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ELECTROPHORETIC SEPARATIONS IN A THERMOELECTROGRAVITATIONAL COLUMN

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ELECTROPHORETIC SEPARATIONS IN A

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ABSTRACT

Electrophoresis, a method of separation of charged species in solution by applying an electrical field has been extensively used on the analytical level since the introduction of Tiselius apparatus. Only recently has there been an increase in interest shown for preparatory electrophoresis. A review of the literature shows that most of the workers in this field have developed convection-free apparatus, with the exception of Kirkwood et al. who utilized the principle of a Clusius-Dickel Column to develop a batch electrophoresis convection cell with reservoirs for the separation of proteins. In the present work, a new electrophoresis cell termed the "Thermoelectrogravitational Electrophoresis Cell without Reservoirs" was constructed and operated using both electrical and thermal fields.

A mathematical theory was also developed using the Furry, Jones and Onsager procedure in thermal diffusion to explain the separations obtained in the column by the application of electrical and thermal fields. This yielded a transport equation which could be used to describe both transient batch and continuous-flow operations of the column. The effect of the heat generation on the velocity and temperature profiles has been accounted for. Only systems

containing one single mobile component in dilute solution have been considered in the development of the theory.

Experimental data were obtained for the transport of bovine albumin in water solution using boric acid - borax buffer at pH 8.6 and phosphate buffer at pH 6.0. The column was 145 cm. high, 10.2 cm. wide and used two membrane spacings of 0.3018 cm. and 0.1354 cm. Experimental flow rates ranged from 0 to 10 gm. per minute of 1 gm./100 ml. albumin solution. Separation factors (defined as the ratio of bottom to top concentration) ranging from 11 to 1.05 were obtained at four different field strengths ranging from 0.0423 to 0.423 volt per centimeter and for three different temperature differences of 0°C, 8.5°C and 16°C respectively. The data showed that meaningful electrophoretic separations could be obtained using thermoelectrogravitational columns.

Qualitative experimental confirmation of the theory was found for both steady state batch and continuous-flow as well as transient batch operations of the electrophoresis column. The experimental data obtained for steady state batch and continuous-flow as well as transient batch operations of the electrophoresis confirmed the quantitative relationship developed in the theory for the parameters: flow rate, field strength, membrane spacing and pH of the solution. In case of the parameter of temperature difference, however, no qualitative agreement was found between theory and experiment.

Quantitative agreement between theory and experiment

was not satisfactory. This might be because of the comparatively large membrane spacings used. It is interesting to note that FJO has been shown to be not quantitative for large spacings in thermal diffusion.

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CHAPTER I

INTRODUCTION

In the field of physical as well as biological sciences, the problem of separation of a mixture into its individual components is very often most important to the understanding of the process under investigation. As the research in fine structure and function of biochemical, biological, and physiological substances becomes sophisticated and more involved, the investigator in the field demands more purity and larger concentration of the system under his study. Moreover, when a study is to be made of molecular and cellular nature of species, the researcher would want these species in the form in which they exist in nature. In the biological field especially, both the separation and the speed of separation are important. Some problems encountered in such separations are discussed by Mel (43).

Several separation processes such as evaporation, distillation, extraction, ion exchange, crystallization, and sedimentation have been advantageously utilized both in

laboratory and in industry. These conventional physical processes are, however, not applicable in many situations in which separation of substances of similar nature is to be achieved. Examples are the separations of organic dye mixtures, biologicals and colloids. Use of other separation techniques less commonly employed for preparatory purposes, therefore, has assumed a great significance.

When ordinary physical processes such as distillation and extraction are not feasible, advantage is taken of the fact that certain force fields cause migration at different velocities of solutes or suspends in liquid solutions and of individual molecules in gaseous mixtures. This fact can be advantageously used to effect separation of the components from their admixture. Such force fields which can be used either alone or in combination are known to be:

(1) Pressure diffusion force

(2) Thermal diffusion force

(3) Gravitational and centrifugal force

(4) Magnetic force

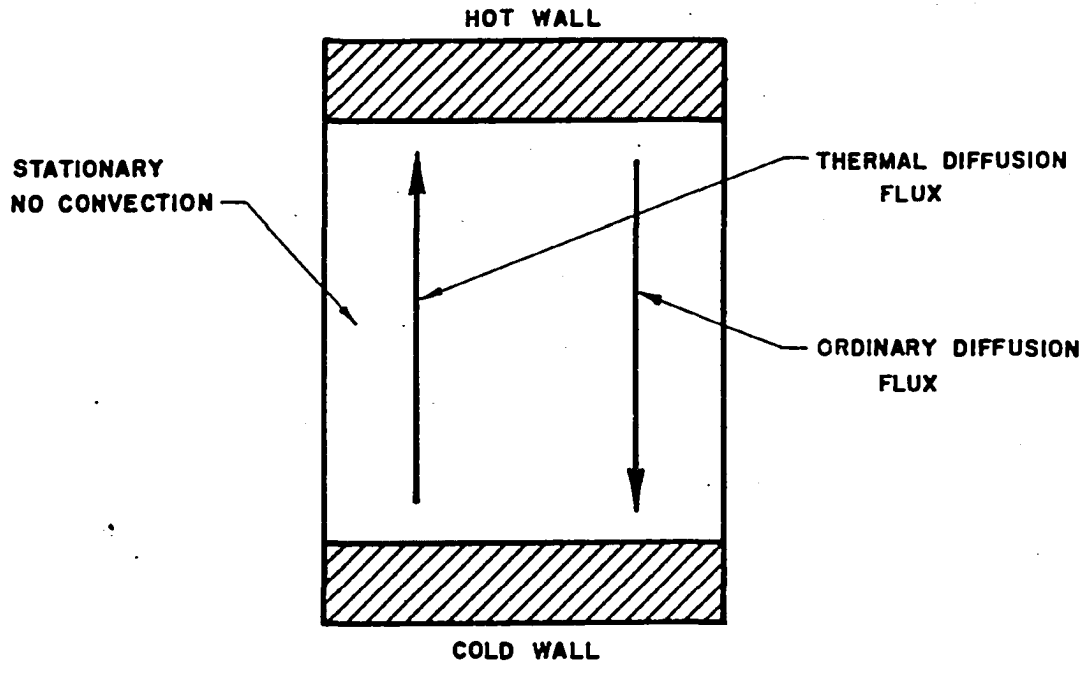
(5) Electric field force

Of these, the pressure diffusion (49), gravitational and magnetic force fields are very weak in effecting separations and have very limited application. Large centrifugal force fields can be produced (49) by special equipment, yet have scant application because of the difficulties involved in continuous operation. Most interest, therefore, centers in the use of either thermal or electrical force fields.

The thermal diffusion separation technique is very well illustrated by a static thermal diffusion cell. In this static method, a temperature gradient is applied in such a manner that no convection currents arise and, in addition, there is no bulk flow. In general, a device for the static method consists of a simple container (Figure 1-1) or cell filled with a mixture to be separated. The bottom of the cell is maintained at some temperature lower than that at the top of the cell. After a period of time, a concentration difference results between the top and bottom of the cell, since the lighter molecules move toward the hot side of the cell. However, the degree of separation obtained in such a cell is usually small and the time required to obtain a steady state can be very long.

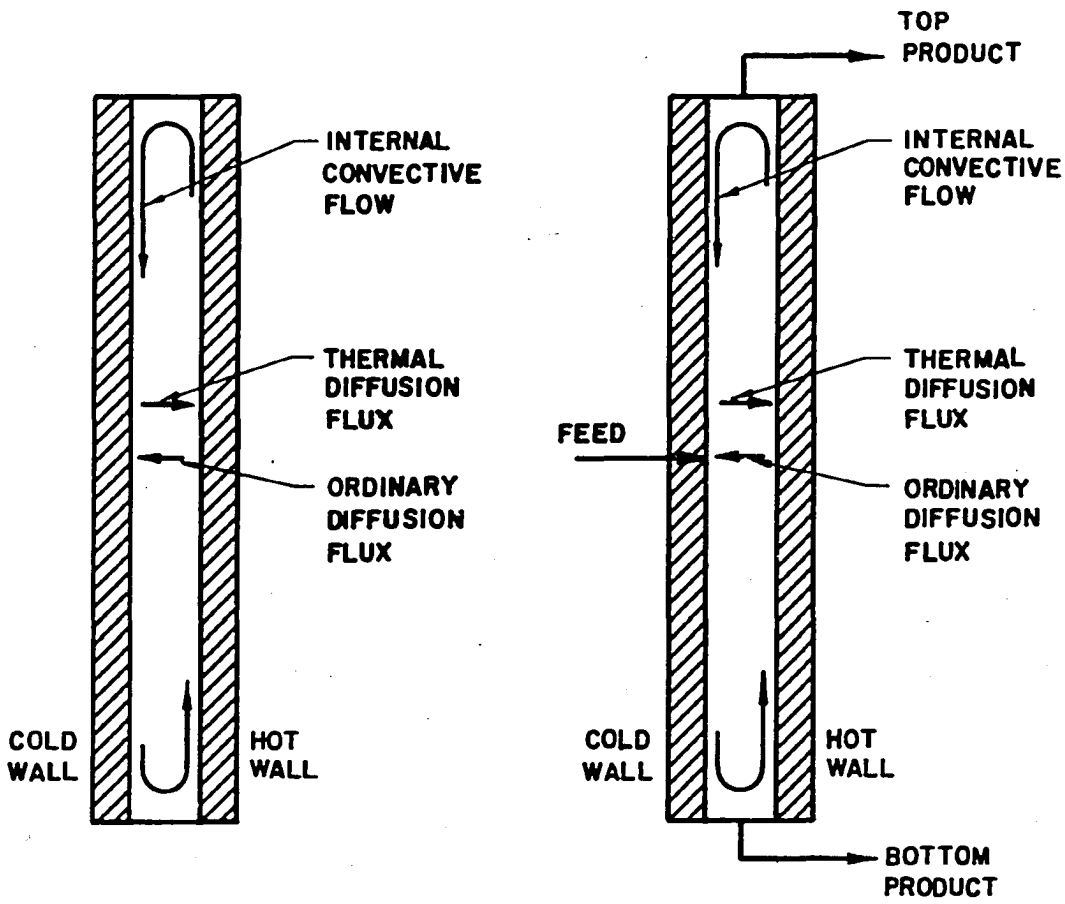
An improvement in the operation of the thermal diffusion method was introduced by Clusius and Dickel in 1938, (9). An apparatus utilizing their method multiplies the separation by means of convection currents in a manner similar to the way a counter-current extraction column produces concentration differences many times greater than the difference for a single stage. This principle is termed the principle of thermogravitational reflux. The apparatus is commonly called a thermogravitational column or Clusius-Dickel column (Figure 1-2). Either batch or continuous operation is easily obtained with such a column.

