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A Critical Evaluation of Electromigration in Stabilized Electrolytes

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A CRITICAL EVALUATION OF ELECTRONMIGRATION
IN STABILIZED ELECTROLYTES

By
Edward Philip Marbach

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy
June
1954

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APPROVAL SHEET

The dissertation submitted by Edward P. Marbach has been read and approved by five members of the faculty of the Stritch School of Medicine, Loyola University.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

May 18, 1975

Date

[Signature]
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Edward Philip Marbach was born in Chicago, Illinois, on November 13, 1926. He was graduated from Spalding High School, in Chicago, in June, 1946. From September, 1946 to June, 1950, he attended Loyola University from which he graduated with the degree of Bachelor of Science in June, 1950 with a double major in Chemistry and Mathematics. He began his advanced studies in the Graduate School of Loyola University, Department of Biochemistry, in September, 1950. He obtained the degree of Master of Science in Biochemistry in June of 1952 and continued his studies for the degree of Doctor of Philosophy.

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He is co-author of the following publications:

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CHAPTER I

INTRODUCTION

The separation of a complex biochemical system, such as a mixture of proteins, into its constituent fractions, remains one of the most difficult analytical operations. The difficulty arises from the inherent lability and interaction of the components, and the close similarity of the constituents of a group to one another.

In the separation of complex mixtures, as well as in the study and measurement of biochemical substances and systems, the technique of ionography has for the most part been used as a qualitative tool. The technique is based on the differential electromigration of substances in electrolytes stabilized with paper and similar materials. The objective of this thesis is the development of the quantitative aspects of ionography. They may be divided into two main groups: (a) the determination of a conversion factor for converting mobilities determined in paper-stabilized electrolytes to those determined in non-stabilized electrolytes (moving-boundary method of electrophoresis), (b) the
theoretical development of the fundamental theory underlying the determination of materials spread over a surface area, e.g., paper, by means of a scanning densitometer. This latter problem will be developed and illustrated by the estimation of lipoprotein concentrations in normal and pathological human sera.

It is of interest to note that several workers, as early as the first decade of this century, realized that electromigration in stabilized media was applicable to problems of a biochemical or clinical chemical nature. The studies of Field and Teague in 1907 were concerned with the electromigration characteristics of diphtheria toxin and antitoxin (15) as well as of tetanolysin and antitetanolysin (16) in agar-stabilized electrolytes. The work of Teague and Buxton (72), also in 1907, on the electromigration behavior of hemolytic amboceptors, hemoglutinins, and complement in agar jelly, may also be mentioned. The earliest investigations on electromigration on filter-paper sheets wetted with electrolytes, deal with biochemical studies of snake venom and was reported in a paper by von Klobusitsky and König published in 1939 (31). Since then, over 400 papers have been published on various aspects of this technique. Review articles dealing with the field of electromigration in stabilized electrolytes have been published by McDonaald et al. (44-6, 49), by Tiselius and Flodin (74,75), by Wunderly (81) and by Kunkel (35).
A description of the Ionograph with the procedure for its operation has been published (49,55) however, a brief description of the instrument is appropriate here. In Figure 1, is shown a schematic diagram illustrating the principal features of an Ionograph. Paper strip, F, is held horizontally in frame, J; the end clamps, H, are movable, thus allowing for variable length paper strips or wide sheets; if desired the end clamps may each be reversed in position and glass plates of any desired length placed on the frame. In any case, the ends of the paper strips or sheets dip into the buffer solution contained in the buffer vessels, E. These, in turn, are connected by means of the inverted U-tubes, C, to the electrode vessels, D. The U-tubes are filled with agar-stabilized electrolyte solution. Platinum electrodes, A, which are connected to a regulated source of electrical power, dip into the electrode vessels. The solution level in each of the buffer vessels, E, is maintained at a constant height throughout a run, by a glass siphon tube, L, of small bore whose ends dip into the buffer solution. Except for the electrode vessels, D, and salt-bridges, C, all components of the apparatus are inclosed within the container, K, which is covered by the lid, B. The migrant may be added to the strips by inserting a micropipet through the openings, G. The open space within the container is maintained at a minimum level by proper design of the apparatus, and by pouring water into the container K, up to the level of the buffer vessels. For experi-
ments involving mobilities, the air is normally displaced by water-saturated helium.

The definitions of terms which will be used frequently throughout this dissertation would also be helpful here. The term "Ionograph" refers to the instrument itself; the term, "ionography", to the technique and the term "ionogram" to the paper strips containing the materials under experimentation. The material which is applied to the filter paper strips will be referred to as the migrant.

General Consideration of the Conditions Necessary for the Determination of Mobilities

Two important applications of the technique of ionography are (1) the empirical fractionation of complex mixtures of labile materials, such as blood plasma proteins, and (2) the determination of mobilities of individual components of such a mixture. It will be shown later that the optimum conditions for carrying out the first operation differ in important details from those requisite for the second operation.

In determining mobilities of a particular migrant in non-stabilized media, e.g. in the moving-boundary electrophoresis apparatus, the pH, the ionic strength of the buffer, the type of buffer, and the temperature must be stated along with the value of the mobilities reported if they are to be meaningful. This must be done because the mobility of the migrant is a function of these variables. If they are kept constant or re-established
FIGURE 1
A SCHEMATIC DIAGRAM OF THE TAUT HORIZONTAL STRIP METHOD
by a second investigator, a mobility having the same numerical value may be obtained.

The situation is somewhat different when determining the mobility of a migrant in a stabilized medium. Several influencing factors, in addition to those mentioned above, must be taken into account, if investigators in different laboratories are to obtain comparable results. Mobility values obtained in stabilized media are, to begin with, usually lower than those observed in free solution. It is evident that when using paper as the stabilizing agent, the type of filter paper used must also be stated. It has also been observed that the determination of the mobility of a particular migrant through a given type of paper, when determined by two observers, on the same type of apparatus, using the same buffer at the same pH, ionic strength and temperature, do not necessarily agree with each other, or with the value determined in free solution. From these facts, it is apparent that the mobility of a migrant in a stabilized medium is influenced by another factor in addition to those which are maintained constant when investigating mobilities in non-stabilized media. It will be shown later that this important factor is the "wetness" of the paper.

Definition of Mobility

In the measurement of the electrical conductivity of a solution, it is well recognized that the motion of the charges carrying the current is merely a drift superimposed on a random
motion that is already present in the absence of an electrical field. In a metal, for example, the free electrons are already executing a random motion; when a voltage is applied, a drift velocity is super-imposed on this movement. In a dilute electrolyte each positive ion and negative ion, in the absence of a field, executes a random movement among the solvent molecules; when a field is applied, the positive ions have a tendency to drift in one direction while the negative ions tend to drift in the opposite direction. In other words, the movement of an ion can be pictured as "directed Brownian motion". However, if it is forgotten that the flow of a current is due to a random motion which was already present before the field was applied, that is, if the random motion is entirely disregarded and the assumption is made that each and every ion in the uniform field of unit strength moves with the same steady velocity, then the distance traveled by each ion in unit time may be defined as the "mobility" of the charged particle. In other words, the "mobility" of a charged particle is the linear displacement of a particle in unit time acted upon by an electrical field of unit strength.

There is no doubt that the ion or particle in question moves with a random motion; however, its linear displacement is the quantity used in calculating its mobility. No correction is normally made for this random motion in calculating mobility values, as was done by Hunkel and Tiselius (38), because the
definition of mobility does not include the random motion. It is true, perhaps that in a buffer system containing paper fibers, that the ion or charged particle has to move with greater deviation from a straight line displacement due to the present of the paper fiber; however, rather than correcting the mobility obtained by making use of the random motion, it would be better to consider the ion or particle as having a decreased thermodynamic activity due to the interaction of the ion or particle with the paper.

Since the mobility of an ion or charged particle is defined as the linear displacement in unit time when acted upon by a field of unit strength, it can be defined mathematically from the following equation:

\[ U = \frac{d}{V/L} \]

where \( U \) is the mobility,

\( d \) is the linear displacement in cm of the migrant in \( t \) sec.

\( V \) is the potential in volts impressed across a distance of \( L \) cm.

**Conditions Necessary for Obtaining Mobility Values in Stabilized Systems**

When determining the mobility of a substance in a stabilized electrolyte, several factors become important. They are (1) no significant change of the migrant or buffer should
occur and (2) no water shifts due either to evaporation or siphoning of water through the stabilized medium can be allowed.

The main cause of evaporation of water from the paper strip is the passage of current through the strip. Evaporation may become excessive if too high a current flows through the strip. It can be decreased in several ways, such as having the strips in an enclosed chamber saturated with water vapor, decreasing the ionic strength of the buffer, decreasing the voltage gradient, lowering the temperature of the strips and surroundings, and conducting the generated heat away by a gas having high specific heat-conduction, such as hydrogen or helium. Of these controllable factors, having the strips in an enclosed chamber saturated with water vapor is of prime importance. Decreasing the potential gradient has a considerable effect because of the fact that a two-fold decrease in potential gradient causes a four-fold decrease in evaporation. Decreasing the ionic strength of the buffer is also very effective. Two important causes for the lack of equilibrium conditions, as regards the water content of the paper strips, can be traced to the lack of a sealed chamber around them, or to not allowing enough time to pass after the strips have been wetted, and before the migrant is applied. The siphoning of buffer solution through the paper or other stabilizers is due to, mainly, non-equal solution levels in the two buffer vessels into which the ends of the paper strips dip. This siphoning through the strips can be minimized simply
by maintaining the buffer levels at both ends of the strips at the same height, by means of a small-bore siphon tube in parallel with the strips, and by establishing equilibrium conditions as regards the water in the paper before the migrant is applied.

Criteria for Obtaining Mobilities in Stabilized Solutions

Certain criteria for determining the conditions necessary for obtaining mobility measurement are: (a) constant voltage and constant current during the course of the experiment; (b) linear displacement of three bands of migrant per strip; (c) linearity of movement of the migrant with time at constant potential gradient; (d) linearity of movement of the migrant with respect to potential gradient, with time constant.

If voltage and current are constant during the experiment, assuming reversible electrodes are used, no significant change of the buffer can occur, such as a change in ionic strength due to evaporation of solvent or solute. If evaporation does occur, it can be detected readily simply by applying three zones of migrant to the strip of paper; one at a point midway between the ends; one about 10 cm from the cathode end of the strip and one about 10 cm from the anode end of the strip. If all three zones move the same distance, the effect of evaporation from the strip can be disregarded; if they do not move the same distance, the effect of evaporation must be taken into consideration. The effect of evaporation on the mobility of the migrant originally placed at the mid-point of the strip is at a
minimum while the other two zones are greatly affected. This can readily be shown by assuming that evaporation occurs uniformly from the strip. Under these circumstances, the solvent must progress through the paper strip from the ends which dip into the buffer vessels. Therefore, the closer the migrant zone is to the ends of the strips, the greater the effect of evaporation on the displacement of the zone. This effect decreases down to zero at the midpoint of the strip. If the migrant has a negative charge and moves to the positive pole, the zone nearest that pole will move slower and in cases of severe evaporation, it will even move in the wrong direction since the water flow is moving in a direction opposite to that of the migrant. In the same sense, the zone nearest the negative electrode will move faster since the water shift is in the same direction as the movement of the migrant. However, since the effect due to evaporation is added to the migration of one zone and subtracted from the migration of the other end zone, one half of the difference of the two end zones is equal to the movement of buffer due to evaporation of solvent. Furthermore, the average of the two displacements of the two end zones comes very close to the true movement.

Linearity of movement of the migrant with respect to time, at constant potential, along with linearity of movement with respect to potential gradient, with time constant, are important criteria for achieving mobility data. If these two
conditions are met, it is obvious that the size or design of a particular apparatus does not influence the mobility determination. It should be noted here that the attainment of linearity of movement of a migrant with respect to time, at constant current is not in itself a sufficient criterion for the determination of mobility data. The criterion is sufficient only if, upon keeping the current constant, the potential gradient remains constant, assuming reversible electrodes. This can easily be shown if one considers the case when the current is kept constant and the strip begins to dry out. As the strip starts to dry, the velocity of the migrant decreases; however, the current flowing also tends to decrease, but this cannot occur because a constant current source is used, therefore, the voltage applied increases in order to keep current constant. This then causes an increase in the velocity so that linearity with time can be achieved. Assume that in the course of the experiment the resistance doubles due to the drying out of the strip. Since the current is kept constant, the voltage applied will double because it is equal to the product of the current and the resistance; the potential gradient will also double. Now in the calculation of mobility, the displacement of the migrant zone must be divided by the applied potential gradient, but in this case, even though linearity of movement with respect to time is achieved, the potential gradient is not constant. It is therefore, impossible to choose a representative value for the applied
potential gradient.

Some practical factors important in achieving linearity of movement of the migrant with time include; (1) the use of reversible electrodes to reduce polarization to a minimum, (2) the employment of agar filled salt bridges to separate the products of electrode reactions from the buffer vessels into which the ends of the paper strips dip. This precaution is especially important when buffer solutions of low ionic strength are used. (3) The establishment and maintainance of a uniform and constant liquid level in the buffer vessels. This can be achieved, as described above, by allowing a siphon with a bore of small diameter to remain in place between the buffer vessels during the course of an experiment. (4) The maintainance in a horizontal plane of that portion of the paper strip throughout which electromigration of the migrant under study takes place. In carefully controlled experiments, it has been observed that nonlinear movement of the migrant with respect to time is obtained when the center of the paper strip or sheet is elevated appreciably above, or lowered much below the level of the ends of its suspended portion (9,14). (5) The use of a water saturated helium atmosphere around the strips and the reduction of the free gas space to a minimum. Due to the low molecular weight of helium and the consequent high relative velocity of its molecules, it exhibits a head conductivity at 0°C. approximately six times that of air. It serves therefore, as an excellent conductor of
heat and consequently aids in dissipating the heat developed in
the strips due to the application of a potential gradient, and
in minimizing the loss of water. When evaporation from the paper
strips is reduced to a minimum and the liquid level in the buffer
vessels is maintained constant, thus offsetting bulk water shifts
or surges through the paper, chromatographic interference is
largely eliminated.

