the two buffer vessels the same. It is necessary to keep the levels the same to prevent hydrostatic siphoning of the buffer through the paper. It will become obvious later in this thesis that a continuous leveling device is necessary because of a continuous flow of buffer solution from the anode to the cathode during the run (electroosmosis).

Plate IV shows the new apparatus which was designed to employ all the advantages on the non-polar liquid type, and to alleviate the difficulties found in the apparatus of Consden and Stanier.

The main principle of the design is that the buffer solution comes in direct contact with the non-polar liquid at an interface. The two liquids are separated only by the interfacial tension (which is relatively high). The density of the non-polar liquid used keeps it above the denser buffer solution.

The way in which the new apparatus alleviates the four difficulties found with the apparatus of Consden and Stanier is respectively:

1. The paper is immersed in liquid at all points
PLATE IV
NEW APPARATUS

ELECTRODE

SALT BRIDGE

HOLDING RODS

PAPER

NON-POLAR LIQUID

LEVELING TUBE

BUFFER

SAT. KCl SOLUTION

-18-
and is never exposed to the air.

2. Tension is kept in the strip by applying a downward force on the holding rods, whose lower ends are attached to the ends of the paper strip. This stretches the strip over the bars and keeps it tight. The rods are clamped on ring stands. One center bar is used in the center of the tank to assure that no catenary sag will occur.

3. The electrode reaction products are kept from the paper by means of KCl-agar salt bridges.

4. The two buffer reservoirs are kept at the same level by means of a leveling tube which connects them. It was found in the laboratory that if the tube was filled with buffer, and the stopcock shut off, some current still leaked through the tube. However, by keeping the inside of the tube dry, and keeping a large air space in the center, the stopcock could be left open without any current leakage. The pressure equilibration was still effective with the air space in the tube.

The tank was assembled from single thickness window glass. The surfaces to be joined were roughened by rubbing them with a suspension of carborundum in paraffin. After considerable searching for a suitable cement (one which would be impervious both to aqueous and organic solvents, and which would hold the glass joints firmly together), an epoxy resin was found suitable.
The resin was supplied by the Schenectady Varnish Company through Dr. H.E. Sheffer.

A constant voltage output AC rectifier was provided by Dr. G.W. Ewing who constructed the instrument.

The electrodes used were both platinum wire electrodes with mercury seals.
EXPERIMENTAL RESULTS

In working with the new apparatus, it was necessary to obtain experimental results which would prove whether or not a linear relationship of migration could be achieved at constant voltage with the apparatus. Since the apparatus was designed so that the paper would be as close to horizontal as possible, and that no evaporation of the buffer solution from the paper would occur, it is obvious that if a non-linear relationship did occur, it would probably be due to buffer interference, assuming constant voltage, etc. This might occur, for example, by the buffer solution shifting, or flowing non-uniformly through the paper during the run, thereby moving the migrant with it.

The method used by Woods and Gillespie (26) to detect buffer flow in the paper was to place spots of glucose at regularly marked intervals along the strip. Since glucose is soluble in the buffer solution, and is almost completely unionized in the pH ranges used, it will not move as a result of the applied electric field. Then any movement of the glucose spots will be due to the movement of the buffer solution.

A run was made using spots of C.P. glucose solution placed every three centimeters along the strip. Three spots were placed side by side at each location in order to obtain checks. The position of application was marked on the paper by cutting small notches on opposite edges of the strip.
The positions could not be marked with pencil because the graphite would act as secondary electrodes, thus distorting the field in which the ions migrate.

Buffer used: Veronal, pH 8.6, ionic strength 0.05
Paper used: Watman no.1.
Field strength: 7.90 volts/cm.
Non-polar liquid: Petroleum ether, b.p. 100-110°C.
Time of run: 5,500 sec.

After the run was completed, the spots were made visible by heating the strip in an oven at 110°C until the glucose charred. The spots appeared clearly and well-defined in their new positions along the strip.

It was seen, upon measuring the total length of the dried strip and comparing it to the original dry length, that the shrinkage was negligible, and therefore no correction factor was necessary. Also, the stretching of the strip while wet introduced an error due to increased path length of about 1%. The movements of the spots were measured and are tabulated below.