Since the introduction of the Clusius-Dickel column, considerable work, both theoretical and experimental, has



PRINCIPLE
OF A
STATIC THERMAL DIFFUSION CELL

FIGURE I-I



CLUSIUS-DICKEL THERMOGRAVITATIONAL COLUMN

FIGURE 1-2

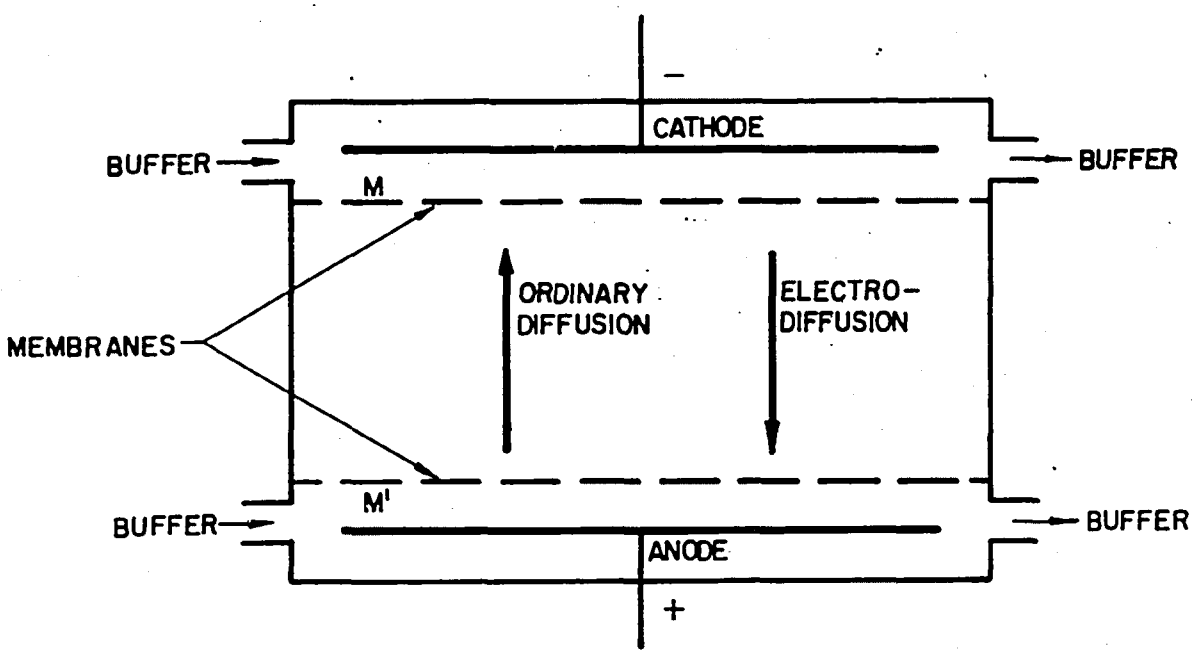
been and is being done concerning the thermogravitational thermal diffusion column, in spite of the fact that the thermal diffusion is very weak, and that thermal diffusion can be a very expensive process. A reader interested in this field is referred to the work of Furry, Jones and Onsager (18), that of Powers and his collaborators (48,7,17) and that of Von Halle (56). A list of recent important publications in this field is included in the list of references (65-77).

Since the separation in a Clusius-Dickel column greatly depends upon the magnitude of the thermal diffusion force applied, it was felt that if a different type of force of greater strength were to be utilized (to have an increased horizontal diffusion velocity of the component molecules) while retaining the advantages offered by the thermoelectrogravitational reflux, it would be possible to obtain increased separation by Clusius-Dickel columns. The use of an electric field force is an obvious choice because large electrical fields can easily be applied to increase the velocities or mobilities of the components.

Use of the electric field force, however, by its very nature, sets a very important limitation on its general application because it can only be used to effect the separation of charged particles in a solution. This restriction, however, is not too severe since, in addition to ions, colloids and large molecules which adsorb ions (and therefore act as though they were charged particles) can be effectively separated and concentrated by using an electrical field.

This process is known as "Electrophoresis" which refers to the movement of charged particles between electrodes under the influence of electric field. It is a very elegant method of separation and has a number of advantages over thermal diffusion in both mode and speed of operation. First, it uses a very much stronger diffusion field force. Second, it offers greater flexibility in conditions of operation because the relative movements of the component molecules to be separated can be regulated to a great extent by adjusting the pH of the solution. In certain cases the two components may be made to move in opposite directions, thus helping to obtain increased separation. In some cases one of the components may be neutralized by adjusting the pH of the solution at its isoelectric point so that only the other components are mobile species. By applying significant electric fields, one could expect to obtain drastic reduction in the size of the equipment necessary to carry out a required separation.

The principle of electrophoresis is explained by the consideration of a static electrophoresis cell in Figure 1-3. The solution of the components to be separated is placed between two semipermeable membranes M and M', behind which are two suitable electrodes. When an electric field is applied across the electrodes, the components will tend to move towards either of the electrodes depending upon the nature of the charge on the component molecules. Convection can be avoided by choosing polarities of the two electrodes in such a manner that the mobile component moves towards the



PRINCIPLE
of a
BATCH ELECTROPHORESIS CELL

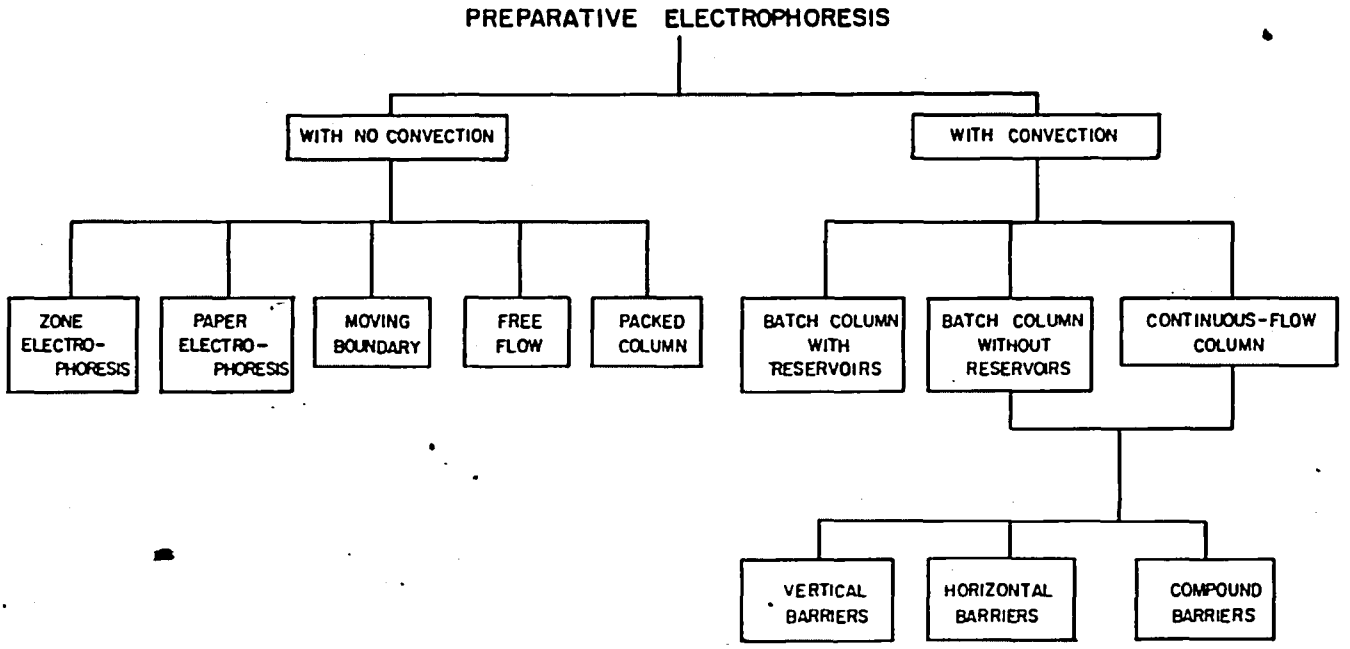
FIGURE I-3

bottom electrode and will therefore be concentrated at the surface of the lower membrane. In addition, the convection can be stabilized by maintaining the bottom of the static electrophoresis cell at a lower temperature than that of the top of the cell. The analogy between a static thermal diffusion cell and the static electrophoresis cell is obvious.

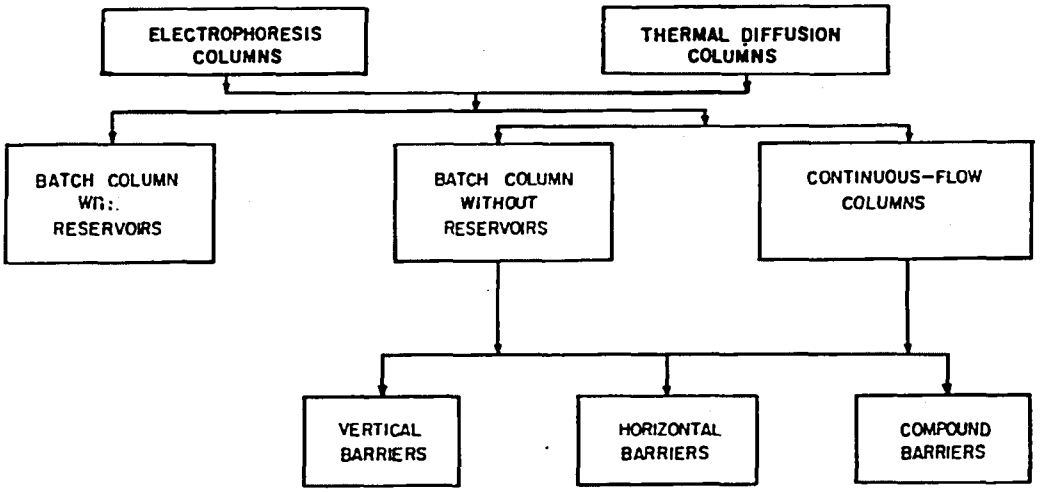
"Electrophoresis" since its suggestion as a separation method has been widely used especially in the fields of biology, physiology and the separation of proteins and colloidal charged particles mostly on a laboratory scale. It would not be much of an exaggeration to say that there are almost as many variations in the nature of equipment designed and used as there are workers in the field. A detailed account of the most important work in the field of preparatory "Electrophoresis" will be given in the next chapter. Most of the researchers have used both batch and continuous apparatus without convection. One major objective has been to avoid opposing density gradients to suppress convection mixing. Also, the heat generated during the process of electrophoresis has to be removed as fast as possible to avoid temperature gradients, and thereby convection, and also to avoid the spoilage of material. Therefore, the apparatus of this type has to be provided with very efficient cooling systems and batch as well as continuous electrophoresis apparatus have been operated below room temperature.