When the electrical energy developed in a strip ex­
ceeds about 0.005 watt per square cm of radiating surface at
25°C., the use of helium is recommended. However, as the temper­
ature is reduced from room temperature, the need for helium de­
creases rapidly, so that many experiments may be carried out in
the neighborhood of 0°C. with good results using a simple air
atmosphere. When ionographic measurements are to be used, for
mobility studies, the ionic strength of the buffer solution used
is generally 0.05 or less.

If the various precautionary measure outlined above
are enforced, it is found that, with all other conditions being
constant the rate of electromigration of the migrant is directly
proportional to the voltage impressed across the ends of the
paper strip or to the potential gradient along the strip (42,55).
If the movement of the migrant is linear with respect to both
time and potential gradient, it is obvious that the size or
design of a particular apparatus no longer exerts an influence
on electromigration rate, and the latter quantity can legiti­
mately be stated simply in terms of cm/sec per volt/cm.

Critical Evaluation of the Types of Apparatus Presently Used and Their Applicability for Mobility Determinations

The various types of apparatus now in use can be divided into four main groups based on the manner in which the paper is held in place:

1. ridge pole suspension of the paper;
2. sandwiching of the paper between two glass plates;
3. immersion of the paper in a non-conducting, non-miscible solvent;
4. horizontal suspension of the strip in a water-saturated gas.

On first consideration, the four different types of apparatus would seem to lend themselves equally well to the determination of mobilities, but on closer examination, this is found not to be so. Consider the ridge pole suspension type of apparatus where the center of the paper is elevated about 13 cm above the ends of the paper as shown in Figure 2. The apparatus has been used by several investigators (9,10,13,14,17,23-25,29,56-58) for fractionation work, but it is not suitable for mobility determinations. Due to the action of gravity, it is almost impossible to obtain an even layer of buffer over the entire length of the paper even before a potential is applied. Due to this fact, it would be impossible to obtain the potential gradient simply by dividing potential applied by the length of the paper, since the potential
FIGURE 2
A SCHEMATIC DIAGRAM OF THE RIDGE POLE SUSPENSION METHOD
gradient is inversely proportional to the cross-sectional area of the paper strip. Again, at the apex of the paper, the least amount of buffer is present and also, the smallest cross-sectional area, and hence, the greatest potential gradient. Near the ends of the paper, the greatest amount of buffer is present, and likewise, the greatest cross-sectional area, and hence, the lowest potential gradient. If the migrant is applied at the apex of the strip, as it is done in practice, the migrant travels in a potential field that is continuously decreasing as the migrant approaches the end of the paper. However, this is not the only difficulty. When the potential is applied to the strip, heat is generated as a result of the passage of current through the paper strip. The heat is proportional to the current, squared, times the resistance of the paper-buffer system. Since the apex of the strip has the least amount of buffer, it will have the greatest amount of resistance as compared with the rest of the paper strip, and therefore, the greatest amount of heat will be generated in this region which is already the driest. The increased generation of heat will cause increased evaporation of the buffer from the region at the apex. Here, a great deal of difficulty arises. First, if the buffer is composed of salts which are not volatile, as is usually the case, there is evaporation of the water in the buffer with the concomitant concentration of the buffer salts. This then causes an increase in the ionic strength of the buffer in this region. This in-
crease in the ionic strength of the buffer causes the buffer to become a better conductor and hence, the potential gradient will become lower.

It can readily be seen that the ridge-pole suspension method for determining mobilities is a poor choice then, for the following reasons: (a) the potential gradient would not be uniform throughout the strip and would change with time making it difficult to define; (b) the ionic strength of the buffer would not be uniform throughout the strip and would also change with time; (c) the ratio of paper to buffer would not be uniform throughout the paper strip. Since, as will be shown later, the mobility of a particle is a function of this ratio, the set-up is not a satisfactory one; (d) as the water in the buffer saturating the paper strips evaporates away, buffer solution will flow from the vessels in which the ends of the paper strips dip to replace the water which is evaporated from the strips; this would cause the migrant to travel through a water current which is increased as the migrant approaches the ends of the paper strips and which will cause the migrant to move slower and slower with respect to time until the point is reached where the movement of the migrant is just offset by the upward flow of water.

With the second type of apparatus, the inherent difficulties may not be as great as in the ridge-pole suspension type, but they do exist. In this variation of the technique, the filter-paper is sandwiched between two glass plates as shown in
Figure 3. This apparatus has been used by several investigators (12, 18-20, 32, 33, 36-39, 68, 71) with considerable success, when dealing simply with empirical fractionations of complex systems. For mobility determinations, however, it is not altogether satisfactory for the following reasons: (a) the migrant must be applied before the system is at equilibrium, that is, before the top plate of glass is put into position. Any movement on the part of the buffer in an attempt to reach a position of equilibrium will cause the migrant to move even before a potential is applied. This difficulty can be overcome to some extent by coloring the migrant so that any movement prior to the application of the potential can be noted. However, coloring the migrant might change its nature in such a way that the mobility determined does not apply to the migrant before it is colored. (b) Another difficulty that arises is the disturbance of the migrant zone when the glass plates are put into position and when they are removed. (c) The cross-section area of the buffer-paper system is a function, to some extent, of the force with which the two glass plates are pressed together. In other words, where the pressure is greatest, the cross-sectional area is at a minimum, the potential gradient is at a maximum, and the ratio of paper to buffer is at a maximum. Just the opposite is true where the force holding the two plates together is at a minimum. One investigator has overcome this rather serious problem to some extent by using a complicated clamp which
FIGURE 3
A SCHEMATIC DIAGRAM OF THE SANDWICHED PAPER METHOD
applies an even pressure throughout (35).

The immersion of the paper strips in a non-miscible, non-conducting organic solvent, such as chlorobenzene or carbon tetrachloride, to control evaporation would seem to have certain desirable advantages. This type of apparatus, shown in Figure 1, has been used by several investigators either for fractionation of biological materials or for mobility determinations (3, 4, 5, 60, 21, 63). The advantages of this type of apparatus are offset due to the fact that the organic solvent may interact with the materials being fractionated, as for example, the blood plasma proteins (5). To overcome this difficulty, Cremer and Tiselius placed the strips of paper between glass plates. The undesirable features, which were pointed out in the first two mentioned types of apparatus, also apply even if the strips are immersed in an organic solvent.

With the horizontal suspension technique a uniform layer of buffer is obtained throughout the paper strip and there are no insurmountable complicating factors as in the two previously mentioned types of apparatus. In this technique, the paper is suspended in a taut horizontal fashion, as is shown in Figure 1. This type of apparatus also has been used by several investigators either to fractionate or to obtain mobilities of biological materials (22, 44, 55, 64). Evaporation from the paper surface can be controlled by suitable adjustment
FIGURE 3

A SCHEMATIC DIAGRAM OF THE IMMERSID
PAPER METHOD
of the ionic strength of the buffer solution, by lowering the temperature and by the use of a helium atmosphere, as explained earlier in this thesis.
CHAPTER II

THE DEVELOPMENT OF A CONVERSION FACTOR BETWEEN
MOBILITIES IN STABILIZED AND NON-STABILIZED MEDIA

Previous Work Reported in the Literature

In a paper by Kunkel and Tiselius, the electro-migration path of an ion or particle is viewed as a sort of meandering tunnel or wormhole through the paper (38). The assumption is made that, for a given paper, all migrants follow along the similar paths, and that since the path is much longer than the actual length of the paper, the potential gradient actuating the migrating particle will be less than the value given by simply dividing the potential difference across the paper strips by the length. It is also assumed that the distance the migrant moves is not simply the distance that is measured from the starting point, but something greater than this because the migrant does not move in a direct course, but rather meanders in and out and up and down. On this basis, then, after applying a correction factor for electroosmosis (using dextran as an indicator of the amount of electroosmotic movement), they apply
a second correction factor for "added migration path length", which appears to bring the mobility of the migrant up to that observed in free solution. They state that this latter correction factor, for a given type of paper, can then be used for mobility calculations under widely varying conditions and types of migrants. In other words, the correction factor for "added migration path length" is a function principally of the paper and not of the migrant.

If the view of Kunkel and Tiselius is to be accepted, the validity of Ohm's law is questioned and the concept of ion movement in free solution is also challenged. Even if these features of their theory are excluded, it still does not explain the difference in mobility values for a given migrant obtained by different observers using the same type of paper.

To show that their theory of "added migration path length" challenges the concept of ion movement in free solution, consider the following quotation from the paper by Kunkel and Tiselius:

The determination of mobilities on paper involved several new considerations because the formulas used in free solution

\[ U = \frac{dl}{V} \]

and

\[ U = \frac{dqk}{li} \]

in which \( V \) is the voltage, \( l \), the length of the channel, \( q \), the cross-sectional area, \( k \), the conductivity, and \( i \),
the current, are not applicable to liquid in a highly porous supporting medium. It could readily be shown that the expressions for field strength, \( V/l \) and \( i/qak \) were not equal. This was due to the fact that \( l \) did not represent the true distance of voltage drop through the paper. This can best be seen from a consideration of Figure II, in which a tortuous channel is envisioned in the paper (1'). This, of course, represents a great simplification of the intricate channeling of liquid that actually exists in the paper. In accordance with the diagram the protein particle takes the tortuous path \( d' \) which follows \( l' \) and is a fraction thereof and not the observed distance.

The implications here are many. First it is implied that an ion in free solution travels in a straight line. This can only be true if first one extrapolates to infinite dilution of migrant and then extrapolates to zero solution concentration, that is one in a vacuum. In this case, there is no doubt that the particle moves in a straight line. In a solution having a concentration of 1% of migrant, the particles collide with many other particles of migrant and with solvent molecules. If a correction for "added migration path length" must be made in a paper-buffer system, then to be logical and consistent, a correction ought to be made for "added migration path length" in a buffer system alone. Only in a vacuum would no correction for added migration path length be required. The mobility of a particle in free solution is expressed as the linear displacement a particle moves per unit time per unit potential field, even though the particle, in actuality, takes a "tortuous path" as has been stated in Chapter I. It is admitted that the "tortuous
path" becomes more "tortuous" when paper is added to the buffer system. The difference between a free solution system and paper-buffer system is nevertheless one of degree, not of kind.

To show that the theory proposed by Kunkel and Tiselius challenges the validity of Ohm's law, consider the following: according to them, the expression for field strength, that is, potential gradient, namely

\[ \frac{V}{L} \text{ and } \frac{i}{qaK} \]

are not equal in paper electrophoresis. Here \( V \) is the voltage applied across the length of paper, \( L \), and \( i \) is the current flowing through the paper with an effectual cross-sectional area of \( qa \), saturated with a buffer whose specific conductivity is \( K \). The non-equality is stated to be due to the fact that \( L \) does not represent the true distance of voltage drop through the paper. There is no need to explain the inequality by using a "tortuous channel", \( L' \). Instead, it should simply be stated that there is no justification for expecting an equality when using \( K \) the specific conductivity of buffer solution instead of \( K_p \), the specific conductivity of the system over which the potential gradient is to be determined, that is, the "paper-buffer system".

In other words, two different systems are compared, one, the system of buffer only, and the other system of paper-buffer. This can be readily shown by combining the equation of Ohm's law

\[ V = iR \]
and the equation defining resistance

\[ R = \frac{L}{qaK_p} \]

to give the equation

\[ V = \frac{iL}{qaK_p} \]

If this, in turn, is substituted into the expression for field strength, \( V/L \), the equation

\[ V/L = \frac{i}{qaK_p} \]

is obtained. From this equality, it is evident that there is no justification for \( 1/qaK \) to equal the potential gradient because even though the expression \( 1/qaK \) is an expression for field strength, it does not refer to the system under study. It refers to a column of buffer, of length \( L \) and cross-sectional area, \( qa \), whose specific conductance is \( K \) and not to the paper strip saturated with buffer of length \( L \) and cross-sectional area, \( qa \), whose specific conductivity for the paper-buffer system is \( K_p \).

The correct expression for field strength for a system containing buffer and paper fiber is either \( V/L \) or \( 1/qaK_p \), both of which are equivalent. Also, since it is easier to measure the voltage drop across the strip and the length of the paper strip from buffer surface to buffer surface, \( V/L \) is the most convenient expression to use for field strength. Even though \( 1/qaK_p \) is equivalent to \( V/L \), it is very difficult to use the
former expression because of the difficulty in evaluating the quantity, \( q_a \), that is, the "effective cross-sectional area".

The mobility, \( U \), represents the linear distance a particle moves in unit time per unit field strength, that is,

\[
U = \frac{d}{Vt}
\]

Therefore, in mobility experiments, potential gradient (volts/cm) must be kept constant so that the conditions for the definition of mobility can be fulfilled. It is absurd to hold current constant and let potential gradient vary when calculating mobilities. It can only be held constant when it causes the potential gradient to be constant. This is when the specific conductance does not change.

However, potential gradient can only be directly measured when the conductor is of uniform cross-sectional area and hence, of uniform resistance. In moving-boundary electrophoresis, the U-tube across which the voltage is impressed is not of uniform cross-sectional area throughout, and the field strength cannot be obtained simply by dividing the voltage applied by the length of the U-tube. To overcome this difficulty in standard electrophoresis, the section of the U-tube through which the particle actually migrates must be of uniform cross-sectional area. Since

\[
V = IR
\]

where \( i \) is current and \( R \) is resistance through the solution, and
\[ R = \frac{L}{K_a} \]

for a conductor or solution of uniform cross-sectional area, \( a \), and length, \( L \), \( K \) equals the specific conductivity of the solution. It follows that if \( V \) is replaced by its equivalent, in the expression for field strength \( V/L \) the following relation is obtained:

\[ V/L = \frac{iR}{L} = \frac{iL}{K_a L} = \frac{i}{K_a} \]

Assuming \( K \), the specific conductivity of the solution does not change (and this is not necessarily a true assumption in a paper-buffer system), current, \( i \), is kept constant to obtain a constant potential gradient. It is not necessary in determining potential gradient to make these substitutions, when the conductor is of uniform cross-sectional area throughout and it is possible to measure \( V/L \) directly as in the technique of ionography. However, if \( K \) is not constant and changes throughout the entire strip, the true potential gradient cannot be obtained by dividing the potential applied across the paper strip divided by its length. Therefore, the mobility cannot be calculated for this reason.