As we have noted above, the checks were obtained on one strip of paper and from one run. The slight variations in the values of the different checks are due to difficulty in measuring the distances to the center of the spots, and possibly also to paper irregularities and/or distortions of the electric field along the edges of the paper strip.
\[ s = \text{the distance from the cathode at which the spot was originally applied, in cm.}\]

\[ d = \text{the distance which the spot moved toward the cathode, in cm. Negative values indicate movement toward the anode.}\]

<table>
<thead>
<tr>
<th>( s )</th>
<th>check 1</th>
<th>check 2</th>
<th>check 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-3.1</td>
<td>-3.1</td>
<td>-3.1</td>
</tr>
<tr>
<td>7</td>
<td>-2.1</td>
<td>-2.0</td>
<td>-2.1</td>
</tr>
<tr>
<td>10</td>
<td>-1.6</td>
<td>-1.7</td>
<td>-1.7</td>
</tr>
<tr>
<td>13</td>
<td>-1.2</td>
<td>-1.1</td>
<td>-1.2</td>
</tr>
<tr>
<td>16</td>
<td>-0.8</td>
<td>-0.5</td>
<td>-0.7</td>
</tr>
<tr>
<td>19</td>
<td>-0.2</td>
<td>-0.1</td>
<td>-0.2</td>
</tr>
<tr>
<td>22</td>
<td>+0.5</td>
<td>+0.6</td>
<td>+0.6</td>
</tr>
<tr>
<td>25</td>
<td>+0.7</td>
<td>+0.8</td>
<td>+0.8</td>
</tr>
<tr>
<td>28</td>
<td>+1.2</td>
<td>+1.4</td>
<td>+1.3</td>
</tr>
<tr>
<td>31</td>
<td>+1.6</td>
<td>+1.8</td>
<td>+1.7</td>
</tr>
<tr>
<td>34</td>
<td>+2.0</td>
<td>+2.0</td>
<td>+2.0</td>
</tr>
<tr>
<td>37</td>
<td>+2.5</td>
<td>+2.8</td>
<td>+2.7</td>
</tr>
<tr>
<td>40</td>
<td>+3.2</td>
<td>+2.9</td>
<td>+3.1</td>
</tr>
<tr>
<td>43</td>
<td>+3.1</td>
<td>+3.3</td>
<td>+3.2</td>
</tr>
<tr>
<td>46</td>
<td>+3.4</td>
<td>+4.0</td>
<td>+3.7</td>
</tr>
</tbody>
</table>
If these data are plotted as they appear in the table a very nearly straight line results. However, in order to illustrate the phenomenon more clearly, one can plot distance from the geometric center of the strip to where the spot was applied versus distance the spot moved toward the geometric center. It is then possible to draw certain conclusions from the graph thus obtained (Plate V, curve a).

The graph is one which has a V shape, and shows clearly that at a position about 5 cm. to the cathode side of the geometric center, the spots did not move at all. All the other spots on either side of that position moved in toward it. The farther from this position of zero buffer flow, the greater the distance was which a given spot moved. Had there been no buffer flow, the curve would have been the x-axis itself. The probable reason for the shift of the point of zero buffer flow from the center toward the cathode will be explained shortly.

The reason for the movement of the buffer toward the center of the paper strip, evidently, was that there was not enough buffer solution in the paper when the run was started, and buffer had to flow into the paper from both ends in order to bring the buffer-to-paper ratio into equilibrium.

The method of wetting the strip, up to this time, had been to saturate the fresh paper strip with buffer solution, then to blot it lightly with another piece of filter paper to remove the excess liquid. The spots were then applied, the
paper placed in the apparatus, and the non-polar liquid poured in. Apparently this blotting removed too much of the buffer solution from the paper.

Therefore, a new method of wetting was introduced. The reservoirs of the tank were filled with buffer and the paper strip was placed in position, but the non-polar liquid was withheld. Buffer solution was sprayed on the paper with an atomizer until the paper appeared to be well wet and uniformly covered. Then the spots of migrating material were placed on the paper and the non-polar liquid was poured in. When a run was made, using this method of wetting, and the data thus obtained were plotted, the same type of V curve was obtained (Plate V, curve b).