The electrophoresis batch cells, like their thermal

diffusion counterparts, are essentially characterized by low capacity, low separation and efficiencies and long duration of operation. Therefore, interest in continuous electrophoresis arose during the last 15 years for preparative purposes. Some progress has been achieved in this field as evidenced by the works of Bier (4), Dobry and Finn (12) and Mel (43). Some of the various ways in which apparatus for preparative electrophoresis can be constructed are shown in Figure 1-4a. The analogy between the thermal diffusion process and the electrophoresis has been illustrated diagrammatically in Figure (1-4b) wherein are shown the various ways in which the thermal diffusion and electrophoresis apparatus can be constructed and operated in similar ways. Though electrophoresis columns can be constructed in a manner similar to thermal diffusion equipment, investigators in the field of preparative electrophoresis have restricted themselves to use and investigation of convection-free apparatus, and in most cases the advantage to be gained by utilizing the reflux action of vertical convection has been lost. The work of Kirkwood and his collaborators (24-25) is the only available literature reference dealing with a combination of thermogravitational and electroconvectional reflux with electrophoresis. He applied this principle of thermogravitational reflux to a batch type electrophoresis cell with reservoirs. It can be said, therefore, that the application of thermogravitational and electrogravitational principle in combination with electrophoretic separation has not received so far



(a)



(b)

ANALOGY BETWEEN
ELECTROPHORESIS AND THERMAL DIFFUSION

FIGURE I-4

the attention it deserves, even though the close analogy between thermal diffusion and electrophoresis would suggest otherwise. In particular, one should expect that the combination of thermogravitational reflux or electrogravitational reflux with electrophoresis should increase separation and capacity, especially when applied to continuous-flow columns. This objective can be easily achieved by extending the analogy between thermal diffusion columns to electrophoresis columns where the principle of gravitational reflux is employed. Such continuous electrophoretic columns could be employed in the fields of biology, physiology, medicine and physical chemistry.

One concludes, therefore, that in spite of the possible potential of the continuous-flow electrophoresis columns, no work has so far been reported on the design, construction and operation of such columns. Further, no theoretical analysis is available to predict their separation performance.

Theoretical and experimental analyses of any process are essential to guide the development of practical equipment. Such analyses might provide a major advance in the field of continuous-flow preparative electrophoresis and would produce completely new information of great value. The present work has therefore, been undertaken as the first phase of a study of continuous flow electrophoresis using the combination of gravitational reflux and an electric field. The main objectives of the immediate work to be described in the following

pages were:

(1) to extend the analogy between the batch thermal diffusion columns with reservoirs to the case of batch as well as centrally fed continuous-flow thermoelectrogravitational electrophoresis columns. This requires the formulation of a mathematical model of such electrophoresis columns.

(2) to test the applicability of the resulting theory by constructing and operating a center-fed continuous-flow thermoelectrogravitational electrophoresis column. The experimental work, using the column, would determine the effect upon separation of field strength, pH of solution, membrane spacing, temperature difference and flow rate.

CHAPTER II

REVIEW OF LITERATURE ON ELECTROPHORESIS

Introduction

Both the electrophoresis and the ionophoresis, ever since their discovery by Reuss (52) in 1808, have been used in several fields of study. Ionophoresis relates to the movement of crystalloids while the term electrophoresis is used to refer to the movement of charged colloids or macroscopic particles between the electrodes under the influence of a direct current electric field. The particles move not only in different directions, but at different velocities depending upon the values of their electrical mobilities in the given medium. The usual objectives of electrophoresis experiments are to obtain information on electrical double layers surrounding the charged particles, the analysis and characterization of mixtures of charged particles, and their separation into individual components.

The electrical mobility of a charged particle is its velocity per unit time under the influence of a field strength of one volt per centimeter. It is a function of the viscosity of the medium, size, shape and concentration of the particles or ions in solution, and the concentration of the electrolytes

in the solution. Therefore, electrophoretic separation depends upon the difference in the rate and the direction of migration of different components. Electrophoresis is an excellent method of separation since it can separate substances which are either chemically similar or have very low mobilities under the influence of other force fields such as pressure and thermal diffusion. As a result, the widespread application of electrophoresis as an analytic and separation tool covers such diverse fields as biology, biochemistry, pathology and physical chemistry. Outstanding contributions have been made in the analysis and characterization of fluids such as blood, serum plasma, egg and milk components and several types of tissue components.

So much work has been done and so numerous are the variations in the techniques (mostly analytical) that it is inconvenient to review here all the work done in detail. Therefore, this review will be restricted to preparative electrophoresis, a term applied to the electrophoretic preparation of purified components from a mixture. The interested reader is referred to more general surveys already available, (78-80).

Although considerable advantage of electrophoresis has been taken for analytical purposes, the progress in efficient utilization of electrophoresis for preparatory purposes has been very slow. Only a few proteins or enzymes are prepared by electrophoresis in preference to other methods. In protein chemistry for instance, considerable work

has been done in various salt and solvent fractionation schemes which are time-consuming, cumbersome and expensive. The salt and solvent fractionation work is out of proportion to the effort put into the development of preparative electrophoretic methods, even though a satisfactory electrophoretic method would, in principle, be an excellent approach to many preparative problems from the point of elegance, cost and electrophoretic purity.

The slow progress in the field of preparative electrophoresis is surprising since one of the most basic discoveries in this field was made in 1924. This was the observation of the phenomenon of electrodecantation by Pauli (44). Only in recent years with the work of Gutfreund (20) Kirkwood, Cann and associates (24-35) Polson (47) and Bier (4) has interest risen in this method and its various analogies.

The various methods that are used for preparative electrophoresis can be grouped under the following subtitles:

(a) Density gradient electrophoresis

(b) Forced flow electrophoresis

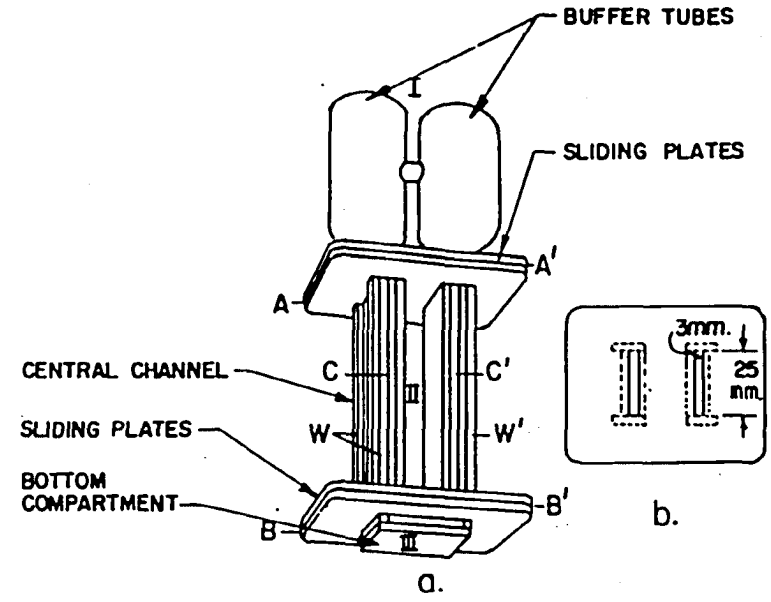
(c) Electrodecantation and sedimentation

(d) Electrophoresis convection method

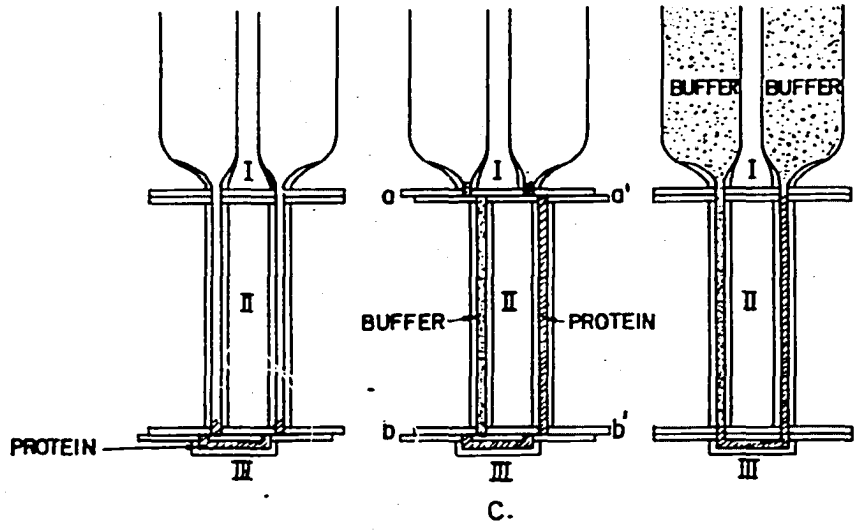
Svensson (59) has reviewed the literature on preparative electrophoresis up to 1948 and recent advances have been partially reviewed by E. Durrum (13). More recent work in the above field will now be reviewed.

Density Gradient Preparative Electrophoresis

This method avoids convection currents in solution and stabilizes the density gradients. The simple Tiselius apparatus is widely used for both analytical and small scale preparatory purposes. A widely used form of the Tiselius cell is shown in perspective in Figure 2-1. The tubes I are connected to the two sides of the middle portion and carry buffer solutions. An electrical field is applied by means of electrodes situated in separate electrode vessels (not shown in figure) which can be connected to the buffer tubes I. w and w' denote the width of the channels. It consists of three sections that slide over each other along the planes A-A′ and B-B′. The U-shaped channel provided by the cell is of rectangular section and is presented in Figure 2-1b. Its principle of operation is diagrammatically shown in Figure 2-1c. In the first step, protein is added to the bottom chamber and slipped to one side trapping the protein in the small lower chamber. Buffer is added to one side (left) and protein to the other (center drawing). The entire assembly is then shifted to close the channels at the top and buffer added to both reservoirs. Both sections are then returned to establish contact among the solutions as shown in the right hand drawing. The electrical field is applied and distinct horizontal zones form for each protein in the protein field (right side) channel. The height of the center section is 92 mm. which, with horizontal plates of 3 mm. thickness, leaves 86 mm. clear height for observation. The channel cross



THE TISELIUS ELECTROPHORESIS CELL



DIAGRAMS ILLUSTRATING THE INITIAL FORMATION OF THE BOUNDARIES IN A TISELIUS ELECTROPHORESIS CELL

FIGURE 2-1

section is 3 x 25 mm., and about 11 ml. of protein solution are required to fill one side of the center section and the bottom section denoted by II and III respectively in the figure.