**Theoretical Development of the Problem**

The fact that mobilities determined in stabilized electrolytes are usually lower than those determined in non-stabilized electrolytes, make imperative, the conclusion that apparently the stabilizer has some effect upon the migrant. This conclusion is further substantiated by the fact that
different mobilities can be obtained for the same migrant under otherwise identical conditions, when the wetness of the paper is not maintained at a constant level in each case. In general, it was found that the wetter the paper, the higher the mobility.

With this as a basis, the effect of the "paper concentration" upon the mobility of the migrant was investigated. It was reasonable to assume that in non-stabilized electrolytes, the paper concentration was zero, and the fastest mobility would then be obtained. In stabilized electrolytes, the paper concentration was some positive value and the mobility observed would be lower than that determined in free solution. In the determination of mobilities as the paper concentration approached zero, the mobilities should increase and approach the value in free solution. In other words, the wetter the paper, the higher the mobility might be expected to be reaching, as a limit, the value in free solution.

It will be assumed and proven later, that the presence of paper causes a decrease in the thermodynamic activity of the migrant, that is, the greater the paper concentration, the greater the decrease in the thermodynamic activity of the migrant and hence, the lower the mobility. Furthermore, once the decrease in the thermodynamic activity of the migrant is calculated or is known as a function of the paper concentration, it should be easy to convert the mobility as determined in a paper stabilized system to the value determined in free solution. It must
be brought out here that the values will be "converted" and not "corrected". The word "corrected" implies that the mobility as determined in a paper stabilized system is in error while the word "converted" implies that the mobility as determined in a paper stabilized system is a valid value although not equal to that in free solution because the conditions are not identical. The experimental conditions in the two cases are dissimilar because of a difference in paper concentration. On the assumption then that the paper causes a decrease in thermodynamic activity of the migrant, the addition of paper to a free solution experiment would cause the mobility of the migrant to decrease, and if the paper concentration was the same as that which existed on a run when using the ionograph technique, the two mobilities would be the same. This point is here belabored because according to Kunkel and Tiselius, the presence of paper does not affect the thermodynamic activity of the migrant and hence cause a lower mobility; rather it is considered that the presence of paper causes an "error" in determining the potential gradient and when this "error" is corrected, the mobility of the migrant is the same, regardless of whether the paper is present or not. As a consequence of their theory, Kunkel and Tiselius apply a "correction factor". In the case of the theory proposed in this thesis, a "conversion factor" is applied to convert mobilities determined in paper stabilized electrolytes to those determined in non-stabilized electrolytes. For ease in referring
to this new view of the mechanics of electromigration, it will be referred to as the "barrier theory". The basis for this nomenclature is that the paper can be thought of an interposing obstacles or barriers in the migration path of the migrant. As a consequence of this barrier effect, the thermodynamic activity of the migrant is decreased thus causing a decrease in its mobility.

Practical Attack on the Problem

To determine what function the mobility of a particular substance is of the paper concentration or wetness, an apparatus was devised in which different wetnesses of paper could be produced and the corresponding mobilities determined. The special apparatus was constructed from a Lucite sheet, approximately 1/4 inch in thickness. A channel about 3 mm deep, 9 mm wide, and 25 cm long, was cut into the Lucite plate. A cover was made out of another 1/4 inch thick piece of Lucite sheet and two small holes were drilled at each end of the channel. The farthest hole on each end was fitted with a small glass U-tube which contained the agar salt bridge. The other two holes were used for two purposes: (1) to introduce a known amount of buffer; (2) two platinum wires were introduced so that the potential applied across the strip could be obtained. A fifth hole was drilled in the Lucite cover so that it came through the cover exactly in the center of the channel. This hole was used to introduce the
migrant after the buffer came to equilibrium. This center hole was covered when not in use by a microscope slide treated with silicone stopcock grease. A transparent rule was glued to the top of the cover so that the displacement of the migrant could be measured directly. The migrant chosen for study was brom phenol blue partly because it has an intense blue color above pH 5, thus obviating the necessity of staining. Another reason for the choice was based on the fact that it has a high electrophoretic mobility. A veronal buffer was used, at a pH of 6.6 and at an ionic strength of 0.05. The temperature of all the experiments was 23° - 27°C. The potential gradient applied was 2-6 volts/cm. The paper used in each experiment was Eaton-Dikeman No. 613, cut so that it would fit into the channel. The paper was weighed and placed in the channel; the cover was greased and placed on top of the channel; the agar salt bridges were then put into place and a measured amount of buffer was added. The current was turned on. One half hour was allowed for the system to come to equilibrium. A 1 per cent solution of brom phenol blue was made using the buffer and one to two microliters were applied to the center of the strip. After an appropriate time interval had passed, the movement and potential gradient was noted and the mobility calculated for that particular wetness of paper.

Results and Conclusions:

The results are plotted in Figure 5. Mobility is
plotted against the ratio of buffer to paper. It is apparent that the results of this set of experiments are inconclusive. No trend is even apparent.

The difficulty apparently arises from the fact that the filter paper has a particular equilibrium wetness and that any attempt to make it wetter results in a two phase system, that is, saturated paper on one hand and excess buffer on the other. Furthermore, the cross-sectional area of the channel was not uniform due to the wetting of the cover in some points and therefore, no true potential gradient could be calculated because of this non-uniformity. From the results of this set of experiments, it was apparent that an indirect approach to the problem was necessary; an approach where all the influencing factors could be controlled.

Use of Decrease in Conductivity as a Means of Measuring the Effect of Paper Upon the Migrant

Since the decrease in thermodynamic activity involves the mobility of the charged ions on particles in solution, use could be made of conductivity measurements to determine the decrease. A conductivity cell, shown in Figure 6, was constructed out of Lucite in the form of a rectangular box. The inside dimensions were 9.4 mm wide, 13.6 mm high and 33.1 mm long. Platinum electrodes covered with platinum black were embedded in opposite walls so that only one surface, the inside, of each electrode would be exposed to the solution. Furthermore, each
EFFECT OF WETNESS ON THE MOBILITY OF BROM PHENOL BLUE

FIGURE 5
platinum electrode covered the entire wall into which it was embedded. This was done so that the cell would behave as an ideal cell, that is to say, the cell, when filled to a particular fraction of the total volume, would exhibit that particular fraction of the total conductivity. A cover of glass made from a microscope slide was used and a clamp was constructed so that the cover could be held tightly upon the cell. The weight of the cell, cover and clamp was approximately, 22.2 gms. The cell when filled with solution, weighed approximately, 26.6 gms, therefore, the weight of solution necessary to fill the cell was about 4.4 gms or about a volume of 4.4 ml. The cell constant was determined with 1/50 normal KCl, and was found to be 2.50. The behavior of the cell as an ideal cell was then next determined. It was filled with 1/50 normal KCl and the resistance measured with a Leeds and Northrup Wheatstone bridge. An Aminco Electronic Null Indicator was used to indicate when the circuit was in balance. The sensitivity of this indicator was 1 millivolt through 1,000 megohms. The cell was then filled to some fraction of the full cell and the resistance measured. This was repeated until 80 per cent of the solution was displaced. The results obtained are plotted in Figure 7. Conductivity times the reciprocal of the fraction of solution present in the cell is plotted against the fraction of solution present in the cell. The cell deviates from ideality when it is approximately 75 per cent empty. However, it never had more than 25 per cent of its
CALIBRATION OF CONDUCTIVITY CELL
USING 0.02000N KCl

EXPERIMENTAL
THEORETICAL

FIGURE 7
solution displaced in the experiments described below. The procedure used in the rest of the conductivity experiments was as follows: The conductivity cell was first weighed empty and that weight was recorded. The cell was then filled with the solution to be studied, it was again weighed and this value was recorded. The conductivity was determined and recorded. The cell was then opened and some of the solution removed. A weighed amount of the desired type of filter paper cut previously, so that each piece could be layered into the cell parallel to the bottom of the cell was added. The cell was refilled and the glass cover carefully replaced so that no air bubbles were trapped inside of the cell. The clamp was then placed in position and firm pressure was applied so that the cover was tightly held to the conductivity cell. The cell was weighed again and the conductivity was re-determined. The above procedure was then repeated until no more paper could be added to the cell. A data sheet for each experiment was prepared similar to that shown in Table VI. In preliminary experiments, the cell was not weighed after each addition of paper to determine the amount of solution present, but was calculated by assuming a constant density for the paper added. This assumption was not correct because the density as measured by the volume of paper displaced ranged from 1.46 to 1.00 gm/ml. This change of density was thought to be due to the presence of minute air bubbles trapped in the paper. This was proven to be the case by placing the cell
in a vacuum desiccator half-filled with the solution with which the cell was to be filled after each addition of paper. Suction was applied and the air bubbles "boiled out" of the paper fibers. With this technique, the paper assumed a constant density of about 1.40 gm/ml; however, it was impossible to determine the conductivity with reproducible results due to the presence of solution on the outside of the cell and contamination of the solution inside of the cell. Furthermore, it was thought that this represented an artificial condition which did not exist when the mobility of the migrant was determined in the ionograph; that is, the air bubbles were not removed in the latter case, but were removed in the former. It was decided that the amount of solution present in the cell would be determined by weighing the cell after each addition of paper. Since the empty weight of the cell, the weight of paper present, and total weight were known, the weight of solution could be determined by subtracting the first two quantities from the last quantity. It would appear that the trapped air bubbles in the paper fibers would affect the conductivity measurements. This is true, but when the conductivity is plotted against the ratio of paper present to the weight of solution present, the effect of the air bubbles on the conductivity would be cancelled out; that is, more air bubbles were trapped in the cell, the conductivity would decrease but also the ratio of paper to solution would increase due to the air bubbles displacing some of the solution. Having an ideal
cell, all conductivity measurements could be referred to the full cell simply by multiplying the conductivity determined by the reciprocal of the fraction of solution present.

The operational procedures are more clearly demonstrated in the following experiments.

A 4 per cent solution of bovine serum albumin was prepared and was brought to a pH of 7.0 by the addition of 0.1N NaOH. The cell was filled with this solution and the conductivity recorded; at the same time, the cell was weighed to within a tenth of a mg. The cell was then opened and a known weight of paper, in this case, Eaton-Dikeman No. 248 was added. The cell was refilled, the cover replaced, and held tightly in place with the clamp. The cell was again weighed and the conductivity again determined. This was repeated until no more paper could be added to the cell. A linear relationship was found to exist between the conductivity and the paper added if the obtained conductivity is plotted against the ratio of paper to solution.

The results obtained are plotted in Figure 8. When the conductivity of a solution in the cell is measured with paper present, two effects come into play. First a decrease in the conductivity due simply to a volume displacement of the solution caused by the volume of the paper fibers present and also to the minute air bubbles trapped between the paper fibers, and secondly, the effect the paper fiber has directly upon the
CORRECTED FOR DISPLACEMENT OF SOLUTION

DECREASE DUE TO THE PRESENCE OF PAPER

4% BSA, PH 7.0
E & D 248 PAPER

FIGURE 8
migrant solution which affects the mobility of the ions present and hence, the conductivity. The sum of the two effects is represented by the open circles in Figure 3. The first effect can be cancelled out nicely by using an ideal cell, then multiplying the conductivity so obtained by the reciprocal of the fraction of solution present. The conductivity obtained is always referred to the cell filled with solution and is represented by the closed circles in Figure 3.

A few words are in order here about the reference state for the measurements. This reference state is the conductivity cell filled with buffer solution having varying amounts of filter paper present. It is then clearly a fictitious state because the paper present has no effect, either due to its own volume or to the air bubbles which are trapped in its fibers on displacing the solution from the cell. Since the different solution to be analyzed here had different conductivities, the conductivity measurements were referred to a "relative thermodynamic activity state" by dividing all of the conductivity measurements multiplied by the reciprocal of the fraction of solution present in any one series of experiments, by the conductivity of the solution containing no paper. This was done so that the different solutions could be compared on the same basis. This reference state, the relative thermodynamic activity state, then is a measure of the decrease in the thermodynamic activity of the migrant solution as measured by conductivity, due to the
interaction of the migrant solution with the paper fibers present and is not affected by the amount of buffer solution which is displaced. The meaning of a fictitious state as used above, simply signifies a state which cannot be physically obtained but is rather obtained by mathematical procedure. It does not mean that the reference state is without practical value. It is analogous to the use of the Standard Free Energy State, used in the field of chemical thermodynamics which often refers to an experimentally fictitious state, but is nevertheless of great importance.

In Figure 9, the effect of Eaton-Dikeman paper No. 248 on a 4 per cent bovine serum albumin solution, at a pH of 7.0, is represented by plotting the ratio of paper to solution against the relative thermodynamic activity. It can be seen that when the ratio is equal to zero, that is, the amount of paper present is zero, the relative thermodynamic activity is equal to 1.00, and no correction is necessary for the activity nor for the mobility of the migrant. This condition refers to a mobility determined in free solution. However, when the ratio is 0.444, the relative thermodynamic activity is 0.748. This means in other words, that the activity of the migrant and its mobility is 0.748 of its activity when no paper is present. In order to convert its activity or mobility to free solution, the mobility at a ratio of 0.444 paper to solution must be multiplied by an activity coefficient, \( A \), which is equal to \( a_{0/8} \).
RELATIVE THERMODYNAMIC ACTIVITY

RATIO OF PAPER TO SOLUTION

4% BSA, pH 7.0
E&D 248 PAPER

FIGURE 9
where $a_0$ is the relative thermodynamic activity in free solution and is set equal to one and $a$ is the relative thermodynamic activity when paper is present; in this case, it is equal to 0.748. Therefore, the activity coefficient is equal to $1/0.748$ or 1.31.