It was decided that the next step was to watch the movements of charged particles in the apparatus with respect to time. The materials to be used were dinitrophenyl derivatives of various amino acids. There are two reasons why these compounds were selected. They were: 1) they are intensely yellow colored, and 2) they attain high electrophoretic velocities. Because of the color of these compounds, their migrations can be observed during the run without stopping. In this way, any number of points can be obtained for a curve of migration versus time, making only one run, and using the same strip of paper. With colorless migrating materials, a run must be made, the strip removed and dried, and the spots dyed by some method (or detected instrumentally).
All of this work would yield but one point for the curve. To obtain another point for the curve, the whole process must be repeated. By using different strips of paper, one might introduce errors due to different textures of paper.

Using the second method of wetting the paper, a run was made with DNP amino acids. The compounds were supplied by Mr. Gilbert Gier (Union College, Class of 1956) who prepared them from the corresponding amino acids.

Six spots were placed on the strip; three of DNP-DL-isoleucine, and three of DNP-DL-arginine; one of each near the anode end of the strip, one of each near the center, and one of each near the cathode end of the strip.

Buffer used: Veronal, pH 8.25, ionic strength 0.05
Paper used: Watman no. 1.
Field strength: 7.90 volts/cm.
Non-polar liquid: Petroleum ether, b.p. 100-110°C.

The readings which were taken are tabulated below. The values given are for distances moved from the point of application toward the cathode. Movements toward the anode are negative.

When these data are plotted on a graph of migration versus time, some interesting curves result (Plate VI).

Three interesting facts can be immediately observed from the graph. They are:

1. The DNP-DL-arginine spot near the anode moved first toward the cathode, then stopped, and moved toward
<table>
<thead>
<tr>
<th>time (sec.)</th>
<th>DNP-DL-isoleucine</th>
<th>DNP-DL-arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>near cathode</td>
<td>near center</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>700</td>
<td>-1.6</td>
<td>-0.7</td>
</tr>
<tr>
<td>1700</td>
<td>-2.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>4050</td>
<td>-4.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>5400</td>
<td>-4.6</td>
<td>-1.3</td>
</tr>
<tr>
<td>7200</td>
<td>-5.5</td>
<td>-1.5</td>
</tr>
</tbody>
</table>

The anode. The other curves all start out with greater slopes than they have after a longer time lapse.

2. The curves all become nearly linear after about 3,000 seconds.

3. The slopes of the curves representing the motion of the arginine spots all become nearly equal after a considerable time lapse, as do the slopes of the curves for the isoleucine spots.

The first fact can be explained easily. As we have seen from the results of the glucose movement runs, the buffer solution moves onto the paper from both ends. Since both these amino acid derivatives are on the basic side of their isoelectric points, they exist as anions, and will move toward the anode. The arginine near the anode wanted to move closer toward the anode because of its charge, but was pushed onto the paper (toward the cathode) by the buffer which
was flowing onto the paper. Evidently the buffer solution stopped flowing later, and the arginine was then able to move toward the anode. With the two spots near the cathode, they wanted to move toward the anode, and the buffer which was flowing onto the paper pushed them farther along in the same direction. Thus the slopes of the curves are greater at the beginning than they are later on. The same is true with the curves of the spots near the center, but to a much less degree. This is because the spots are near the point of zero buffer flow, and there is little effect on them due to buffer solution flow.

The second fact is explained by the fact that the buffer solution flow onto the paper from both ends is a process which will reach equilibrium. Evidently, the equilibrium is established within 3,000 seconds. Then the curves all become linear, because all the conditions for linear migration have been satisfied.

The third fact that migration rates of different spots of one compound become equal is reasonable because if linear relationship exists, and the compounds are all pure, then their migration rates should be the same regardless of where they are located on the strip. Then, also, as has been pointed out earlier, migration rates of different compounds will be different, and the rate curves of different compounds will not be the same.

These observations led to the conclusion that the best way to deal with the problem of buffer solution flow would be 1) to immerse the paper with the spots on it and the field
applied for about 3,000 seconds and let equilibrium be established, then 2) to take readings on the spots.

A run was made with three spots of DNP-DL-isoleucine; one near the cathode end, one near the center, and one near the anode end.

Buffer used: Veronal, pH 7.98, ionic strength 0.05
Paper used: Watman no. 1.
Field strength: 7.90 volts/cm.
Non-polar liquid: Petroleum ether, b.p. 100-110°C.