The cells are constructed of glass or quartz plates except the tubes of section I. A more complete description of the cell and its operating technique can be found elsewhere, (5).

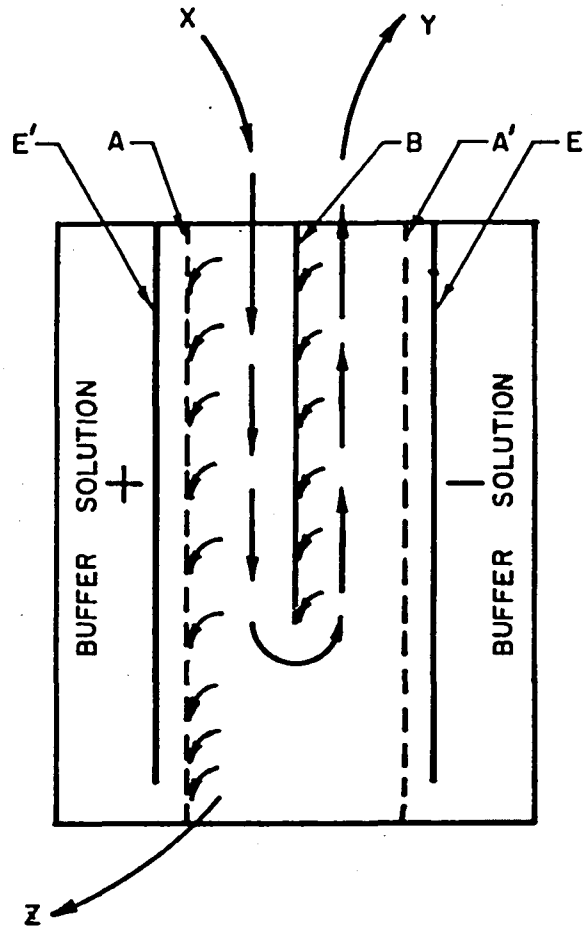
The Tiselius Cell is not particularly suitable for preparation work because of its inability to completely separate the components of a mixture into distinct zones. Separation into zones always requires stabilization by other means such as the use of paper, powder and gels. When stabilization of density gradients is obtained this way, the process is termed free boundary zone electrophoresis. Improved apparatus has been described by Philpot (46) and Ericson and Nihlen (15), Sorof and Ott (56) and Kolin (36-39), Svensson and Valmet (58). However, these designs are not at present developed for fractionation of large volumes of solution.

Forced Flow Electrophoresis

The main objective in the field of preparatory electrophoresis has been to obtain good separation at rapid speed. The capacity of an electrophoresis apparatus has now become an important aspect. For this purpose it has been considered desirable to have a free solution condition and no convection. It is very difficult, as has been stated by Raymond (50-51)

and later by Dobry and Finn (12), to establish and maintain proper rates of flow and steady flow patterns. . Some apparatus in which an attempt is made to obtain such conditions have been reported. In such equipment a flow pattern is imposed mechanically on the liquid which flows through the electrophoresis cell. The apparatus described by Bier (4) Dobry and Finn (13) and later by Mel (43) can be grouped under the heading "Continuous Forced Flow Boundary Electrophoresis". These will be described here in detail because they represent a great advance in the field of continuous flow electrophoresis. One of the objectives in the design and the operation of these apparatus was to obtain a stabilized laminar flow without convective mixing.

A method in which two fractions of a solution are separated immediately after leaving the channel of an electrophoresis cell was proposed by Bier (4). The operational principle of this apparatus is illustrated in Figure 2-2. In essence, the electrophoresis cell consists of two outer semipermeable membranes A and A', held stretched in a plexiglass frame (not shown in diagram). The cell is immersed in a circulating cooled buffer, and a direct current electric field established across the membranes. The two membranes are parallel, 3 to 4 mm. apart, while a third semipermeable membrane B is inserted between them, partway into the cell. The cell is thus divided into three compartments, two at the top and a common one at the bottom. All three compartments have means for continuous input or withdrawal of the colloid



A, A′ – Semipermeable membrane
B – Filter paper membrane
E, E′ – Electrodes

PRINCIPLE OF FORCED FLOW ELECTROPHORESIS
BY
MILAN BIER

FIGURE 2-2

solution.

The operating principle of the apparatus is as follows:

If the solution to be fractionated contains two proteins which differ in their isoelectric points, then the pH of the solution and the outside buffer is adjusted close to the isoelectric point of one of the proteins, rendering it an immobile component of the solution. The solution is continuously fed into one of the top compartments and the polarity of the current is so selected that the mobile protein migrates towards the outer membrane of the compartment as indicated in Figure 2-2. Products are withdrawn from the bottom at z and at the top at y. The other protein does not move and hence remains uniformly distributed throughout the solution.

The feed solution is thus separated into two fractions. The bottom fraction contains the migrating proteins as well as the immobile component in original concentration. The fraction at the top contains the other protein obtained in high electrophoretic purity. The method has an advantage in that the rates of flow of the protein solution at the top and bottom can be controlled independently of each other. The method can be used for the preparation of electrophoretically pure proteins or for concentrating them.

Under proper operating conditions, a definite flow pattern is established in the cell depending upon a large number of variables such as rates of inflow and outflow, feed temperature, temperature gradient within the cell, and the field strength.

The performance of a number of cells of different dimensions and under a variety of experimental conditions was studied by Bier (4). Most of the work was carried out with a single mobile component protein system in the hope of optimizing cell design and studying the principles involved. He obtained data on various cells as a function of flow rate, current density and the position of the center membrane with regard to its extension in the cell. Different types of membranes such as a hardened wet-strength filter paper Whatman No. 52 or 54 were used and found satisfactory. Several arrangements of the central membrane were studied. These included center membranes extending over the entire length of the cell and having openings of various geometrical patterns, such as vertical or horizontal slits or perforations. Moreover fully permeable membranes were studied such as cotton, silk and nylon cloth. Only the filter paper was found satisfactory.

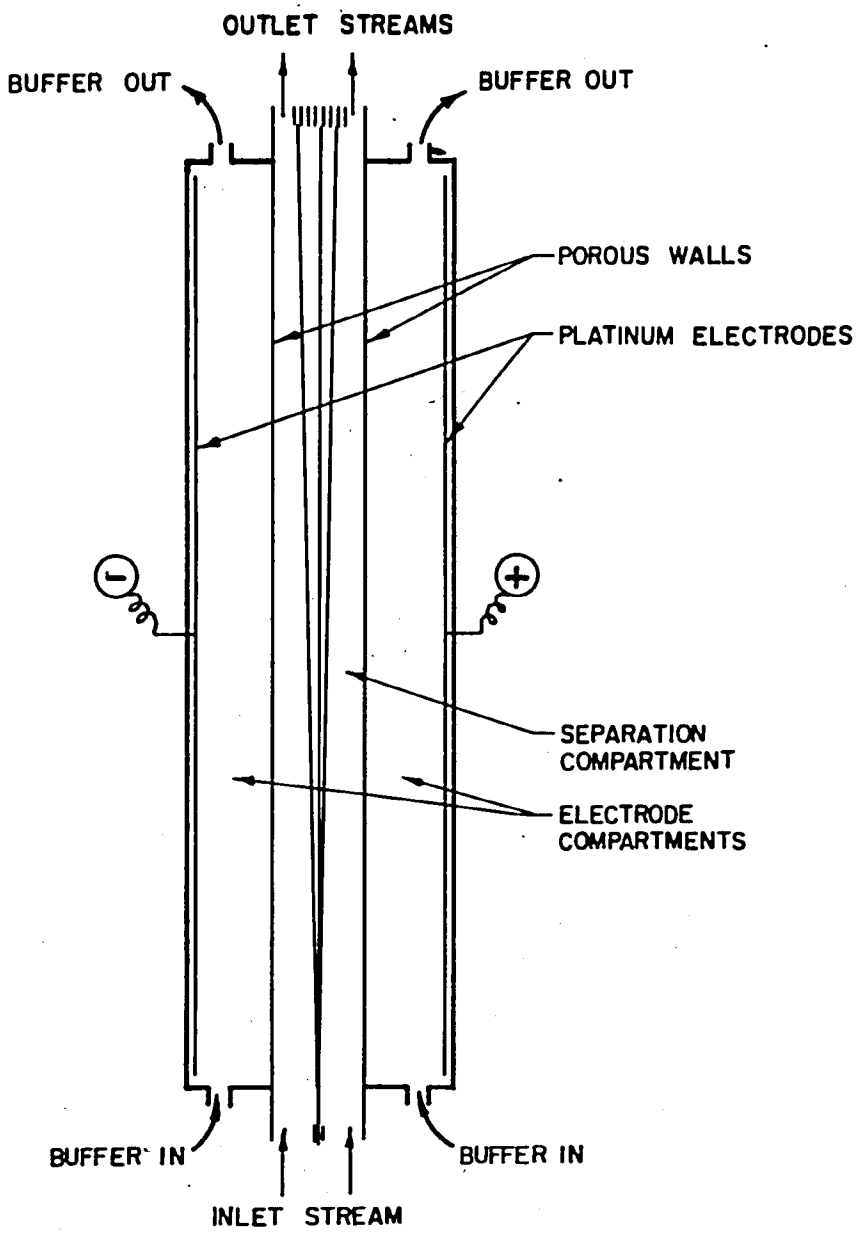
Since its suggestion, Forced Flow Electrophoresis has been successfully used to isolate gamma-globulin in electrophoretic purity (4). Seibert (54) applied it for further purification of well characterized polysaccharides of tubercle bacilli. Other numerous applications have also been reported.

The 4 ml./min. output of Bier's apparatus for processing a given solution was many times greater than the output of the hanging-curtain paper electrophoresis instruments then employed. It can also be favorably contrasted with the electrophoresis convection method, which yields about 100/ml. per

batch, the processing of which may require 24 hours or more (68). The output by Bier's method could also be conveniently increased by arranging a series of cells in parallel. Accordingly flow rates might be achieved which would be important for the industrial preparation of electrophoretically pure proteins.