It was noted in preliminary experiments that when a dilute solution of migrant was used (usually 2% or less) and the specific conductivity fell below $4.0 \times 10^{-4}$ mhos, that the conductivity of the solution upon the addition of paper did not decrease. Upon the addition of paper, the conductivity increased instead of decreasing. It appeared as if the paper was a better conductor than the solution. This behavior can be explained by the presence of salts in the paper, and an experiment was performed to test whether a salt or some equivalent electrolyte was present in the paper. A very dilute solution of KCl (0.0010 Molar) was used so that a very small amount of electrolyte, if present in the paper, could be detected. Eaton-Bikeman paper No. 613 was used. Some of the paper was used without leaching, and some of the paper was leached for one hour using doubly distilled water. This was repeated three times. The paper could not be washed or handled vigorously without disintegrating. The results of this experiment are shown in Figure 10. It will be noted that the specific conductivity of the 0.0010 Molar KCl is $1.5 \times 10^{-4}$ mhos, and with the addition of unwashed paper, the conductivity doubled when the ratio of paper to
Figure 10: Specific conductivity ($10^{-4}$ mhos) vs. ratio of paper to solution for 0.0010M KCl. Solid circles represent unwashed E&d 613 paper, and open circles represent washed E&d 613 paper.
solution was 0.36. The conductivity rose only to \(1.6 \times 10^{-4}\) mhos when the washed paper was used. Actually, the conductivity should have decreased in a manner similar to the way 0.020 Molar KCl behaved as is shown in Figure 11. However, it did not decrease due to the presence of some salts which were not washed out. Accordingly, in all of the conductivities reported here, when it is not otherwise stated, the paper was leached for two weeks with a daily change of double distilled water and then dried for two days at 50°C. Furthermore, since it was felt that all of the salts could not be extracted, even with this prolonged treatment, the concentrations of the substances to be studied were so chosen that the specific conductivity of the solution would usually be much greater than \(3.0 \times 10^{-4}\) mhos.

The Effect of Migrant Concentration

Of prime importance is the effect of migrant concentration itself on the decrease in the thermodynamic activity of the migrant. A 2 per cent and 4 per cent solution of bovine serum albumin, at a pH of 8.6 was used. The paper added was Eaton-Dikeman No. 613. The results are presented in Figure 11. The decrease in the activities are similar for the two solutions until the ratio of paper to solution becomes 0.23, and then the decrease in the conductivity for the 2 per cent solution was much less than for the 4 per cent solution. This can be explained by the fact that when the ratio of paper to solution became 0.23, the specific conductivity approached the low value where
Figure 11

- 2% B.S.A., PH 8.6
  PAPER - E&D 613

- 4% B.S.A., PH 8.6
  PAPER - E&D 613

Relative Thermodynamic Activity

Ratio of Paper to Electrolyte
the small trace of electrolytes present in the washed paper starts to affect the conductivity measurement. From the result of this experiment, since both solutions' activities decreased with the same slope at the beginning, it is felt that the effect of migrant concentration upon the relative thermodynamic activity of the migrant-paper system is of second order importance, within a reasonable range of migrant concentrations.

The Effect of Different Types of Paper Upon the Thermodynamic Activity of the Migrant

The next question of importance is: How do different papers affect the thermodynamic activity of the migrant? Three papers were chosen. They were Eaton-Dikeman Nos. 248, 613 and Cremer-Tiselius Munktells. The migrant was a 4 per cent solution of bovine serum albumin at a pH of 7.0. The results are plotted in Figures 12 and 13. It can be seen that over all, the different papers do not vary greatly, and in the case of Eaton-Dikeman No. 248 and C. T. Munktells, the difference is very small. However, the different papers do have a significant difference. This can be explained by the reasonable assumption that the different processes involved in making different types of filter paper changes the nature of the cellulose fiber either physically or chemically.

The Effect of the Same Paper on Different Types of Migrants

Figure 14 illustrates the results obtained when 0.00200 Molar KCl, 0.01 Normal NaOH, 0.1 per cent glycine at a pH of
4% BOVINE SERUM ALBUMIN
PH 7.0

- MUNKTELL'S
- E&D 613
- E&D 248

FIGURE 12
$4\% \text{BSA, PH 7.0}$

- [ ] E&D 613 PAPER
- [ ] E&D 248 PAPER
- [ ] C.T. MUNKTELLS

**FIGURE 13**

**RELATIVE THERMODYNAMIC ACTIVITY**

**RATIO OF PAPER TO SOLUTION**

1.0

0.9

0.8

0.7

0.6

0.5

0.4

0.3

0.2

0.1

0.0
FIGURE 14

- 0.0200M KCl
- 0.1% GLYCINE, PH 10.91
- 0.01M NAOH
- 4% B.S.A., PH 8.6

PAPER - E&D 613

RELATIVITY THERMODYNAMIC ACTIVITY

RATIO OF PAPER TO SOLUTION
10.91 and bovine serum albumin at a pH of 8.6 were used. The paper in all cases was Eaton-Dikeman No. 613. From the first approximation, considering the molecular size, it was thought that the paper would have the least effect on the salts, a greater effect upon an amino acid and the greatest effect upon a protein molecule. These were not the results obtained. The amino acid, glycine, was the least affected, next were the strong electrolytes, KCl and NaOH, and the greatest effect was on the protein, bovine serum albumin. Apparently, no simple predictable relationship exists between migrant size and its interaction with the cellulose fiber, but perhaps, some factor like the surface density charge on the migrant plays a role.

The Interaction of Paper and a Protein at Different Values of pH

Figure 15 illustrates the results of the decrease of the thermodynamic activity upon the migrant using a protein, bovine serum albumin, at different pH's. Bovine serum albumin at a pH of 5.2 (which is very near its isoelectric point) has the least decrease in its thermodynamic activity when paper is introduced. This decrease is increased when the protein is displaced on either side of its isoelectric point. The results can be made more apparent when the slopes of the decrease in the thermodynamic activity is plotted against pH as is done in Figure 16. Since it is assumed that the paper surface bears a net negative charge due to the selective absorption of hydroxyl ions, one would expect an increase in interaction as the protein...
Figure 15

Relative Thermodynamic Activity

Ratio of Paper to Solution

- PAPER - E&D 613
- 4% BSA, PH 3.5
- 4% BSA, PH 5.2
- 4% BSA, PH 7.0
- 2% BSA, PH 8.6
FIGURE 16

THE EFFECT OF pH ON BSA-PAPER INTERACTION
is displaced to the acidic side of its isoelectric point. The protein then bears a net positive charge. However, its increased interaction on its basic side is not too apparent when one considers that the protein bears a net negative charge. Perhaps with a charge redistribution on the basic side of the protein's isoelectric point, the charges are redistributed so that the molecule is now forced to migrate perpendicularly to its long axis or at some angle greater than zero. Thus, the paper fibers interfere to a greater extent than could be predicted.

The Application of the Conversion Factor for Converting Mobilities Determined on Paper to Those Determined in the Absence of Paper.

If no other phenomenon occurs when paper is added to a buffer solution, the free solution mobility can be obtained if the mobility of a migrant determined with paper present, is corrected for the migrant's decrease in thermodynamic activity, caused by its interaction with paper. The results are shown in Table I. In all the experiments, bovine serum albumin was the migrant. The buffer used was veronal, at a pH of 8.6, and an ionic strength of 0.0125. The paper on which the mobility was determined was in all cases, Eaton-Dikeman No. 613. All the runs were made at 10°C, and all the mobilities reported are expressed as $1 \times 10^{-5}$ cm$^2$/volt-sec. The instrument used was the Ionograph which employs the horizontal suspension of the filter-paper strips. A photograph of this instrument is shown
**TABLE I**

DETERMINATION OF FREE SOLUTION MOBILITY USING EATON-DIKELMAN NO. 613 PAPER; A VERONAL BUFFER OF IONIC STRENGTH 0.0125 AT A pH OF 8.6.

APPLYING THE FOLLOWING EQUATION: \( u_f = A \mu \)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Migrant</th>
<th>Ratio of</th>
<th>&quot;Wetness&quot; L/R</th>
<th>Activity coefficient A</th>
<th>Mobility Obtained ( \times 10^5 )</th>
<th>Mobility Calculated ( u_f \times 10^5 )</th>
<th>Mobility Actual ( \times 10^5 )</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>11G</td>
<td>BSA</td>
<td>0.429</td>
<td>2.33</td>
<td>1.58</td>
<td>-5.00</td>
<td>-7.90</td>
<td>-11.24</td>
<td>29.7</td>
</tr>
<tr>
<td>13G</td>
<td>BSA</td>
<td>0.440</td>
<td>2.27</td>
<td>1.61</td>
<td>-4.50</td>
<td>-7.25</td>
<td>-11.24</td>
<td>35.5</td>
</tr>
<tr>
<td>12G</td>
<td>BSA</td>
<td>0.427</td>
<td>2.34</td>
<td>1.58</td>
<td>-5.36</td>
<td>-8.31</td>
<td>-11.24</td>
<td>26.1</td>
</tr>
<tr>
<td>20G</td>
<td>BSA</td>
<td>0.431</td>
<td>2.32</td>
<td>1.59</td>
<td>-6.12</td>
<td>-8.14</td>
<td>-11.24</td>
<td>27.6</td>
</tr>
<tr>
<td>26G</td>
<td>BSA</td>
<td>0.437</td>
<td>2.29</td>
<td>1.60</td>
<td>-4.62</td>
<td>-7.39</td>
<td>-11.24</td>
<td>34.3</td>
</tr>
<tr>
<td>24G</td>
<td>BSA</td>
<td>0.457</td>
<td>2.19</td>
<td>1.65</td>
<td>-4.02</td>
<td>-6.65</td>
<td>-11.24</td>
<td>40.9</td>
</tr>
</tbody>
</table>
In experiment 110, the wetness was 2.32, that is the ratio of solution to paper was 2.32. The wetness was determined before and after each run and if it deviated more than one percent, the experiment was discarded. The reciprocal of the wetness is 0.429 and the decrease in the thermodynamic activity is 0.653; therefore, the activity coefficient, \( A \), is 1.58. If the mobility determined, namely 5.00 is multiplied by the activity coefficient, the free solution mobility should be obtained. Actually, the mobility calculated is 7.90 instead of the 11.24 which is the mobility in free solution. There represents a difference of 32 per cent. In the other experiments shown, the difference is of the same magnitude.

It is apparent that another factor in addition to those considered above, enters in when paper is added to the buffer solution. This factor is electroosmosis.

The Factor of Electroosmosis

Due to, perhaps, the selective absorption of hydroxyl ions from the buffer solution, the surface of the filter paper fiber possesses a net negative charge, and if it were free to move, the buffer solution moves as if it were positively charged and moves to the negative pole. The cause of electroosmosis can be explained by two theories, the classical theory of the Helmholtz double layer and the theory of water transport by hydrated ions.
Figure 17

The Ionograph
On the classical grounds, it has been pointed out that in electromigration in stabilized electrolytes, the mobility of positively charged ions might be expected to be increased while that of negatively charged ions might be decreased, due to the phenomenon of electroosmosis (44, 52). In the case of electrolytes stabilized with paper, this conclusion is based on the observation that the paper surface generally appears to bear a negative charge, while the aqueous solution in contact with it acts as if it were positively charged. Consequently, the resultant velocity vector of the positive ions would be expected to be the sum of the simple electromigration velocity and the electroosmosis velocity of the solution in which they are being buoyed along. Negative ions, on the other hand, have been pictured as breasting the electroosmotic velocity and the electroosmotic velocity of the liquid.

There is a tendency, nowadays, to discount the role played by a Helmholtz double layer in the phenomenon of electroosmosis. This school of thought may perhaps be exemplified by the work of Darmois (6) who explains the transport of water associated with electroosmosis on the basis of transport of water molecule by ions. His work on ultra-violet absorption studies led him to the conclusion that the number of water molecules electrically bound to a chloride ion is of the order of 1000 in a solution of 0.005 Normal NaCl and that the great difference in the hydration of cations and anions can explain the electroosmo-
sis phenomenon. In general, positive ions appear more highly hydrated than negative ions, and hence, there would be a tendency for a net flow of water toward the negative electrode. He derives an equation, in fact, purporting to relate in a quantitative way, the rise of solution at one electrode due to electroosmosis, the potential, the number of ions of one sign per milliliter, the viscosity of the media, the mobilities of the positive and negative ions and the number of moles of water of hydration associated with the positive and negative ions, respectively.

Whether the phenomenon of electroosmosis is viewed from the classical or modern viewpoint, however, does not invalidate the idea that there may be some effect on the mobility of a migrant, and from the standpoint of this net effect, both theories can be shown to lead to essentially the same conclusions.