The following readings were taken, with the usual definition of signs holding true.

<table>
<thead>
<tr>
<th>time</th>
<th>near cathode</th>
<th>near center</th>
<th>near anode</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 sec.</td>
<td>0.0 cm.</td>
<td>0.0 cm.</td>
<td>0.0 cm.</td>
</tr>
<tr>
<td>1450</td>
<td>-1.4</td>
<td>-0.8</td>
<td>-0.9</td>
</tr>
<tr>
<td>3400</td>
<td>-1.4</td>
<td>-1.7</td>
<td>-1.6</td>
</tr>
<tr>
<td>8000</td>
<td>-3.2</td>
<td>-3.5</td>
<td>-3.8</td>
</tr>
<tr>
<td>10200</td>
<td>-3.9</td>
<td>-4.4</td>
<td>over edge.</td>
</tr>
</tbody>
</table>

When these data are plotted on a graph of time versus movement of the applied materials as usual, three curves are obtained (Plate VII). This graph is plotted with movement toward the anode upward as the ordinate. The curves show the expected irregularity before 3,000 seconds, but unusually good linearity from then on.

Before the run had been completed, but after 3,000 seconds,
PLATE VII

MIGRATION OF DNP-DL-ISOLEUCINE

SPOT NEAR CATHODE
SPOT NEAR CENTER
SPOT NEAR ANODE

MIGRATION CM

← Anode
three spots of glucose were applied to the strip in order to detect any further buffer flow. The total time which the glucose spots were on the paper during the run was 4,340 sec. Upon heating, it appeared that all three spots had moved toward the cathode a small amount (opposite in direction to the movement of the isoleucine spots). All three spots had moved by nearly the same amount, and the average movement was 0.66 cm.

This uniform movement can be accounted for by the existence of electroosmotic flow. (27)(28).

The veronal buffer anion is probably highly hydrated, (by hydrogen bonding, van der Waals' forces, etc.) and if it traveled to the anode, it would carry a large amount of water with it.

\[
\begin{align*}
&\text{C}_2\text{H}_5 \\
&\text{C}_2\text{H}_5 \\
&\text{O} \\
&\text{O} \\
&\text{H} - \text{N} \\
&\text{N} - \text{N} \\
&\text{O} \\
&\text{O}
\end{align*}
\]

However, it is probably fairly strongly adsorbed on the hydroxy groups of the paper. On the other hand, the sodium ion which is one of the cations of the buffer is also highly hydrated, being the second smallest element in the alkali metal group, and will travel toward the cathode, carrying some water with it. This will result in a general and uniform cathodic water flow.

In calculating the mobility of a compound, the movement
due to electroosmotic flow must be corrected for.

Thus it has been proven that the new apparatus does
give linear migration of charged particles, if the proper
procedure is followed.

A new approach to the application of paper electrophoresis
was tested in an attempt to separate the dextro form from the
levo form of DL mixtures of compounds by using a buffer which
was prepared from a dextro acid. The buffer used was
D-tartaric acid buffered with potassium acid D-tartrate.

Attempts were made to resolve some DNP-DL-arginine,
but the spots were hard to see and their outlines indefinite
so that it was impossible to tell whether the spots which had
been applied in a round shape, had elongated to any extent.

Therefore, it was decided to use the pure amino acids
and stain the spots by spraying with ninhydrin after the run
was made. The compound used was DL-aspartic acid in the
tartrate buffer at pH 2.0, ionic strength 3.00. The compound
was applied to the paper in a thin straight line across the
paper. After staining, two of the three applications showed
some small degree of elongation, but hardly enough to prove
any separation. On another trial at a longer time of run,
no better results were obtained.

Due to the lack of time, this problem could not be
investigated any further at this time.
CALCULATIONS

From the experimental results, a calculation may be made of the electrophoretic mobility of dinitrophenyl-DL-isoleucine, which might be useful at some future date.

We have seen that mobility is defined as:

\[ u = \frac{V}{X} \]

where \( u \) = the mobility of the ion.
\( V \) = the electrophoretic velocity of the ion.
\( X \) = the field strength.