Dobry and Finn (12) added either dextran or methocel to increase the viscosity of the solution and thereby to stabilize the forced laminar flow in their apparatus. The principle of this apparatus is schematically shown in Figure 2-3. The solution moved upwards between two Flexolith porous walls. The height of the apparatus was about 5 feet. The width of the cell was 3 inches and the two membranes were separated by a distance of 2 inches. Feed was introduced into the apparatus through the feed ducts provided at the bottom. The cathode was made of Hastelloy C and the anode of stainless steel. The products were withdrawn at the top through several ducts. The buffer compartments were fitted with electrodes across which a very high electric field could be applied. The cell was constructed of Plexiglass. The buffer compartments were provided with gas vents. In the operation of this column, the substances to be separated must be prevented from reaching the porous Flexolith membranes since these are permeable to proteins.

A basic desire on the part of many workers in carrying out electrophoretic separations is to eliminate remixing of the partially separated fractions or layers. Such remixing



PRINCIPLE
OF
ELECTROPHORESIS APPARATUS OF DOBRY AND FINN

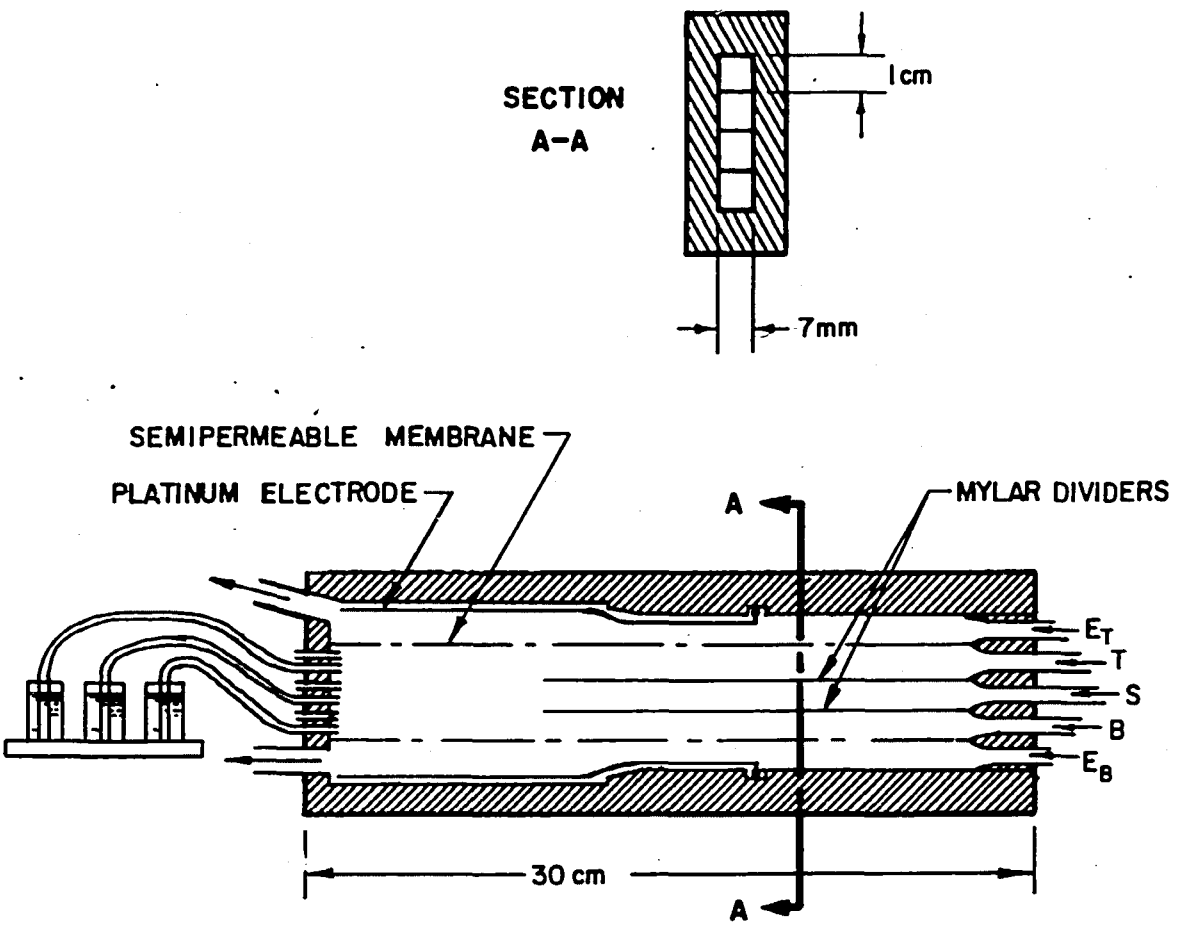
FIGURE 2-3

of the components takes place because of the convection which is brought about by either local density differences or by thermal gradients due to uneven heating.

Some degree of success has been achieved in maintaining a free flow, stabilized against convection in the apparatus described by Mel (43). A schematic diagram of Mel's apparatus is given in Figure 2-4. The apparatus is essentially an electrophoresis cell in a horizontal position which was fitted with two semipermeable membranes M and M′. The solution flowed freely between these two membranes across which the electric field was applied. E_T and E_B were the inlets for buffer solution. The solution to be separated entered at S between the membranes. T and B can be used either as inlets for feed or buffer solution as desired. Several fractions of the separated solution were collected at the several outlets at different levels.

Stabilized free flows are achieved by virtue of laminar flow conditions, density differences between the various layers, mechanical stabilization by mylar dividers and by the closed hydraulic nature of the entire system.

Samples were pumped at constant rate through one or more inlets with the remaining inlets carrying buffer or other solutions. The author claims that perfectly laminar flows without convective mixing were obtained with flows of 1.2 ml. per minute per outlet or 14.4 ml. per minute total. This apparatus has been successfully used in concentrating dyes, dilute enzyme solutions, protein separations with and



A SKETCH OF THE
FREE FLOW ELECTROPHORESIS APPARATUS OF MEL

FIGURE 2-4

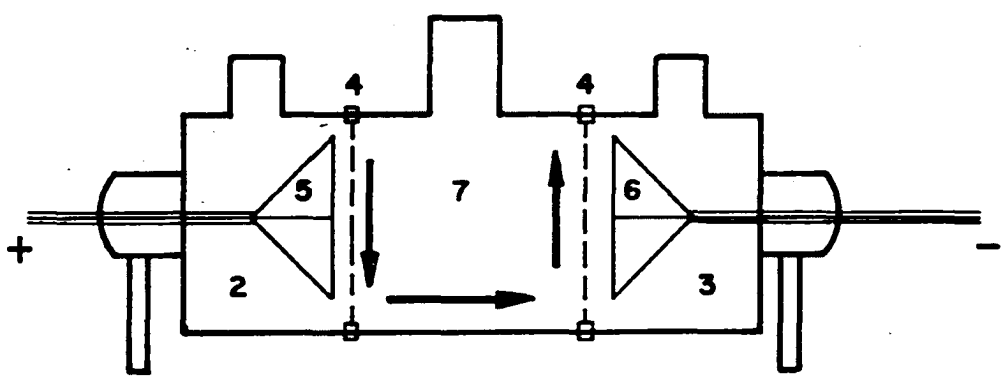
without interactions, as well as yeast cell separations.

Electrodecantation Apparatus

The phenomenon of electrodecantation was first discovered by Pauli (44). He observed that when a quiet colloid solution was subjected to a direct current field, stratification appeared, the solution separating after a time into two distinct layers. The bottom layer contained most of the colloidal matter. The electrodecantation cell of Pauli is illustrated in Figure 2-5.

This cell consisted of three chambers, one for the solution to be stratified and the other two for background buffer solutions. In the figure, the central chamber was the electrodecantation compartment. The central compartment was separated from the buffer compartments by means of semipermeable membranes 4. The direct current electric field was applied by electrodes 5 and 6. Pauli used his apparatus to stratify proteins, and many inorganic sols.

The phenomenological theory of electrodecantation was explained by Blank and Valko (6). Under the influence of the electric field, the colloidal particles migrate toward one of the semipermeable membranes separating the central compartment from the electrodes as shown in Figure 2-5. In the vicinity of this membrane, the concentration of colloid increases, the local density rises and hence the solution sinks to the bottom of the compartment. At the same time, solution at the other membrane is reduced in colloid concentration and



5–6 – Electrodes
4–4 – Semipermeable membranes
7 – Separation compartment
2–3 – Background buffer compartments

A SKETCH OF PAULI'S ELECTRODECANTATION CELL

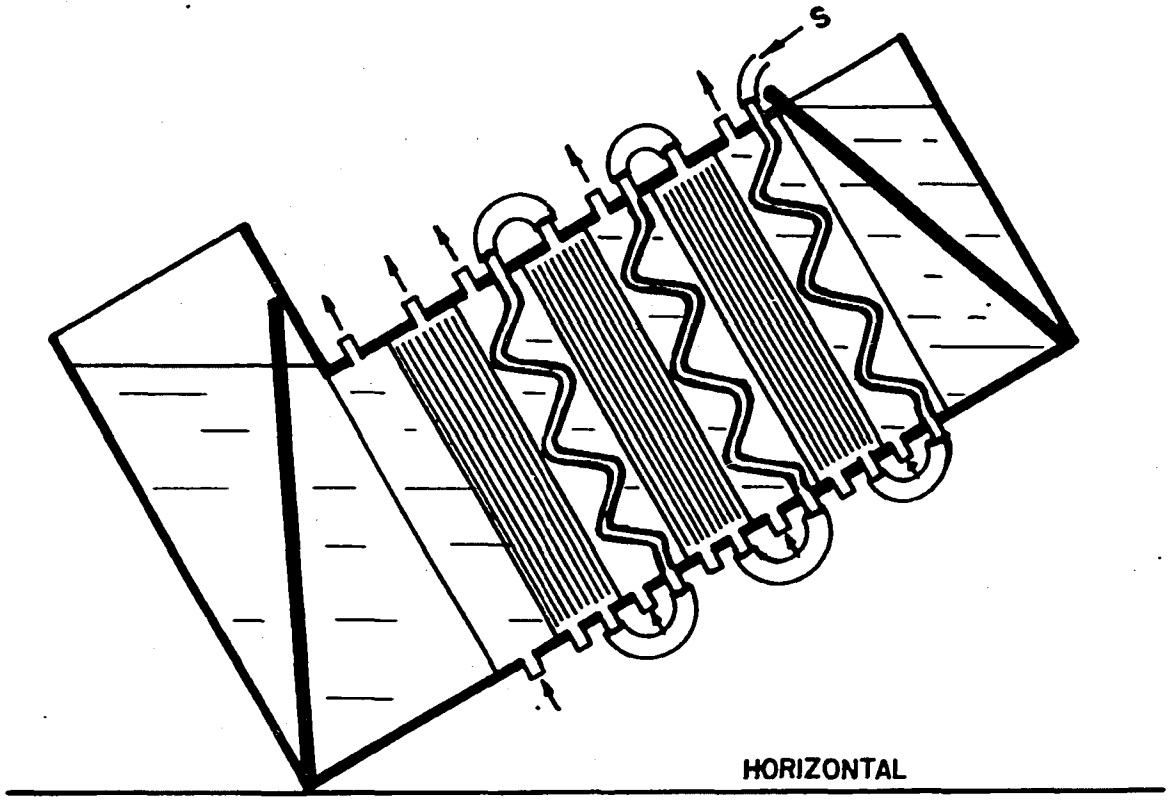
FIGURE 2–5

rises to the top. The bottom and top layers are stabilized by density gradients. This explanation was later confirmed by Verwey and Kruyt (64).