Using the treatment of thermodynamics of irreversible processes, (7,8) one can write as phenomenological equation, the relations,

\[ I = L_{11} \Delta \Phi + L_{21} \Delta P \]  
\[ J = L_{21} \Delta \Phi + L_{22} \Delta P \]  

and

\[ L_{12} = L_{21} \]

where \( I \) represents electric current density

\( J \) represents water flow

\( \Delta \Phi \) represents the potential gradient

\( \Delta P \) represents the pressure difference existing in the two
buffer vessels causing a difference in buffer solution levels, and the coefficients \( L_{11} \) and \( L_{22} \) are related to the electrical and hydrodynamical resistance, respectively. The coefficient \( L_{12} = L_{21} \) is connected in kinetics theories with the so-called zeta potential and the double layer properties. Then electroosmosis, defined as the flow of matter per unit electrical potential, in the state with fixed uniform pressure can be expressed by means of equation 1 and 2 as follows:

\[
\frac{J}{\Delta \phi} = L_{21} + L_{22} \frac{\Delta \phi}{\rho}
\]

and if the two end vessels are connected by a siphon so the pressure difference is zero, equation 3 reduces to

\[
\left(\frac{J}{\Delta \phi}\right)_{\Delta \rho = 0} = L_{21}
\]

As has been stated previously, the coefficient \( L_{21} \) is connected with the so-called zeta potential and the double layer properties. Consider equation 3. As the pressure level in the negative buffer vessel builds up due to transport of water by electroosmosis, the \( \Delta \rho \) in the second term on the right hand of the equation becomes a larger negative value, approaching such a value that in the limit, the second term cancels out the first term on the right hand side of the equation, leaving

\[
\frac{J}{\Delta \phi} = 0
\]

This means that the pressure or level in the negative buffer vessel will approach such a value, causing the electroosmosis to
become zero. However, in the mobilities determined in Table I, a siphon was used to connect the two buffer vessels so that the equation, \( k \), applies. This equation states that electroosmosis proceeds at a maximum rate independent of time. In other words, in the measurement of the mobilities in Table I, electroosmosis was constant and at its maximum rate. Therefore, a constant factor should be added to each value for the mobilities determined from experiment to experiment. This would be true if the wetted paper strips were of uniform cross-sectional area, that is if the paper was at the same wetness. Consequently, a conversion formula will have to be developed which will take care of the variable mentioned previously, that is, the wetness of the paper, the decrease in thermodynamic activity of the migrant and the factor of electroosmosis as modified by the wetness of the paper.

**Development of a Conversion Formula for Converting Mobilities Determined in Stabilized Solutions to Those in Non-stabilized Solutions.**

If electroosmosis is present, it is obvious that the observed displacement of the migrant is equal to its displacement due to electromigration plus its displacement due to electroosmosis. This can be written mathematically in the following equation:

\[
de = d_e + d_{eo}
\]  

(6)
where \( d_e \) represents the displacement of the migrant observed, 
\( d_e \) represents the displacement of the migrant due to electromigration, 
\( d_e_0 \) represents the displacement of the migrant due to electroosmosis.

But
\[
d_e = \frac{d_f}{A} \tag{7}
\]
where \( d_f \) equals the displacement of the migrant in free solution, and \( A \) equals the activity coefficient of the migrant. Assume that the activity coefficient for the migrant will be the same whether the migrant is moved either by a potential gradient or by a flow of buffer due to electroosmosis. Then

\[
d_e_0 = \frac{D}{A} \tag{8}
\]
where \( D \) equals the displacement of buffer which is the result of electroosmosis, which is something greater than the displacement of the migrant due to electroosmosis. Then by combining equations 6, 7 and 8,

\[
d_f = A d_e_0 - D \tag{9}
\]
is obtained. However, \( D \), the electroosmosis, is a function of the wetness of the paper. It has been noted in the fractionation of plasma proteins that gamma globulin, which has a low free solution mobility, actually moves in a reverse direction in a paper-buffer system, to what it does in free solution.
Furthermore, it has been noted, in a qualitative way, that the drier the paper, the greater the displacement in the wrong direction. Therefore, it will be assumed that $D$ will be the following function of wetness:

$$D = \frac{w_1}{w_2} P$$

(10)

where $p$ is proportional to the coefficient $L_{21}$, which is a constant, and was discussed previously. It is a constant however, only in each defined system. This equation is applicable only when a siphon connects the two buffer vessels. However, once the system is defined, $p$ is a constant and is not a function of wetness or migrant. Likewise, $W_1$ is defined as the weight of water in the primary water layer between the paper fiber and the buffer, which is not in the primary water layer. It should be noted that the sum of $W_1$ and $W_2$ equals the ratio of weight of buffer to the weight of paper or the wetness of the paper, and is the reciprocal of the paper concentration, $R$.

That is,

$$\frac{1}{R} = W_1 + W_2 \text{ or, } W_2 = \frac{1}{R} - W_1$$

(11)

Now if equations 9, 10 and 11 are combined, the following relationships results:

$$DF = AD_0 - \frac{w_1 P}{\frac{1}{R} - w_1}$$

(12)

If equation 12 is divided through by the time in seconds and the potential gradient, the following equation is obtained:
$$M_s = AU - \frac{W \cdot L}{W - W_1} \tag{13}$$

where $u_f$ is the free solution mobility for the migrant,

$U$ is the mobility for the migrant in the paper-stabilized solution,

$L$ is the maximum mobility the water molecules have in the primary water layer and is proportional to $L_{12}$.

To apply this equation, a particular system has to be defined. This system will be a veronal buffer at a pH of 8.6 and ionic strength of 0.0125 at a temperature of 1°C. using Eaton-Dikeman paper No. 613. If the free solution mobility is known, along with observed mobility in the paper-buffer system at a known paper concentration, $A$ and $R$ can be calculated. This leaves one equation with two unknowns, $W_1$ and $L$. However, $W_1$ and $L$ can be determined if the observed mobility in the paper-buffer system is known at two different wetnesses because then one has two equations with two unknowns. Furthermore, since the coefficient, $A$, is a linear function of the paper concentration, $R$, the equivalent of $A$ in terms of $R$ can be used in equation 13. Since $A$ equals $a_0/a$, then $a$ is equal to $mR + 1$ where $m$ is the slope of the straight line where relative thermodynamic activity is plotted against $R$. Therefore, $A$ equals $\frac{1}{mR + 1}$.

That is, for a particular migrant, the activity coefficient, $A$, is a function only of the paper concentration, $R$. Then equation 13 reduces to
Using the first two observed mobilities for bovine serum albumin reported in Table I, to determine \( W_1 \) and \( L \) and Figure 6 to determine the slope, \( m \), the following values are obtained for the defined paper-buffer system.

\[
W_1 = 1.96 \\
L = 0.630 \\
M = 0.860
\]

And equation 14 for this particular system reduces to

\[
M_f = \frac{U}{W_1 + 1} - \frac{L}{\sqrt{R} - W_1}
\]  

(15)

Equation 15 shows that the mobility obtained in a paper-buffer system is solely a function of \( R \), the paper concentration, expressed as the ratio of paper to buffer. To calculate the free solution mobilities from those observed in the paper-buffer system, equation 1 is used. The free solution mobility so calculated is reported in Table II. It should be noted that the values obtained agree to within an average of 0.44 mobility units.

To test the validity of equation 15, bovine plasma gamma globulin was used. Gamma globulin was chosen due to the fact that it moves, in the paper-buffer system, in a direction opposite to that in free solution. Its free solution mobility was determined to be -2.98, while its observed mobility in the paper-buffer system is 0.91 at an \( R \) value of 2.29. It must be
TABLE II

DETERMINATION OF FREE SOLUTION MOBILITY USING DIAPO-UN ICEMAN NO. 613 PAPER AND A VARIOUS BUFFER OF IONIC STRENGTH, 0.0125 AT A pH OF 6.6 AT 1°C. APPLYING THE FOLLOWING EQUATION

\[ \mu = \frac{U}{1 + mr} - \frac{1.24}{r} - 1.96 \]

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Migrant</th>
<th>Ratio of paper to buffer &quot;R&quot;</th>
<th>Value of &quot;m&quot;</th>
<th>Mobility Observed ( \times 10^5 )</th>
<th>Mobility Calculated ( \times 10^5 )</th>
<th>Mobility Actual ( \times 10^5 )</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>11G</td>
<td>BSA</td>
<td>0.429</td>
<td>-0.360</td>
<td>-5.00</td>
<td>-11.25</td>
<td>-11.24</td>
<td>11-</td>
</tr>
<tr>
<td>13G</td>
<td>BSA</td>
<td>0.440</td>
<td>-0.860</td>
<td>-4.50</td>
<td>-11.25</td>
<td>-11.24</td>
<td></td>
</tr>
<tr>
<td>12G</td>
<td>BSA</td>
<td>0.427</td>
<td>-0.660</td>
<td>-5.26</td>
<td>-11.57</td>
<td>-11.24</td>
<td>0.33</td>
</tr>
<tr>
<td>20G</td>
<td>BSA</td>
<td>0.431</td>
<td>-0.660</td>
<td>-5.12</td>
<td>-11.58</td>
<td>-11.24</td>
<td>0.34</td>
</tr>
<tr>
<td>26G</td>
<td>BSA</td>
<td>0.437</td>
<td>-0.360</td>
<td>-4.62</td>
<td>-11.15</td>
<td>-11.24</td>
<td>0.09</td>
</tr>
<tr>
<td>24G</td>
<td>BSA</td>
<td>0.457</td>
<td>-0.860</td>
<td>-4.03</td>
<td>-12.04</td>
<td>-11.24</td>
<td>0.80</td>
</tr>
<tr>
<td>26G</td>
<td>Gamma-Globulin</td>
<td>0.437</td>
<td>-0.700</td>
<td>0.91</td>
<td>-2.41</td>
<td>-2.98</td>
<td>0.57</td>
</tr>
<tr>
<td>29G</td>
<td>Gamma-Globulin</td>
<td>0.427</td>
<td>-0.700</td>
<td>0.57</td>
<td>-2.46</td>
<td>-2.98</td>
<td>0.52</td>
</tr>
</tbody>
</table>
remembered that both $W_1$ and $L$ are functions only of the paper-buffer system, while $m$ is a function only of the migrant used. Therefore, $W_1$ and $L$ remain the same but the value of $m$ changes because a different migrant is used. Using the data from Table IX in the appendix, the value of $m$ for gamma globulin is found to be -0.700. Then using equation 15, except with -0.700 instead of -0.360 for the value of $m$, the free solution mobility may be calculated from the data determined in the paper-buffer system. The results are also listed in Table II. The deviation from the actual value determined in free solution is 0.55. This is within the experimental error and substantiates the proposed equation.
CHAPTER III

THE THEORETICAL DEVELOPMENT OF THE FUNDAMENTAL THEORY UNDERLYING
THE DETERMINATION OF MATERIALS SPREAD OVER A SURFACE AREA

To estimate the relative amounts of a given substance in a particular zone, on an ionogram, at least two methods are available. (A) The strip may be cut into sections, the colored material eluted with suitable solvents, and its intensity determined in a standard spectrophotometer fitted with small cuvets (5, 17, 22, 32, 33, 69, 70, 82). On the other hand, a key element in the various fractions for example, nitrogen, may be determined by the micro-kjeldahl method and the concentration of the original components computed. Similar methods rely on the absorption of ultraviolet light, determination of a particular amino acid, etc. (B) The distribution pattern of various substances on a strip can be determined directly by a transmission densitometer (26, 40, 41, 59, 63, 69, 77, 78). In this procedure, the paper is usually treated with some material to render it translucent, for example, a bromonaphthaline dissolved in paraffin oil to give a solution having a refractive index of
1.51 (26), of one of the patented transparentizers, e.g., Keuffel and Esser's "Translux". It is then passed through the densitometer by some geared device which moves the strip forward a mm or so at a time. The impulses from the photoelectric cell then go to a sensitive galvanometer whose deflections are read or automatically recorded.

The patterns obtained by means of the schlieren method when used in conjunction with the moving boundary apparatus, (30,61) and those derived from transmission densitometer readings on paper or other similar materials are usually considered to be the same, but they differ not only in degree but in kind. Due to this intrinsic difference, the two patterns cannot be compared directly.

Differentiation of the densitometer curve yields the schlieren pattern. This can be shown as follows: assume the following hypothetical case, a strip of homogenous paper 1 cm wide to which is applied, evenly, 1 mg of dye per 1 cm$^2$ over 3 cm of paper length. Figure 18, part A, is a diagram of these conditions. If light of proper wave length passes through the paper, and finally is picked up by a photoelectric cell, a densitometer curve can readily be obtained. A curve as shown in Figure 18, part B, will be obtained if optical density is plotted against distance, $x$, moved along the paper length. Assuming Beer's law to hold optical density can be replaced by concentration. As the light passes through the paper containing
LEGEND TO FIGURES

PART A; A schematic representation of a developed ionogram, which is 1 cm wide, containing dye at a concentration of 1 mg per cm length, over a length of 3 cm of a paper ribbon. The total dye present is therefore 3 mg.

PART B; Schematic representation of a densitometer pattern based on the ionogram shown in Part A. The area under the curve, namely 3 cm², represents the total amount of dye applied.

PART C; Schematic diagram of a schlieren pattern based on the ionogram in Part A. The area under each separate section of the curve, namely 1 cm², represents the concentration of dye present.
no dye, that is between the 0 and 1 cm mark, the optical density is set to read zero. Now, if the paper is moved 1 cm, so that the light passes through the paper between the 1 and 2 cm mark, the optical density reading will still be zero because no dye is present. However, when the paper is moved forward between the 2 and 3 cm mark, the dye will absorb a quantity of light. Assume, for convenience's sake, that the constant in Beer's law is 1, when the unit of concentration for the dye are mg per cm length of paper. Then the optical density reading will be 1. The same reading will be obtained over the next two cm because the same amount of dye is present. However, when the light passes through the paper between the 5 and 6 cm mark, the optical density reading will back to zero because no dye is present.

If the curve shown in Figure 18, part B, is integrated, the area under the curve will be obtained, and it can be shown, as follows, to be proportional to the quantity of dye applied:

\[
\text{Area} = \int_{3\text{cm}}^{5\text{cm}} C \, dx = C(3\text{cm}) = (1\text{mg/cm})(3\text{cm}) = 3\text{mg}.
\]

However, in practice, concentration changes with respect to \( x \), therefore, the area cannot be determined practically by integration but rather it must be obtained either by means of a planimeter or by direct weighing on an analytical balance. In the densitometer curve, it can be seen that concentration \( C \), is plotted against distance. However, if one differentiates the
curve, that is, plots $\frac{dC}{dx}$ against the distance, $x$, (this can be looked upon as plotting the slope of the curve against the distance $x$), the classical schlieren pattern is obtained.