However, this holds true only for a particle whose only driving force is the electric field minus the viscous retarding force of the buffer solution. This value has also to be corrected for the electroosmotic flow of the buffer solution. This can easily be done by considering the "apparent mobility" of the glucose spot in the same run.

In the run described on page 31, the field strength was 7.90 volts/cm. Then, since the average migration distance of the glucose spots and the time in which they moved that far are known, the "apparent mobility" can be calculated for the glucose.

\[ u_g = \frac{0.66 \text{ cm.}}{4,340 \text{ sec.}} \times \frac{1}{7.90 \text{ volts/cm.}} \]

\[ = 1.93 \times 10^{-5} \text{ cm.}^2/\text{volt-sec.} \]

For the uncorrected electrophoretic velocity of the
DNP-DL-isoleucine, the average of the values of the slopes of the linear parts of the migration versus time curves (Plate VII) may be used.

When this is done, the uncorrected mobility is found to be:

$$u_{\text{uncorr.}} = 4.90 \times 10^{-5} \text{ cm.}^2/\text{volt-sec.}$$

Then, since the movement of the glucose spots was opposite in direction to that of the DNP-DL-isoleucine, the latter had been retarded by the buffer solution flow, and the above uncorrected mobility is too low. The true or corrected mobility will be the sum of the uncorrected mobility and the "apparent mobility" of the glucose.

$$u_{\text{corr.}} = (4.90 + 1.93) \times 10^{-5}$$
$$= 6.83 \times 10^{-5} \text{ cm.}^2/\text{volt-sec.}$$

for 2,4-dinitrophenyl-DL-isoleucine in veronal buffer of ionic strength 0.05 at pH 7.98 on Watman no. 1 paper at 24°C.
SUMMARY

The foregoing studies have shown that the new paper electrophoresis apparatus which was designed, provides a simple method of operation, and gives reproducible results. Then it was shown that if enough time was allowed to pass, with the potential applied across the paper strip, the buffer-to-paper ratio equilibrium was established, and the apparatus gave results which show linearity of migration of charged particles.

With the new apparatus, one can obtain and publish results with confidence that his results can be reproduced in other laboratories where the variables of pH, ionic strength of buffer, field strength and current can be controlled.

The mobility of DNP-DL-isoleucine under the conditions stated was calculated from the experimental results, as it might be of use in the future.

Near the completion of this project, a reference was discovered (29) to unpublished work which described an apparatus which was somewhat similar to the one described in this thesis. That apparatus, however, was of such design that the author doubts that reproducible results can be obtained with it, because the center of the strip was elevated considerably above the ends of the strip. Under these conditions, as has been pointed out, gravity becomes an added force in the migration of the particles. Also, there was no provision for keeping the tension in the strip, and
catenary suspension would depress the center of the strip. Therefore, although the design of that apparatus is somewhat similar to the apparatus developed in this thesis, the author concludes that the new apparatus is superior in design.
SUGGESTIONS FOR FURTHER WORK

The efforts expended on further investigations into the possibility of separating the D-form from the L-form of various optically active mixtures by paper electrophoresis using an optically active compound for a buffer, might be fruitful. By changing the variables such as pH, type of optically active buffer, ionic strength of the buffer, temperature, and time of run, one might be successful in making this type of separation. The method could have industrial importance in such a process as continuous paper electrophoresis.

One might use the new apparatus to determine the isoelectric points of many of the BNP amino acids. This could be done by determining the mobilities of a compound in buffers at various pH values. It has been shown how this can be done rapidly by the visual method described. Then by plotting the mobilities versus pH values, the isoelectric point is at that place where the curve crosses the zero mobility line. This method would probably be more accurate than the present method of plotting migration values versus pH values, because migration values are usually obtained from a single movement of a single spot, whereas the mobility (which is the migration value for a time of 1 second at unit field strength) is obtained from the slope of a line which is the average of a series of points (determinations).

If the number of applications of paper electrophoresis
keeps increasing at its present rate, paper electrophoresis will soon be a standard and perhaps indispensable method of chemical analysis. Then perhaps, the new apparatus or a future modification of it will prove useful in perfecting and carrying out new applications of paper electrophoresis.
BIBLIOGRAPHY

17. Block, Durrum, Zweig; op.cit., p335.
18. Block, Durrum, Zweig; op.cit., p349.