The problem of heat generated by the electric current passing through the center compartment was partially avoided by use of inorganic colloids. Moreover, experiments were carried out with unbuffered solutions in electrode compartments, thereby having both electrodialysis and electrodecantation occurring simultaneously. Only small current densities were used so that power consumption and heat generation were very small.

Gutfreund (20) used the electrodecantation method to separate hemoglobin and ovalbumin. His apparatus was similar to that of Pauli but he kept one of the proteins at its isoelectric point so that only one protein moved on passage of the current through the solution.

The capacity of Pauli's apparatus has been increased by modifying it to a multi-membrane apparatus by Polson (47). He used the multimembrane principle of Stamberger (57), spacing the cellophane membranes about 1 mm. apart with the use of plastic frames. He thus improved conditions of heat dissipation as well as pH conditions of the buffer solutions. A diagram of such apparatus is shown in Figure 2-6. The cell was oriented at an angle to the horizontal because a greater efficiency of separation was obtained. He used a membrane spacing of 0.75 mm., cell height of 45 cm., width of 9 cm., and a thickness of the multimembrane compartment 2.5 cm.



MULTIMEMBRANE APPARATUS OF POLSON

FIGURE 2-6

Water leakage was avoided by using rubber gaskets between the different compartments. A temperature gradient in addition to the concentration gradient was claimed to reduce the undesired convection currents.

Electrodecantation is largely used in latex technology. It was further found to be of use in the concentration of aqueous dispersions of polytetrafluoroethylene (49). Polson and collaborators (47) used the principle of electrodecantation for separation of proteins.

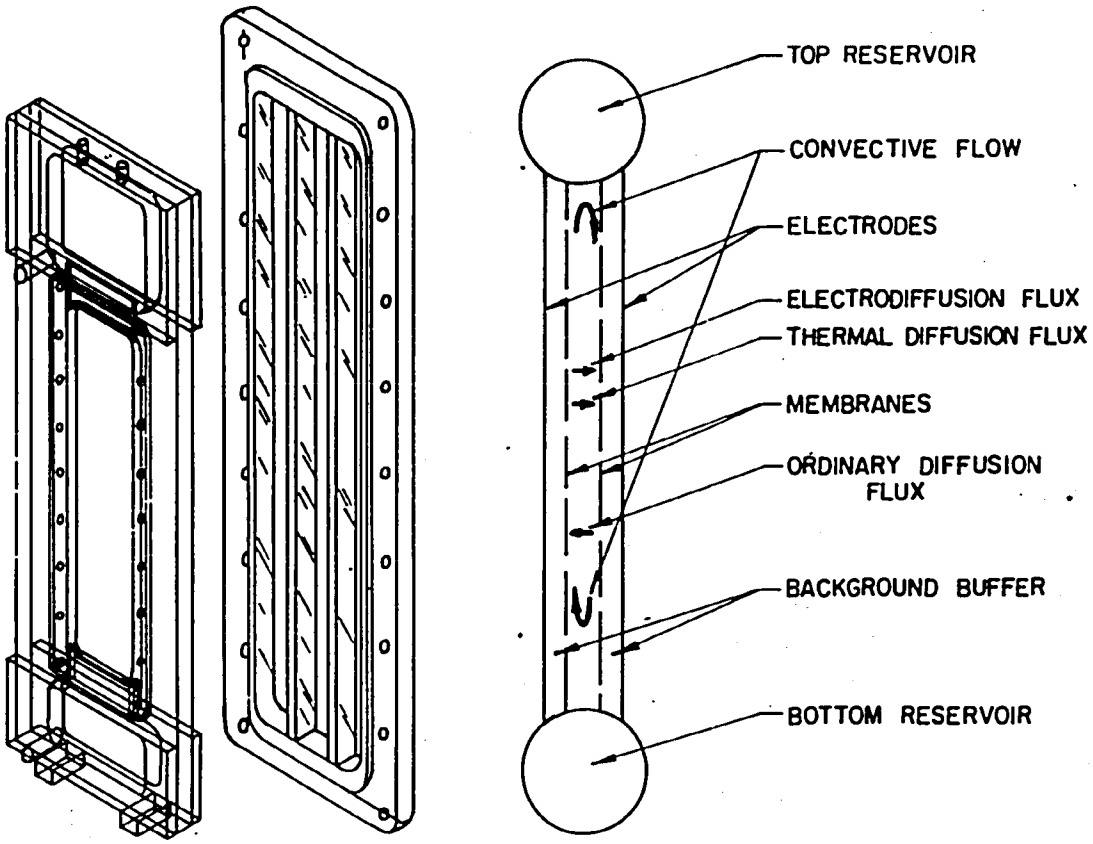
Electrophoresis with Convection

It may be observed in the separation technique and the apparatus reviewed so far that in all these devices (probably Bier's cell is an exception) an effort has been made to avoid convective mixing. Thus the advantage gained by gravitational reflux action of laminar convection in the vertical thermogravitational thermal diffusion column of Clusius and Dickel (9) has not been employed. The only work available in the literature using the principle of thermogravitational reflux is that of Kirkwood who was the first to propose this new method of protein separation in 1941. Termed electrophoresis convection, it was based upon a combination of thermal diffusion and electrophoresis. He utilized a batch column with reservoirs to separate horse hemoglobin, azo-ovalbumin, and bovine albumin. The modified apparatus which was used in most of the later work has been described by Cann et al. (24-35). This modified cell

described by Cann is illustrated in Figure 2-7a, and a schematic diagram of the principle is presented in Figure 2-7b. It consisted of an upper and a lower compartment. These compartments were connected by a narrow vertical channel formed by two parallel semipermeable membranes. These membranes were held in place by two face plate assemblies. The channel was connected to the compartments through narrow slits in the compartments. The parts of the apparatus were made of plexiglass and nylon screws were used for assembly of the plates. The capacities of upper and lower compartments were of the order of 100 ml. and 50 ml. respectively. The height of the channel was 24 cm. and the width 5.1 cm. The spacing between membranes was 0.1 cm. to 0.2 cm. depending on the hydrostatic pressure. During operation, the entire cell assembly was immersed in a cooled circulating buffer. The electric field was applied across the membranes by two platinum external electrodes coextensive with the membranes.

During operation, the apparatus was filled with protein solution to be separated. Circulation of buffer was started and the electric field was then established. At the end of the run, the top compartment was first emptied.

The procedure involved adjusting the pH of the solution so that one of the components was at its isoelectric point and therefore immobile. Under the influence of the electric field, all the mobile components would migrate toward one of the membranes, form a layer of increased concentration and sink into the bottom because of the gravity



(a) A view of a plexiglass apparatus and a faceplate.

(b) Principle

ELECTROPHORESIS CONVECTION APPARATUS BY CANN et al.

FIGURE 2-7

controlled convection. An equal volume would be displaced from the bottom compartment on the other side and become exposed to the electrical field. In this manner, a continuous convective flow was established between the two compartments through the channel formed by the two membranes. At the end of the experiment, involving several hours, the mobile components would be transported to the bottom compartment while the isoelectric protein, unaffected by the electric field, would remain in its original concentration in the upper compartment. It could then be withdrawn in a purified state. Several stages could be used to effect further purification. The isoelectric procedure employed was by far the most efficient, however, only the two terminal protein components could be separated, since all the mobile components with large mobilities would concentrate at the bottom while those with isoelectric points close to that of the isoelectric component would remain in substantial concentration at the top.

If for reasons of stability or solubility of the components, the isoelectric point procedure cannot be suitably employed, one takes advantage of the difference in mobilities of two components. In this second method of operation of the apparatus, all the components are mobile and move toward the same membrane. However, the faster-moving components will be transported from the top compartment at a higher rate than the slower-moving components resulting in a partial separation. The greatest separation of the components

would be obtained by so choosing the operating time that half of the major component would be transported out of the upper compartment. In mixtures containing more than two components, the separation could be achieved in steps by obtaining either the most acidic or the most basic component in pure state.

Another version of the apparatus was constructed by Raymond (51). This cell consisted of a single length of dialysing tubing, bent into U shape. The bottom compartment was formed by the bottom of U shaped tubing, while both the open ends of the tube served as the top compartment. The intermediate portion of the tubing was stretched in a plastic frame forming two channels within which electrophoresis convection took place. This intermediate portion of the tubing was the only one exposed to electric field. The advantage claimed was that the size of the compartment could be varied. The leakage problem was almost eliminated since the entire process was carried out in an intact length of tubing. Other modifications of this cell have been suggested (40,60).

Kirkwood also developed the mathematical theory of electrophoresis convection (24-35) for batch columns with reservoirs.

The method of electrophoresis with convection has been used successfully for purification of proteins such as serum proteins, egg proteins and several others. Papers of Kirkwood and his collaborators (24-35), Cann et al. (24-35), Phelps and Cann (45), Timashelf and Tinoco (61), Mathies (41) and Singer (55) may be consulted for details.

Kirkwood's apparatus, being a batch apparatus, has some disadvantages from the point of view of handling purification of large volumes. These are the long time of separation, poor separation efficiency and low output (100 ml. solution could be purified in 24 hours or more). In spite of this, it has been extensively used for protein purification in laboratories.

Conclusion

On the basis of the literature reviewed in this chapter, the following conclusions are drawn about the various apparatus as described in the literature:

(1) The apparatus use either batch operation with low capacity or continuous operation with very small flow rates.

(2) The apparatus were designed to avoid convection mixing (Tiselius cell) or to get a stable free boundary flow (laminar stable flow) as in Mel's apparatus. In forced flow electrophoresis (Bier's apparatus) a flow pattern was imposed upon the moving protein. Thus most of the authors have not taken advantage of reflux action obtained due to convection. On the contrary, the tendency was to suppress convection.