Here the area is proportional to concentration. This can be shown as follows:

$$\text{Area} = \int \frac{dC}{dx} \, dx = \int \frac{dC}{dx} \, dx = \int dC = C.$$

Since $C$ usually changes with respect to distance, $x$, this expression is very difficult to integrate. Here again, evaluation of the area is therefore accomplished either by a planimeter, by direct weighing on an analytical balance, or by other practical methods.

From the foregoing considerations, one can readily see that areas from the densitometer patterns are proportional to the absolute quantity of material present, while the areas from schlieren patterns are proportional to the concentration of the material. Although the curves from a complex mixture such as blood plasma proteins may, therefore, show a certain correspondence in shape and height, no exact correspondence is to be expected because of the intrinsic, fundamental difference in the nature of the two somewhat similar patterns. This conclusion holds whether the measurements are made directly on the paper strips by means of a transmission densitometer, or are based on cutting the strip into narrow slices, elution of the material, and making use of a spectrophotometer or other device to determine
the concentration present originally in the individual slices.

It should be mentioned here that the above results are obtained if the assumptions made are correct. The first assumption made is that the paper is homogeneous. This is a reasonable assumption insofar as the variation in optical density for Eaton-Dikeman paper No. 613 is no more than 0.02. The second assumption, that the material can be evenly spread over the paper surface, is questionable; when the paper is viewed under a microscope, it appears as a mat composed of cellulose fibers rather than a plane surface. Since the material is not in solution but rather precipitated upon the cellulose fibers, there is a limit to the uniformity with which the material can be spread upon the paper surface. The third assumption that Beer's law applies to a material spread over a surface rather than a material in solution, is restricted to an optical density not much greater than 0.5 (40,41,76). This is due to two causes: (A) this is a limit to the uniformity of spreading of the material over the paper surface and (B) minute pin holes are present in the paper which do not affect the optical density reading when the material is present in low concentrations, but do affect the optical density readings when the concentration of the material becomes large. Therefore, the above theoretical development is restricted to low concentrations of the material spread over the paper surface.
Considering the light slit used in scanning the paper strip, it is obvious that only as the cross-sectional area of the light slit approaches zero, and the light intensity approaches infinity, can the material be estimated in all concentrations with no inherent error. A finite cross-sectional area of the slit causes a deviation from the theoretical development. This deviation, it is true, is small when a small cross-sectional area of the light slit is used. It is obvious that the lower limit of the cross-sectional area of the light slit that can be used is limited by the maximum intensity that can be obtained from the light source.
CHAPTER IV

DETECTION AND ESTIMATION OF LIPOPROTEIN CONCENTRATIONS IN NORMAL AND PATHOLOGICAL HUMAN SERA

Review of Literature

Several analyses of lipids and lipoproteins by the method of electromigration on wet filter-paper surfaces have been reported. The chief difficulties encountered in the study of these substances are in the detection of the bands or zones on the ionogram, in the absorption of the lipid or lipoprotein on the surface of the paper, and in the retention of the lipid stain by the paper itself. The usual technique is to run at least two strips, one of which is stained for lipids and lipoproteins while the other is stained for proteins. In this manner the positions of the lipoprotein bands can be given relative to the protein bands.

Swahn (69) used a semi-saturated solution of Sudan black B in 50 per cent ethanol for the detection of lipids and lipoproteins. Since Sudan black B is not a dye in the technical sense, it does not stain, but colors lipids black by dissolving in them.
The coefficient of distribution for Sudan black B between lipids and 50 per cent ethanol is very favorable to the former. The coloring appears to be quite specific in that at present, the list of substances known to be colorable with Sudan black B include nothing but lipids and lipid-complexes such as lipoprotein (2). Swahn stained the paper strips for 30-45 minutes and then rinsed them with 3-4 changes of 50 per cent ethanol until only a faint blue tone remained on those parts of the paper where there were no lipids. He also reported that with Munktel's No. 20 paper, there was more absorption of the Sudan black B than with Whatman's No. 1 paper. At room temperature, a good separation of the α- and β- lipoproteins was obtained using a 0.05 Molar barbital buffer solution at pH 8.8. Employing an apparatus similar to that which was used by Koiv, Wallenius and Gronwall (33), Swahn studied the distribution and relative amounts of the serum lipids from normal and from pathological patients. Fasoli (13,14) obtained differentiated bands of the α- and the β-lipoprotein fractions using an instrument similar to that employed by Flynn and de Mayo (17). He stained the lipids with a saturated solution of Sudan III in 50 per cent ethanol for 30 minutes at 40°C., then washed them thoroughly in 50 per cent ethanol and subsequently in distilled water.

The conditions for carrying out his separations were: barbital buffer, pH 8.6, ionic strength 0.05, s. and s. No. 598 paper; a potential gradient of about 3 volts/cm for a
duration of twelve hours. Rosenberg (65) in his studies of human serum has confirmed the observations of Swahn (69) and of Fasoli (13,14).

Durrum, et al. (11) separated the α- and β-lipoproteins using Whatman 3 mm filter paper employing a veronal buffer of pH 8.6 and ionic strength 0.05. The lipids and lipoproteins were stained for 16 hours in a bath comprising a saturated solution of oil red 0 in 60 per cent ethanol. The strips were then rinsed with tap water, blotted and dried. The resulting strips showed a red pattern against a pink background. The use of oil red 0 as a lipid or lipoprotein stain is not too practical because the α-lipoprotein does not show up clearly. Later, Smith, Crawford, Jetton and Durrum (67) state that studies by paper electrophoresis of top fractions of serum prepared by the Gofman technique have proven unsatisfactory due to absorption of the material by the paper. Kunkel and Slater (36) used both Whatman No. MM paper and potato starch to obtain lipoprotein patterns of serum with a barbital buffer of pH 8.6 and ionic strength of 0.10. Marbach (42) determined the electrophoretic properties of bovine serum lipoprotein using the technique of ionography. They determined the electromigration rate of bovine serum β-lipoprotein over the pH range from 2.7 to 8.6. The pH versus mobility plot they obtained is similar to that of a protein, except that the β-lipoprotein is a much slower moving substance; its isoelectric point was determined to be approximate-
ly 5.2. The conditions for the experiment were: veronal buffer, pH 2.7 to 8.6; ionic strength 0.015; potential gradient 10 volts/cm; temperature 10°C; time 2-4 hours; atmosphere, helium saturated with water vapor.

Nikkila (62) using Munktell 20 filter-paper, fractionated 0.25 ml of serum in four hours. His analyses on 10 normal and 2 atherosclerotic human sera and one hypercholesterolemic rabbit serum show that all β-globulin is richest in lipid material. A high gamma-globulin lipid value is found but it is believed to be caused by some technical difficulty. In patients with active atherogenesis, there seems to be a shift of lipids from α-globulins to slower fractions.

Experimental

All of the techniques for detecting lipoproteins mentioned in the literature were tested to determine which of the techniques was the most sensitive and practical. None of them proved to have exceptional merit. However, the stain Sudan black B showed the most promise. The optimum conditions for using this stain were then studied.

In order that comparison might be made, two spots containing 0.001 ml of blood plasma each were placed on individual pieces of Eton-Dikeman No. 613 paper. Approximately 500 pieces of paper, each containing two spots, were prepared on the same day.
The first variable studied was the dye concentration. Swahn recommended a 1/2 saturated solution of Sudan black B in 50 per cent ethanol. Four solutions were prepared. All of them contained 50 per cent ethanol, but varying amount of Sudan black B. The concentrations of the stain were 1/4, 1/2, 3/4, and saturated. Each piece of paper was stained at room temperature for 20 minutes, and was then washed twice in 50 per cent ethanol for five minutes. The saturated solution stained the plasma spots a little deeper than the other concentrations of stain.

The effect of ethanol concentration was studied next. A 65 per cent ethanol solution was found to be the most effective. At ethanol concentrations of about 80 per cent and above, the lipid materials were washed out of the paper, and at concentrations lower than 50 per cent ethanol, the stain was so sparingly soluble, the spots did not pick up appreciable amounts of the stain. Sudan black B is insoluble in water.

The effect of temperature was next studied and it was determined that at a temperature of 40°C, the plasma spots were stained the deepest.

Different solvents were studied, besides the ethanol, such as acetone, methanol, carbon tetrachloride, ether and dioxane. Of all the solvents used, none were superior to ethanol.

Different methods of washing were studied, such as, using the different solvents mentioned above, varying the con-
centrations of the solvent in water, varying the time of washing and varying the temperature of the solution used for washing. None of these variations improved the staining of the lipids. The chief difficulty with using Sudan black B was due to the fact that the cellulose fibers picked up stain as well as the lipid spot, and any condition which removed the stain from the paper also removed appreciable amount of the stain from the lipid. However, a later paper by Swahn (70), he mentions that the staining solution was improved by completely removing all suspended particles of the stain from the staining solution. It appeared as if the suspended particles were responsible to some extent for the staining of the paper.

The procedure developed, which was an improvement upon Swann's method, was as follows: one hundred ml of a 75 per cent solution of ethanol was boiled with 0.1 gm of Sudan black B for three minutes. The solution was cooled to room temperature and repeatedly filtered using a thick, hard filter paper. This solution was then kept at 40°C in a constant temperature bath. The paper strips were stained in this solution for twenty minutes, and rinsed at room temperature in 50 per cent acetone for two minutes. This procedure for rinsing was repeated until the paper background was a very light blue. This usually required 3-4 rinses. The resulting stained paper appeared as a light blue colored paper with rather dark spots where the plasma had been applied. Different reagents were used to bleach
the background, such as hydrogen peroxide, commercial bleach, and reducing agents. None had any apparent effect. However, the dark blue spots changed to dark reddish spots when they were placed in a dilute nitrous acid solution.

The conditions for separating the lipoprotein fractions in blood serum were as follows: veronal buffer at room temperature and at pH 3.6 and an ionic strength of 0.05 was used. The potential gradient was 8 volts/cm. One thousandth of a ml of blood serum was applied and was allowed to migrate for three hours. Although the proteins stained quite well using the brom phenol blue reagent (%), the lipids could not be detected, even though they stained quite readily before the fractionation took place. This could be explained by the fact that during the fractionation the lipids and lipid-like materials had spread over a larger area with the result that the lipid concentration was now too low to be detected with the stain.

The quantity of sample applied in the next experiments was doubled, that is, 0.002 ml of blood plasma was applied to each strip. Now the lipids and lipoproteins could be detected, but the coloring was very light, about 0.4 optical density above the background. The quantity of sample applied could not be further increased because sharp separation of the fractions could not then be obtained.

**Results and Conclusions**

An automatic scanning densitometer was used to
determine the protein pattern along with the lipid pattern (76). When scanning for the proteins, a wavelength of 585 millimicrons was used and 595 millimicrons when scanning for lipid materials. A typical pattern is shown in Figure 19. The solid line represents the protein pattern and the broken line, the lipid pattern. It will be noted that the plasma proteins have separated into five fractions, the albumin, $a_1$, $a_2$ $\beta$ and gamma globulins while the lipids separated into two main fractions, the large fraction, which moves the least, contains all the free lipids, and the $\beta$-lipoproteins, and the smallest fraction, which moves the same distance as the $a_1$ globulins, contain the $\alpha$-lipoproteins (69). The ordinate upon which the patterns are plotted is a linear function of concentration up to an optical density of 0.4, so that the area under the curves which do not go above 0.4 O. D. are proportional to the amount of material present providing the stain uptake is proportional to the material present (27,76). In the case of Sudan black B, Swahn has shown this to be so (76). Therefore, the area under the curve, determined with a planimeter, is proportional to the amount of lipid and lipoprotein present in the serum sample. The total area under the lipid curve divided by the area under the $\alpha$-lipoprotein peak is equal to the ratio of total lipid materials to the $\alpha$-lipoproteins which will be defined as the $\alpha$-L ratio. It appears that the $\alpha$-L ratio may possibly be related in some way to the severity of certain forms of heart
FIGURE 19

DENSIOMETRIC PATTERN OF AN IONOGRAM
### TABLE III

THE RATIO OF TOTAL PLASMA LIPIDS TO ALPHA LIPOPROTEINS IN DIFFERENT CASES

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>(\text{L} ) Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>Rheumatic fever with cardiac involvement</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>Rheumatic fever with polyarthritis and mild carditis</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>6</td>
<td>Rheumatic fever with rheumatic heart disease</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.8</td>
</tr>
<tr>
<td>7</td>
<td>Rheumatic fever with rheumatic heart disease</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>8</td>
<td>Rheumatic fever with Mitral Valvuritis and Myocarditis</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
</tr>
</tbody>
</table>
disease, as is shown in Table III. In this Table, the "normals" are listed first, and then the cases with increasing severity of heart disease are then listed. Apparently, the more severe the disease, the higher is the alpha-L ratio. It has been shown by several investigators (79, 80), that attacks of rheumatic fever occur shortly after infection by group A beta-hemolytic streptococci. Of two hemolysins produced by the organism, streptolysin S is apparently responsible for rheumatic illness. When the serum inhibitor of streptolysin S is very weak, the serum phospholipids are low. Therefore, since it is believed that phospholipids are necessary for the conversion of lipids and beta-lipoprotein to alpha-lipoprotein, (1, 79, 80), a serum low in inhibitor of streptolysin S would also be low in alpha-lipoprotein. This would seem to substantiate the results shown in Table III because a decrease in alpha-lipoprotein would cause an increase in the alpha-L ratio.

A possible explanation for the conversion of the free lipids into lipoproteins may be as follows: The most unstable form in which the lipid can exist in the blood plasma is as free lipid in the form of chylomicrons, which have a very large particle weight. However, the body can convert these chylomicrons to either the alpha- or beta-lipoproteins. Of the two lipoproteins, the beta-lipoprotein would be the least stable because it contains 75% lipid and has an average particle weight of 1.3 million while the alpha-lipoprotein contains only 35%
lipid and has an average particle weight of 200,000 (28%). It would then seem that a person with a heart disease such as rheumatic fever, may have some difficulty in converting either the chylomicrons or the beta-lipoprotein into the more stable alpha-lipoprotein.