(3) Kirkwood utilized the principle of thermogravitational and electrogravitational reflux in a batch type electrophoresis apparatus with reservoirs

for the separation of proteins in various protein systems, and established a mathematical theory to evaluate the performance of such batch columns.

(4) There appears to be no attempt as yet to explore the possibility of constructing continuous-flow electrophoresis columns in a manner similar to Clusius-Dickel thermal diffusion columns and to utilize them for the separation of protein or other systems.

(5) No mathematical theory is available which would help to evaluate the performance of continuous-flow electrophoresis columns although there is a possibility that Kirkwood's theory for batch columns with reservoirs could be extended to continuous-flow columns.

A continuous-flow electrophoresis column would allow handling of large volumes of solution per unit time since the separated fraction of the material could be removed from the apparatus continuously.

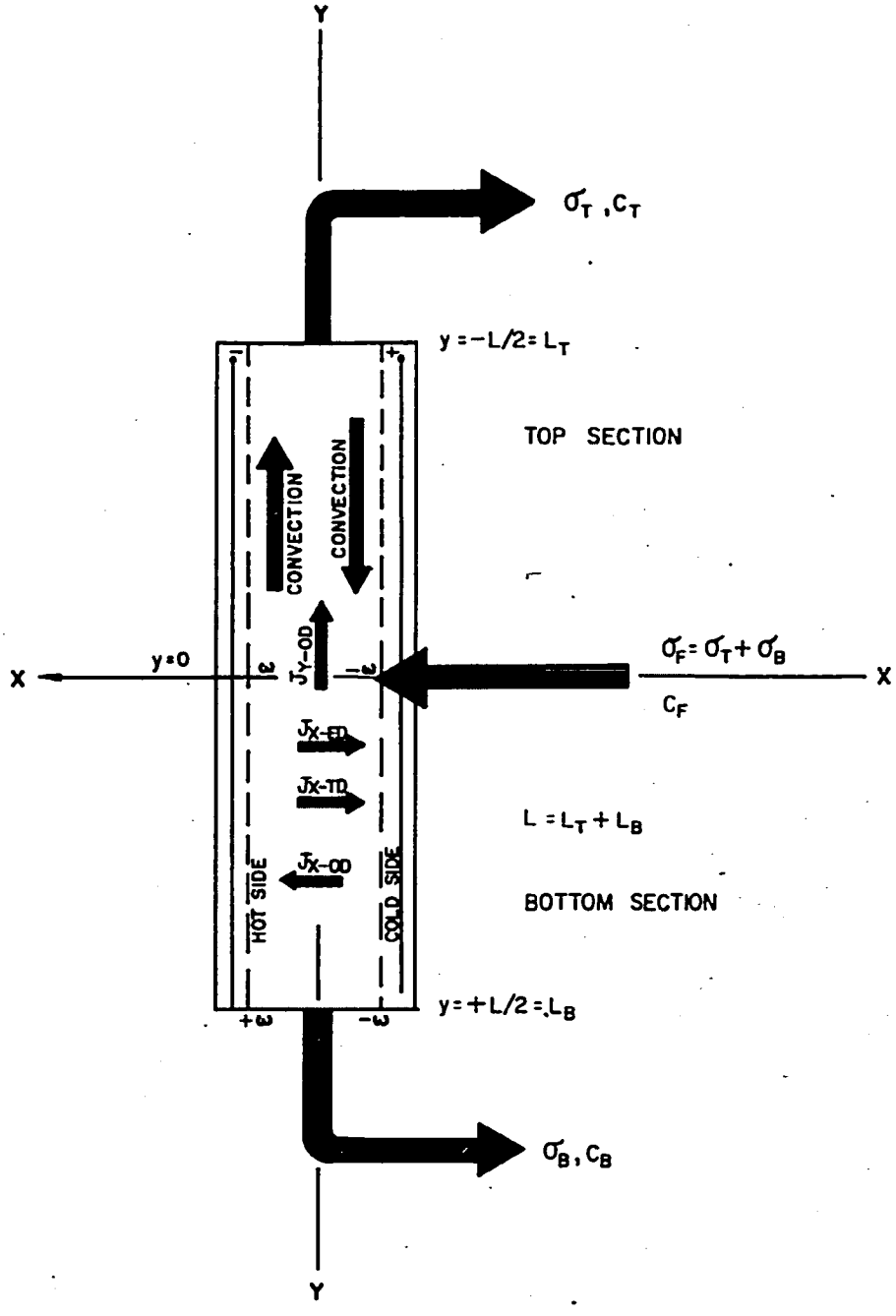
CHAPTER III

THEORETICAL ANALYSIS

The review of the literature about preparative electrophoresis shows no theoretical or experimental work for continuous electrophoresis columns similar to Clusius-Dickel continuous-flow thermal diffusion columns. In the present chapter a theory will be developed to describe such column operation mathematically.

A qualitative description of the proposed column will be given first; structural details are deferred until the next chapter. Figure 3-1 illustrates the principle of a continuous-flow thermoelectrogravitational electrophoresis column. The column consists of parallel membranes (concentric apparatus could also be constructed) separated by a spacer. On the outside of the membranes are the two electrodes and circulating buffers at two different temperatures. This column can be operated either in a batch manner if no products are withdrawn from the column continuously or in a continuous-flow manner (Figure 3-2) in which products are continuously withdrawn at the bottom and at the top of the column.

For continuous operation, the feed can be introduced into the working space and products withdrawn at the bottom



SCHEMATIC DIAGRAM
OF A
CONTINUOUS-FLOW THERMOELECTROGRAVITATIONAL
ELECTROPHORESIS COLUMN

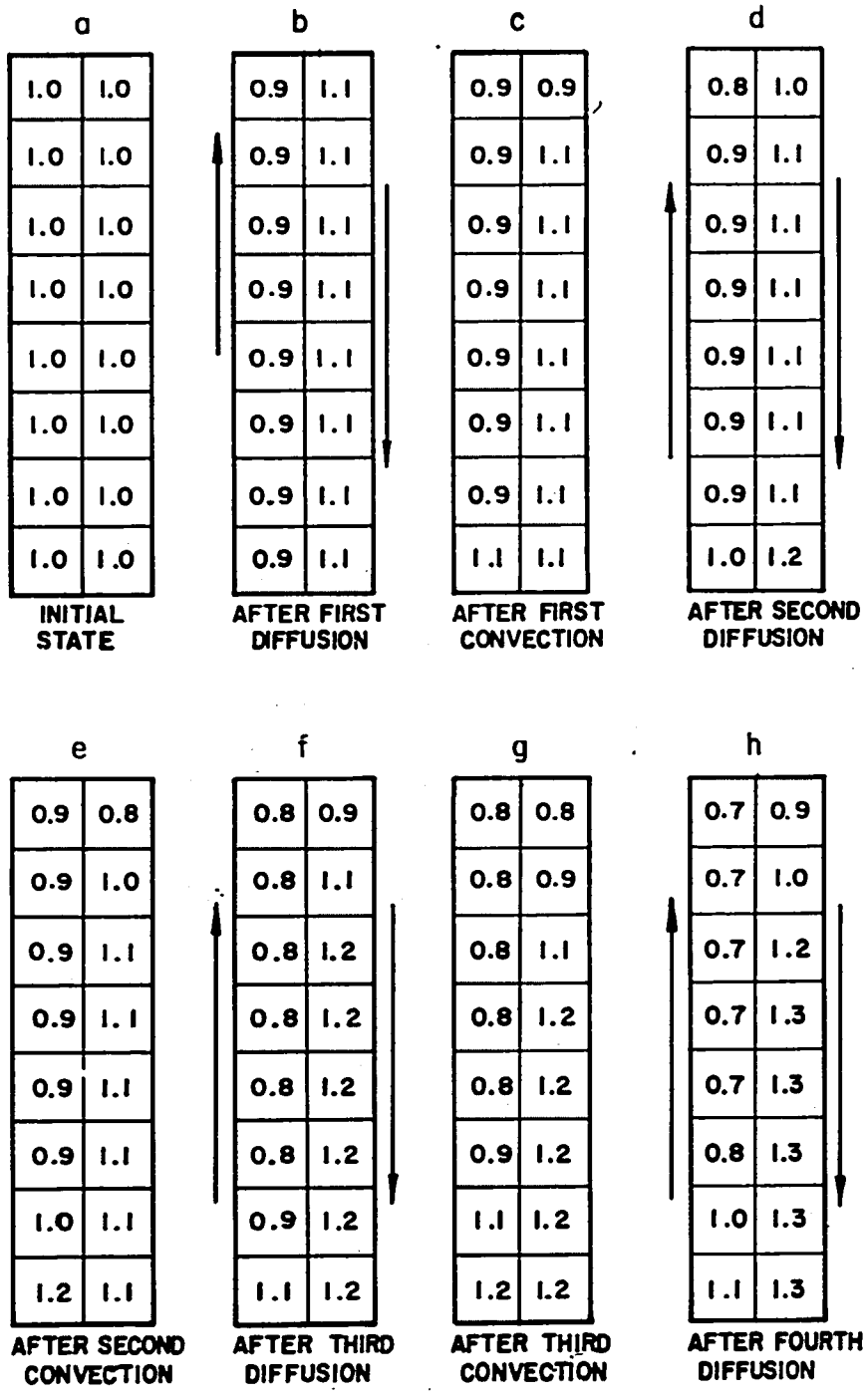
FIGURE 3-1

and top. The rates may be adjusted as desired. In order to maintain the pH of the solution in the working space during electrophoresis, a flow of buffer solution from behind the membranes is required to replace the depleted ions. These buffers are also used as a convenient means of maintaining the temperature difference between the membranes. Another function of the flowing buffer solution would be to remove the heat generated in the apparatus. The direct current electric field is applied across these membranes by means of coextensive electrodes which are outside the membranes. If there were a temperature difference between the membranes, a thermal diffusion field in the horizontal direction can also be established.

When the voltage gradient and the temperature gradient are established, the charged particles will move toward one of the membranes. If the temperature at this membrane is lower than that at the other, the solution will sink to the bottom, because of the higher density at a lower temperature and increased concentration. At the surface of the other membrane, exactly the opposite phenomenon takes place and the liquid rises. A convection current is therefore set up which gives the reflux action enhancing the separation obtained. The semipermeable membrane in electrophoresis is necessary to prevent the moving charged components to be separated from reaching the electrode and depositing on it. The combination of heat transfer and concentration change that is brought about by the presence of the electrical and

temperature fields, multiplies the gravitational reflux action. The column can therefore be termed as "thermoelectrogravitational continuous-flow electrophoresis column".