It must be remembered that the data presented is not complete enough to draw any definite conclusions, but a tendency appears to be present. A much larger number of "normals" and properly diagnosed diseased cases would, of necessity, have to be analyzed in order to evaluate the real significance of the alpha-L ratio.
CHAPTER V

SUMMARY

It was shown that the mobility of the migrant was obtained in a stabilized electrolyte providing certain criteria were fulfilled. These criteria were: (a) constant voltage and constant current during the course of the experiment, (b) linear displacement of three bands of migrant per strip, (c) linearity of movement of the migrant with time at constant potential gradient, (d) linearity of movement of the migrant with respect to potential gradient, with time constant.

The applicability of the four basic types of apparatus to the determination of electromigration mobilities was discussed, and it was shown that the horizontal suspension of the paper strips in a gas space was the most feasible for the mobility determinations.

A Barrier Theory was proposed to explain the interaction of the migrant with the paper. The use of decrease in conductivity as a means of measuring the effect of paper upon the migrant was discussed, and shown to be feasible.

The effect of different types of paper upon the thermodynamic activity of the migrant were studied, and it was shown that the different papers did not alter the thermodynamic activity of the migrant to any large extent.
The effect of different migrants upon the same type of paper was studied. No simple predictable relationship existed between the migrant's size and its interaction with the cellulose fiber, but it was concluded that perhaps some factor such as the surface charge density of the migrant might play a role.

The interaction of paper and protein at different pH's was investigated and it was shown that the least interaction occurs near the isoelectric point of the protein.

A formula for converting mobilities determined in stabilized solution to those in non-stabilized solution was proposed. This formula showed that the mobility observed in a in a stabilized medium was a function solely of the stabilizer's concentration expressed as the ratio of stabilizer to solution. It was also shown that this formula took into account, the decrease in thermodynamic activity of the migrant due to the interaction of the migrant with the stabilizer. The equation also took into account the effects of electroosmosis. The application of the formula to convert the mobilities of proteins as determined in a paper stabilized buffer, to the mobilities of the same materials when determined in free solution showed agreement within experimental error.

A theoretical development of the fundamental theory underlying the determination of materials spread over a surface area was proposed and its limitation indicated.
A modified procedure for staining lipids and lipoproteins using Sudan black B was developed and the conditions necessary for the separation of alpha-lipoproteins from the rest of the lipids in human serum was developed. It was also shown that the ratio of total lipid and lipid-like materials to a lipoprotein could be obtained in a straightforward manner. This ratio was defined as the alpha-L ratio and it appeared that its magnitude might be indicative of the severity of certain forms of heart disease.
AN ATTEMPT TO DIRECTLY MEASURE THE EFFECT OF PAPER WETNESS ON
THE MOBILITY OF BROM PHENOL BLUE IN A-VARIOUS BUFFER AT A PH OF
8.6 AND AN IONIC STRENGTH OF 0.025 USING EATON-DIKEMAN 613 PAPER

<table>
<thead>
<tr>
<th>Ratio of gm of Buffer to gm. of paper</th>
<th>Mobility $\times 10^5$ cm²/volt-sec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.94</td>
<td>15.4</td>
</tr>
<tr>
<td>0.94</td>
<td>16.7</td>
</tr>
<tr>
<td>1.00</td>
<td>14.8</td>
</tr>
<tr>
<td>2.00</td>
<td>24.8</td>
</tr>
<tr>
<td>2.00</td>
<td>23.4</td>
</tr>
<tr>
<td>2.06</td>
<td>15.0</td>
</tr>
<tr>
<td>2.74</td>
<td>14.8</td>
</tr>
<tr>
<td>3.80</td>
<td>17.0</td>
</tr>
<tr>
<td>3.80</td>
<td>17.0</td>
</tr>
<tr>
<td>4.00</td>
<td>14.8</td>
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<tr>
<td>6.04</td>
<td>13.6</td>
</tr>
<tr>
<td>6.05</td>
<td>13.9</td>
</tr>
<tr>
<td>6.85</td>
<td>13.8</td>
</tr>
<tr>
<td>6.85</td>
<td>14.8</td>
</tr>
</tbody>
</table>
TABLE NO. V

EXPERIMENT NO. 1E

THE CALCULATION OF CONDUCTIVITY CELL NO. 2 TO DETERMINE ITS CHARACTERISTICS

AS AN IDEAL CELL USING 0.020M KC1

<table>
<thead>
<tr>
<th>TOTAL WT. OF CELL</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RESISTANCE IN OHMS</th>
<th>CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF RECIP. OF FRACTION OF SOL'N. PRESENT AND COND. x 10^-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.4946</td>
<td>0.8916</td>
<td>0.2115</td>
<td>4355</td>
<td>2.296</td>
<td>10.86</td>
</tr>
<tr>
<td>25.8854</td>
<td>1.2754</td>
<td>0.3025</td>
<td>3020</td>
<td>3.311</td>
<td>10.95</td>
</tr>
<tr>
<td>24.2635</td>
<td>1.6605</td>
<td>0.3930</td>
<td>2283</td>
<td>4.380</td>
<td>11.15</td>
</tr>
<tr>
<td>24.8677</td>
<td>2.2647</td>
<td>0.5371</td>
<td>1667</td>
<td>5.998</td>
<td>11.17</td>
</tr>
<tr>
<td>25.3850</td>
<td>2.7820</td>
<td>0.6598</td>
<td>1357</td>
<td>7.369</td>
<td>11.17</td>
</tr>
<tr>
<td>26.7750</td>
<td>4.1720</td>
<td>0.9890</td>
<td>910</td>
<td>10.990</td>
<td>11.11</td>
</tr>
<tr>
<td>26.8190</td>
<td>4.2160</td>
<td>1.0000</td>
<td>893</td>
<td>11.200</td>
<td>11.20</td>
</tr>
</tbody>
</table>
### TABLE NO. VI

**EXPERIMENT NO. 1**

**DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 0.1% GLYCINE AT A pH OF 10.91 USING VARYING AMOUNTS OF EATON-DIKEMAN PAPER NO. 613**

<table>
<thead>
<tr>
<th>WT. OF SOL.'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL.'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL.'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOLUTION PRES.</th>
<th>RELATIVE RATIO OF PAPER TO SOL.'N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1819</td>
<td>0.0000</td>
<td>4.1819</td>
<td>1.000</td>
<td>17.14</td>
<td>17.14</td>
<td>1.000</td>
</tr>
<tr>
<td>4.2480</td>
<td>0.2512</td>
<td>3.9968</td>
<td>1.046</td>
<td>15.95</td>
<td>16.69</td>
<td>0.974</td>
</tr>
<tr>
<td>4.2995</td>
<td>0.4795</td>
<td>3.8200</td>
<td>1.095</td>
<td>14.99</td>
<td>16.41</td>
<td>0.957</td>
</tr>
<tr>
<td>4.3610</td>
<td>0.6992</td>
<td>3.6618</td>
<td>1.142</td>
<td>14.21</td>
<td>16.22</td>
<td>0.946</td>
</tr>
<tr>
<td>4.4089</td>
<td>0.9111</td>
<td>3.4978</td>
<td>1.196</td>
<td>13.27</td>
<td>15.87</td>
<td>0.926</td>
</tr>
<tr>
<td>4.2000</td>
<td>0.0766</td>
<td>5.1234</td>
<td>1.338</td>
<td>11.42</td>
<td>15.28</td>
<td>0.882</td>
</tr>
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</table>
TABLE NO. VII

EXPERIMENT NO. 2A

DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 4% BOVINE SERUM ALBUMIN AT A pH OF 5.2 USING VARYING AMOUNTS OF EATON-DIKEMAN PAPER NO. 613

<table>
<thead>
<tr>
<th>WT. OF SOL’N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL’N.</th>
<th>RECIPROCAL OF FRACTION OF SOL’N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY X 10^{-4}</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL’N. PRESENT</th>
<th>RELATIVE THERMO-DYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL’N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2400</td>
<td>0.0000</td>
<td>4.2400</td>
<td>1.000</td>
<td>2.73</td>
<td>2.73</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>4.3138</td>
<td>0.2674</td>
<td>4.0464</td>
<td>1.048</td>
<td>2.48</td>
<td>2.60</td>
<td>0.952</td>
<td>0.066</td>
</tr>
<tr>
<td>4.3534</td>
<td>0.4314</td>
<td>3.9220</td>
<td>1.081</td>
<td>2.37</td>
<td>2.56</td>
<td>0.938</td>
<td>0.110</td>
</tr>
<tr>
<td>4.4290</td>
<td>0.7425</td>
<td>3.6865</td>
<td>1.150</td>
<td>2.17</td>
<td>2.50</td>
<td>0.916</td>
<td>0.201</td>
</tr>
<tr>
<td>4.3970</td>
<td>0.9219</td>
<td>3.4751</td>
<td>1.220</td>
<td>2.00</td>
<td>2.44</td>
<td>0.894</td>
<td>0.265</td>
</tr>
<tr>
<td>4.4010</td>
<td>1.1026</td>
<td>3.2984</td>
<td>1.285</td>
<td>1.96</td>
<td>2.52</td>
<td>0.923</td>
<td>0.334</td>
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</table>
### TABLE NO. VIII

**EXPERIMENT NO. 3A**

DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 4% BOVINE SERUM ALBUMIN AT A pH OF 3.5 USING VARYING AMOUNTS OF EATON-DIXEYAN PAPER NO. 613

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY $\times 10^{-4}$</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMO-DYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2463</td>
<td>0.0000</td>
<td>4.2463</td>
<td>1.000</td>
<td>20.75</td>
<td>20.75</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>4.3002</td>
<td>0.1952</td>
<td>4.1050</td>
<td>1.034</td>
<td>19.28</td>
<td>19.94</td>
<td>0.961</td>
<td>0.048</td>
</tr>
<tr>
<td>4.5800</td>
<td>0.4407</td>
<td>3.9193</td>
<td>1.083</td>
<td>17.86</td>
<td>19.34</td>
<td>0.939</td>
<td>0.112</td>
</tr>
<tr>
<td>4.4278</td>
<td>0.6885</td>
<td>3.7393</td>
<td>1.136</td>
<td>16.05</td>
<td>18.23</td>
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<td>0.184</td>
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<td>3.5699</td>
<td>1.189</td>
<td>14.65</td>
<td>17.42</td>
<td>0.840</td>
<td>0.253</td>
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<td>4.5070</td>
<td>1.0675</td>
<td>3.4395</td>
<td>1.234</td>
<td>13.86</td>
<td>17.10</td>
<td>0.824</td>
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TABLE NO. IX

EXPERIMENT NO. 4A

DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 10% GAMMA GLOBULIN

AT A pH OF 9.0 USING VARYING AMOUNTS OF EATON-BIKEMAN PAPER NO. 613

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY ( \times 10^{-4} )</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMODYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
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<tbody>
<tr>
<td>4.2945</td>
<td>0.0000</td>
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<td>15.39</td>
<td>1.000</td>
<td>0.000</td>
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<tr>
<td>4.3348</td>
<td>0.1794</td>
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<td>1.033</td>
<td>14.74</td>
<td>15.21</td>
<td>0.988</td>
<td>0.043</td>
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<td>4.4158</td>
<td>0.4825</td>
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<td>12.84</td>
<td>14.02</td>
<td>0.911</td>
<td>0.123</td>
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<td>4.4629</td>
<td>0.7949</td>
<td>3.7480</td>
<td>1.146</td>
<td>11.77</td>
<td>13.49</td>
<td>0.876</td>
<td>0.191</td>
</tr>
<tr>
<td>4.4947</td>
<td>0.8449</td>
<td>3.6498</td>
<td>1.177</td>
<td>11.21</td>
<td>13.19</td>
<td>0.857</td>
<td>0.232</td>
</tr>
<tr>
<td>4.5289</td>
<td>0.9923</td>
<td>3.5366</td>
<td>1.214</td>
<td>10.69</td>
<td>12.98</td>
<td>0.843</td>
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</tbody>
</table>
**TABLE NO. X**

**EXPERIMENT NO. 6**

**DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 0.0010M KCl**

**USING VARYING AMOUNTS OF EATON-DIXEMAN PAPER NO. 613 UNWASHED**

<table>
<thead>
<tr>
<th>Wt. of Sol'n. and Paper</th>
<th>Wt. of Paper</th>
<th>Wt. of Solution</th>
<th>Reciprocal of Fraction of Sol'n. Present</th>
<th>Specific Conductivity $\times 10^{-4}$</th>
<th>Product of Specific Conductivity and Reciprocal of Fraction of Sol'n. Present</th>
<th>Relative Thermo-Dynamic Activity</th>
<th>Ratio of Paper to Sol'n.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2560</td>
<td>0.0000</td>
<td>4.2560</td>
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<td>1.46</td>
<td>1.46</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>4.3482</td>
<td>0.1303</td>
<td>4.2179</td>
<td>1.009</td>
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<td>1.59</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td>4.4250</td>
<td>0.5547</td>
<td>4.8703</td>
<td>1.100</td>
<td>2.09</td>
<td>2.29</td>
<td>0.143</td>
<td>0.143</td>
</tr>
<tr>
<td>4.4195</td>
<td>1.1185</td>
<td>3.0310</td>
<td>1.290</td>
<td>2.38</td>
<td>3.07</td>
<td>0.339</td>
<td>0.339</td>
</tr>
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</table>
TABLE NO. XI

EXPERIMENT NO. 6B

DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 0.001OM KCl USING VARYING AMOUNTS OF EATON-DIXEMAN PAPER NO. 613 LEACHED FOR ONE HALF HOUR IN 30ml DISTILLED WATER THREE TIMES

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE RATIO OF THERMO-DYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2310</td>
<td>0.0000</td>
<td>4.2310</td>
<td>1.000</td>
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<td>4.2794</td>
<td>0.1749</td>
<td>4.1045</td>
<td>1.031</td>
<td>1.44</td>
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<td>0.043</td>
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<tr>
<td>4.3456</td>
<td>0.3862</td>
<td>3.9604</td>
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<td>1.40</td>
<td>1.49</td>
<td>0.097</td>
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<tr>
<td>4.4607</td>
<td>0.7761</td>
<td>3.6746</td>
<td>1.151</td>
<td>1.35</td>
<td>1.53</td>
<td>0.211</td>
<td>4.4607</td>
</tr>
<tr>
<td>4.5467</td>
<td>1.0460</td>
<td>3.4967</td>
<td>1.210</td>
<td>1.32</td>
<td>1.60</td>
<td>0.300</td>
<td>4.5467</td>
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</table>
TABLE NO. XII
EXPERIMENT NO. 8

DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 10% BOVINE SERUM ALBUMIN AT A pH OF 8.2 USING VARYING AMOUNTS OF EATON-DIKEMAN PAPER NO. 613

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPIROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMO-DYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
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</thead>
<tbody>
<tr>
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<td>23.6</td>
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<td>4.4190</td>
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<td>4.1306</td>
<td>1.055</td>
<td>22.0</td>
<td>22.1</td>
<td>0.936</td>
<td>0.070</td>
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<tr>
<td>4.4855</td>
<td>0.5767</td>
<td>3.9068</td>
<td>1.112</td>
<td>19.7</td>
<td>20.9</td>
<td>0.886</td>
<td>0.147</td>
</tr>
<tr>
<td>4.5280</td>
<td>0.7867</td>
<td>3.7413</td>
<td>1.162</td>
<td>18.0</td>
<td>19.9</td>
<td>0.843</td>
<td>0.210</td>
</tr>
<tr>
<td>4.5080</td>
<td>1.0263</td>
<td>3.4817</td>
<td>1.250</td>
<td>15.4</td>
<td>16.5</td>
<td>0.776</td>
<td>0.295</td>
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<tr>
<td>4.3980</td>
<td>1.1903</td>
<td>3.2077</td>
<td>1.355</td>
<td>13.4</td>
<td>17.2</td>
<td>0.732</td>
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TABLE NO. XIII

EXPERIMENT No. 8B

DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF NaOH

AT A pH OF 11.8 USING VARYING AMOUNTS OF RATON-DIXMAN PAPER NO. 613

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMO-DYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2816</td>
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<td>4.0075</td>
<td>1.070</td>
<td>26.1</td>
<td>28.0</td>
<td>0.940</td>
<td>0.091</td>
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<tr>
<td>4.3205</td>
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<td>21.2</td>
<td>23.7</td>
<td>0.796</td>
<td>0.213</td>
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<tr>
<td>4.4535</td>
<td>0.9515</td>
<td>3.5020</td>
<td>1.222</td>
<td>19.8</td>
<td>24.2</td>
<td>0.813</td>
<td>0.272</td>
</tr>
</tbody>
</table>
### EXPERIMENT NO. 2F

**DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 2% BOVINE SERUM ALBUMIN AT A pH OF 8.6 USING VARYING AMOUNTS OF EATON-DIKEMAN PAPER NO. 613**

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMODYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
</thead>
<tbody>
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<td>6.45</td>
<td>1.000</td>
<td>0.000</td>
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<td>6.28</td>
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<td>0.038</td>
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<td>4.3390</td>
<td>0.4496</td>
<td>4.8894</td>
<td>1.088</td>
<td>5.27</td>
<td>5.74</td>
<td>0.890</td>
<td>0.115</td>
</tr>
<tr>
<td>4.3990</td>
<td>0.7078</td>
<td>3.6912</td>
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<td>4.64</td>
<td>5.32</td>
<td>0.826</td>
<td>0.192</td>
</tr>
<tr>
<td>4.4355</td>
<td>0.8944</td>
<td>3.5411</td>
<td>1.195</td>
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<td>5.09</td>
<td>0.790</td>
<td>0.253</td>
</tr>
<tr>
<td>4.4680</td>
<td>1.0177</td>
<td>3.4583</td>
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<td>0.761</td>
<td>0.296</td>
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<td>3.3659</td>
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<td>4.85</td>
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</table>
### TABLE NO. XV

#### EXPERIMENT NO. 3F

**DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 2% BOVINE SERUM ALBUMIN AT A pH OF 8.6 USING VARYING AMOUNTS OF C.T. MUNKTELLS PAPER**

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY $\times 10^{-4}$</th>
<th>SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMO-DYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
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</thead>
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<tr>
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<td>6.46</td>
<td>1.000</td>
<td>0.000</td>
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<td>0.1110</td>
<td>4.1414</td>
<td>1.020</td>
<td>6.25</td>
<td>6.36</td>
<td>0.978</td>
<td>0.027</td>
</tr>
<tr>
<td>4.3330</td>
<td>0.4855</td>
<td>3.8475</td>
<td>1.098</td>
<td>5.22</td>
<td>5.74</td>
<td>0.983</td>
<td>0.126</td>
</tr>
<tr>
<td>4.3939</td>
<td>0.7620</td>
<td>3.6319</td>
<td>1.163</td>
<td>4.46</td>
<td>5.19</td>
<td>0.797</td>
<td>0.210</td>
</tr>
<tr>
<td>4.4440</td>
<td>0.9658</td>
<td>3.4782</td>
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<td>0.757</td>
<td>0.277</td>
</tr>
<tr>
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<td>1.2073</td>
<td>3.3077</td>
<td>1.277</td>
<td>3.68</td>
<td>4.71</td>
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<td>0.355</td>
</tr>
<tr>
<td>4.5069</td>
<td>1.3533</td>
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<td>1.431</td>
<td>3.34</td>
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<td>0.735</td>
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</table>
### TABLE NO. XVI

**EXPERIMENT NO. 4F**

**DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 2% BOVINE SERUM ALBUMIN AT A pH OF 8.6 USING VARYING AMOUNTS OF EATON-DIXEMAN PAPER NO. 613**

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMODYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2234</td>
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<td>4.3340</td>
<td>1.000</td>
<td>6.60</td>
<td>6.60</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>4.2475</td>
<td>0.1560</td>
<td>4.0915</td>
<td>1.035</td>
<td>6.17</td>
<td>6.38</td>
<td>0.967</td>
<td>0.038</td>
</tr>
<tr>
<td>4.3210</td>
<td>0.3370</td>
<td>3.7840</td>
<td>1.119</td>
<td>5.20</td>
<td>5.92</td>
<td>0.881</td>
<td>0.142</td>
</tr>
<tr>
<td>4.3598</td>
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<td>1.179</td>
<td>4.70</td>
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<td>4.3640</td>
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<tr>
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<td>2.9580</td>
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<td>3.31</td>
<td>4.73</td>
<td>0.717</td>
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<td>4.3410</td>
<td>1.5394</td>
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</table>
TABLE XVII

EXPERIMENT NO. 5F

DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 4% BOVINE SERUM ALBUMIN AT A pH OF 7.0 USING VARYING AMOUNTS OF EATON-DIKEMAN PAPER NO. 613

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^{-4}</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMODYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>7.77</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>4.2850</td>
<td>0.1375</td>
<td>4.1475</td>
<td>1.023</td>
<td>7.38</td>
<td>7.55</td>
<td>0.972</td>
<td>0.033</td>
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<td>0.2755</td>
<td>4.0361</td>
<td>1.061</td>
<td>6.97</td>
<td>7.33</td>
<td>0.943</td>
<td>0.068</td>
</tr>
<tr>
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<td>0.4331</td>
<td>3.9215</td>
<td>1.083</td>
<td>6.50</td>
<td>7.03</td>
<td>0.906</td>
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<td>4.4210</td>
<td>0.7088</td>
<td>3.7122</td>
<td>1.143</td>
<td>5.80</td>
<td>6.63</td>
<td>0.854</td>
<td>0.191</td>
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<tr>
<td>4.4160</td>
<td>0.9655</td>
<td>3.4505</td>
<td>1.230</td>
<td>4.96</td>
<td>6.10</td>
<td>0.785</td>
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TABLE NO. XVIII
EXPERIMENT NO. 6F
DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 4% BOVINE SERUM ALBUMIN AT A pH OF 7.0 USING VARYING AMOUNTS OF RATON-DIKEMAN PAPER NO. 248

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
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<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMODYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
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<tr>
<td>4.2477</td>
<td>0.0000</td>
<td>4.2477</td>
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<td>7.41</td>
<td>7.41</td>
<td>1.000</td>
<td>0.000</td>
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<tr>
<td>4.2730</td>
<td>0.1553</td>
<td>4.1177</td>
<td>1.032</td>
<td>7.07</td>
<td>7.30</td>
<td>0.972</td>
<td>0.038</td>
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<tr>
<td>4.3120</td>
<td>0.3753</td>
<td>3.9367</td>
<td>1.079</td>
<td>6.56</td>
<td>7.08</td>
<td>0.943</td>
<td>0.095</td>
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<td>4.3406</td>
<td>0.5557</td>
<td>3.7849</td>
<td>1.122</td>
<td>6.13</td>
<td>6.88</td>
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<td>0.147</td>
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<td>0.7792</td>
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<td>4.3932</td>
<td>1.0261</td>
<td>3.3671</td>
<td>1.262</td>
<td>4.96</td>
<td>6.26</td>
<td>0.834</td>
<td>0.305</td>
</tr>
<tr>
<td>4.3210</td>
<td>1.5637</td>
<td>2.9573</td>
<td>1.436</td>
<td>3.92</td>
<td>5.64</td>
<td>0.751</td>
<td>0.461</td>
</tr>
</tbody>
</table>
### Table No. XIX

**Experiment No. 7F**

**Determination of the Conductivity of an Aqueous Solution of 4% Bovine Serum Albumin at a pH of 7.0 Using Varying Amounts of C.T. Munkteells Paper**

<table>
<thead>
<tr>
<th></th>
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<td>4.2490</td>
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<td>7.62</td>
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<td>0.000</td>
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<tr>
<td>4.2870</td>
<td>0.1399</td>
<td>4.1471</td>
<td>1.025</td>
<td>7.32</td>
<td>7.50</td>
<td>0.983</td>
<td>0.034</td>
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<tr>
<td>4.3273</td>
<td>0.2888</td>
<td>4.0385</td>
<td>1.052</td>
<td>6.96</td>
<td>7.33</td>
<td>0.961</td>
<td>0.072</td>
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<tr>
<td>4.3700</td>
<td>0.4613</td>
<td>3.9087</td>
<td>1.087</td>
<td>6.67</td>
<td>7.24</td>
<td>0.981</td>
<td>0.118</td>
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<tr>
<td>4.4370</td>
<td>0.6767</td>
<td>3.7603</td>
<td>1.130</td>
<td>6.05</td>
<td>6.84</td>
<td>0.897</td>
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</tr>
<tr>
<td>4.4600</td>
<td>0.9231</td>
<td>3.5369</td>
<td>1.201</td>
<td>5.40</td>
<td>6.49</td>
<td>0.853</td>
<td>0.261</td>
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<tr>
<td>4.5480</td>
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<td>3.0962</td>
<td>1.372</td>
<td>4.12</td>
<td>5.65</td>
<td>0.742</td>
<td>0.404</td>
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</table>
# TABLE NO. XX

## EXPERIMENT NO. 8F

**DETERMINATION OF THE CONDUCTIVITY OF AN AQUEOUS SOLUTION OF 10% BOVINE SERUM ALBUMIN AT A pH OF 5.2 USING VARYING AMOUNTS OF EATON-DIKEMAN PAPER NO. 613**

<table>
<thead>
<tr>
<th>WT. OF SOL'N. AND PAPER</th>
<th>WT. OF PAPER</th>
<th>WT. OF SOL'N.</th>
<th>RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>SPECIFIC CONDUCTIVITY x 10^-4</th>
<th>PRODUCT OF SPEC. CONDUCTIVITY AND RECIPROCAL OF FRACTION OF SOL'N. PRESENT</th>
<th>RELATIVE THERMO-DYNAMIC ACTIVITY</th>
<th>RATIO OF PAPER TO SOL'N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3120</td>
<td>0.0000</td>
<td>4.3120</td>
<td>1.000</td>
<td>6.15</td>
<td>6.15</td>
<td>1.000</td>
<td>0.000</td>
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<tr>
<td>4.5550</td>
<td>0.1728</td>
<td>4.1822</td>
<td>1.031</td>
<td>5.83</td>
<td>6.00</td>
<td>0.977</td>
<td>0.041</td>
</tr>
<tr>
<td>4.4770</td>
<td>0.3627</td>
<td>4.0443</td>
<td>1.066</td>
<td>5.50</td>
<td>5.86</td>
<td>0.954</td>
<td>0.090</td>
</tr>
<tr>
<td>4.4585</td>
<td>0.5876</td>
<td>3.8689</td>
<td>1.114</td>
<td>5.10</td>
<td>5.68</td>
<td>0.924</td>
<td>0.152</td>
</tr>
<tr>
<td>4.5085</td>
<td>0.9469</td>
<td>3.5616</td>
<td>1.211</td>
<td>4.69</td>
<td>5.56</td>
<td>0.905</td>
<td>0.262</td>
</tr>
<tr>
<td>4.5074</td>
<td>1.1719</td>
<td>3.3355</td>
<td>1.293</td>
<td>4.10</td>
<td>5.29</td>
<td>0.861</td>
<td>0.351</td>
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</tbody>
</table>
TABLE XXI

THE EFFECT OF pH ON THE INTERACTION OF A 4% BOVINE SERUM ALBUMIN SOLUTION AND BAYON-DIKMAN NO. 613 PAPER

<table>
<thead>
<tr>
<th>pH</th>
<th>Slope</th>
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<tr>
<td>3.5</td>
<td>-0.665</td>
</tr>
<tr>
<td>5.2</td>
<td>-0.450</td>
</tr>
<tr>
<td>7.0</td>
<td>-0.750</td>
</tr>
<tr>
<td>8.6</td>
<td>-0.860</td>
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