The action of thermogravitational as well as electrogravitational reflux in enhancing the separation in a continuous electrophoresis column over that in a static cell may be illustrated in the same manner as done by Grew and Ibbs (19) for the thermal diffusion column. Consider an electrophoresis column consisting of a pair of vertical flat membranes, initially filled with a solution of a charged colloid 1% in concentration. This situation is illustrated in Figure 3-2. The initial condition of uniform composition is shown in Figure 3-2a, in which, for simplification, it is considered that the initial solution is composed of 16 portions. The separation is initiated by applying the electric and temperature fields to initiate convection. A relatively short time will be required to establish the voltage gradient. Separation by electrodiffusion in a direction normal to the membranes and convective flow of solution parallel to the membranes occur simultaneously; but for this illustrative stepwise procedure, they will be considered to take place alternately. First, electrodiffusion occurs and an equilibrium separation of 0.1 gm./100 ml. is obtained between each pair of the 16 portions (Figure 3-2b). The composition is expressed in terms of the charged species which also will be assumed to be heavier than the solvent as for example, a protein dissolved in a buffer solution. Next, the convection



ENRICHMENT by THERMOELECTROGRAVITATIONAL REFLUX

FIGURE 3-2

initiated by the action of the temperature gradient and electrophoresis transports solution downwards in the cold section a distance equal to the length of one portion, and upward an equal distance near the hot membrane as shown in Figure 3-2c. The continuation of the sequence of Figure 3-2 shows how the charged molecules or particles are transported toward the bottom of the column with each step. This results in a concentration difference between the ends of the column which is greater than the separation obtainable by a static electrophoresis cell using the same electric field. The convection, therefore, is the predominant factor in determining the degree of separation attainable.

Batch and continuous-flow columns could be operated in several ways either for concentrating a solution or for preparing pure components. The electrophoresis column can be operated in three different ways. They are:

(a) Isoelectric Point Procedure

In this method, the pH is adjusted to such a value that one of the components is at its isoelectric point. It is therefore immobile and remains in its original concentration throughout the column.

This procedure, when applicable, is a very efficient means by which separation of two or more proteins may be achieved. In multicomponent systems it is advisable first to isolate the component with either the highest or lowest isoelectric point.

(b) Differential Transport

The isoelectric procedure is, however, not always applicable. This may be due to very low solubility of the component to be separated at its isoelectric point or a very small difference between the isoelectric points of the components to be separated. In such a case, the pH of the solution is adjusted in such a way that a considerable difference in mobilities of the components is obtained. In this, the component with higher mobility will collect at one end of the column at a faster rate than that of the other, and a partial separation would result. Further processing of the concentrated solution would be required to improve the purity. It is possible to overcome the disadvantage of limited solubility of a component at its isoelectric point by the formation of a complex with certain strongly acidic or basic substances. Such complex formation shifts the isoelectric point of the component. This procedure has limited application and has been applied to the purification of proteins.

(c) Reverse Transport Method

The third way in which the column can be operated is the reverse transport method. At a particular pH between their isoelectric points, the two components to be separated may have mobilities in opposite directions. The essential requirement of this procedure is that the bulk of the material must migrate in one direction. This is necessary to

cause a density differential,* otherwise no convection would occur. This procedure has limited application for substances like proteins because it is difficult to obtain mobilities of opposite sign which have a large difference between them.

A few observations regarding the operation of an electrophoresis cell might be worth mentioning here. In thermal diffusion for a two component system, if one component (say of lower density) goes to the hot wall, the other macromolecule must go to the cold wall. When combination of electrophoresis and thermal diffusion are used, care must be taken to see that the charged particle moves in the same direction under the influence of both the electrical field and the thermal field forces, so that they reinforce the effect of each other. Otherwise, the temperature field might be effective in the opposite direction and reduce the effectiveness of the separation.

Another important aspect is the generation of heat by the passage of electrical current through the solution. This heat must be removed to avoid the possibility that the temperature will rise beyond a certain limit and spoil some material such as protein in the solution.

The electrophoresis cell must be provided with an arrangement (such as vents) to eliminate the gases, mainly

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*Temperature difference would cause a density difference but as explained later in Chapter IV, only small temperature differences can be employed and hence one has to rely mostly on the migration of the bulk of the material in one direction to create the density difference.

hydrogen and oxygen, which are produced at the electrodes due to electrolysis. The presence of these nascent gases in solution or working space of the cell is undesirable because they might spoil the material in the solution. Their accumulation in the buffer compartment might also increase the pressure which would be transmitted to the solution space through the porous membranes causing disturbance in the laminar convective flow.

Mathematical Descriptions

With the above qualitative description of the principle and operation of the continuous-flow electrophoresis column, one postulates a phenomenological description of the electrophoresis column. Many mathematical approaches have been employed in the case of thermal diffusion. These do not differ in principle but in rigor of the analysis. The one largely used in thermal diffusion by most workers is the procedure followed by Furry, Jones, and Onsager (18). The basic equation obtained by them for the mathematical description of a thermal diffusion column was a partial differential equation with non-constant coefficients which was difficult to solve analytically. Furry, Jones and Onsager, therefore, considered the operation of a batch thermogravitational column and obtained an approximate expression to describe its behavior. The resulting equation, obtained by reduction of the general nonlinear partial differential equation is commonly called the transport equation. This method was modified

by Bardeen (2) and others and extended to flow columns (78, 48). This transport equation for thermal diffusion was developed by considering the net transport of one component of a binary system passing through a plane perpendicular to the walls of a batch thermal diffusion column. This transport is the net result of the combination of convection and diffusion:

$$\tau_{Th} = \int_{-\omega}^{\omega} B\rho C_1 \, v(x) \, dx - \int_{-\omega}^{\omega} B\rho D \frac{\partial C_1}{\partial y} \, dx \qquad 3\text{-a}$$

where

τ_{Th} - Transport of the component up the column, gm./min.

B - Column width, cm.

v - Velocity, cm./sec.

ρ - Average density of fluid, gm./ml.

D - Molecular diffusivity, $cm.^2/sec.$

C_1 - Concentration of component labelled 1.

Integration of this expression and incorporation of the boundary conditions at the walls, together with several approximations yielded the following expression:

$$\tau_{Th} = H_{Th}\overline{C}_1(1 - \overline{C}_1) - K_{Th} \frac{d\overline{C}_1}{dy} \qquad 3\text{-b}$$

wherein

H_{Th} - A column parameter, gm./min.

K_{Th} - Another column parameter, gm.-cm./min.

$\bar{C}_1$ - Concentration of component labelled 1 at x = 0

with $$H_{Th} = B\rho \left(\frac{g\beta_T \Delta T}{\eta} \right) \left(\frac{\alpha_{Th} \Delta T}{\bar{T}} \right) \frac{(2\omega)^3}{6!} \qquad 3\text{-c}$$

$$K_{Th} = K_{c_{Th}} + K_{d_{Th}} \qquad 3\text{-d}$$

$$K_{c_{Th}} = \left(\frac{B\rho}{D} \right) \left(\frac{g\beta_T \Delta T}{\eta} \right)^2 \frac{(2\omega)^7}{9!} \qquad 3\text{-e}$$

$$K_{d_{Th}} = B\rho D 2\omega \qquad 3\text{-f}$$

where g - Acceleration due to gravity

β_T - Coefficient of thermal expansion

η - Viscosity

H_{Th} - Column constant

$K_{c_{Th}}$ - Column constant

$K_{d_{Th}}$ - Column constant

K_{Th} - Column constant

$\bar{T}$ - Average temperature

ΔT - Temperature difference

2ω - Spacing between the plates

α_{ls} - Thermal diffusion constant

D - Ordinary diffusion coefficient

This is the transport equation as presented by Furry, Jones and Onsager (18) for a thermal diffusion column. In the rest of this discussion, this approach will be referred to as the FJO procedure.

Kirkwood et al. (24) employed a more thorough

analytical approach in the mathematical analysis of an electrophoresis convection column with reservoirs. They developed this theory to account for the convection brought about by the concentration difference occurring because of the electric field and did not consider a temperature difference. In their derivation (Appendix E) a quasistationary state was assumed, in which the velocity and concentrations were functions only of vertical and horizontal position and did not change with time in the vertical channel or column connecting the reservoirs. This transport process was then subjected to a boundary condition allowing a slow accumulation of material in the reservoirs. The solution of these equations described the transport of a mobile component from the top to the bottom reservoir. The assumption of stationary state yields an invariant concentration at the ends, and requires constant net flux down the column. Since the net flux at the ends must be zero at steady state, the net flux everywhere becomes zero. Kirkwood's results cannot accommodate such a condition. For the steady state continuous flow case, the net flow would be constant, not zero, and should collapse to the batch case as the external flow decreases toward zero, and his equations cannot be solved for this condition.

It is therefore concluded that the analysis of Kirkwood cannot be used to describe the steady state batch case or continuous-flow case of an electrophoresis column without reservoirs. The nonlinear equations obtained for a

multicomponent system could however be solved numerically for either case, but this procedure would be very tedius.

On the other hand, the FJO procedure of analysis referred to earlier can accommodate the boundary conditions required by the steady state batch case as well as the continuous-flow case. Moreover, even though it is approximate, it yields for the transport of a component in a column, a nonlinear differential equation, which can be further simplified by additional approximation to an ordinary first order linear differential equation which is capable of not only analytic solution, but of varied manipulations as well, in order to accommodate a variety of cases of column operation. Thus, this procedure has been successfully utilized to describe the behavior of thermogravitational thermal diffusion columns under several conditions. For example it has been successfully used by Powers and his associates in thermal diffusion (7,17,48) to describe the behavior of a transient batch column, and continuous-flow column with or without vertical or horizontal barriers in liquid thermal diffusion. The FJO transport equation is also easily modified to describe concentrated, dilute and intermediate binary solution systems.

The important drawback of the original FJO procedure is the lack of proper method for including the effect of the concentration difference or the so called "forgotten effect" in the equations. In thermal diffusion, this was ignored by several authors (7,17,48) but this effect will be of major

magnitude in the case of electrophoresis because of the largeness of the electric field force. This is not, however, a very severe mathematical restriction for the use of the FJO procedure because methods are now available to include the forgotten effect in the mathematical description of the column. Thus in thermal diffusion, first de Groot and associates (10), and later Baldschweiler (1), Von Halle (66) and Horne and Bearman (73) took into account the forgotten effect and obtained suitable mathematical expressions to account for its influence on the separation in thermal diffusion.

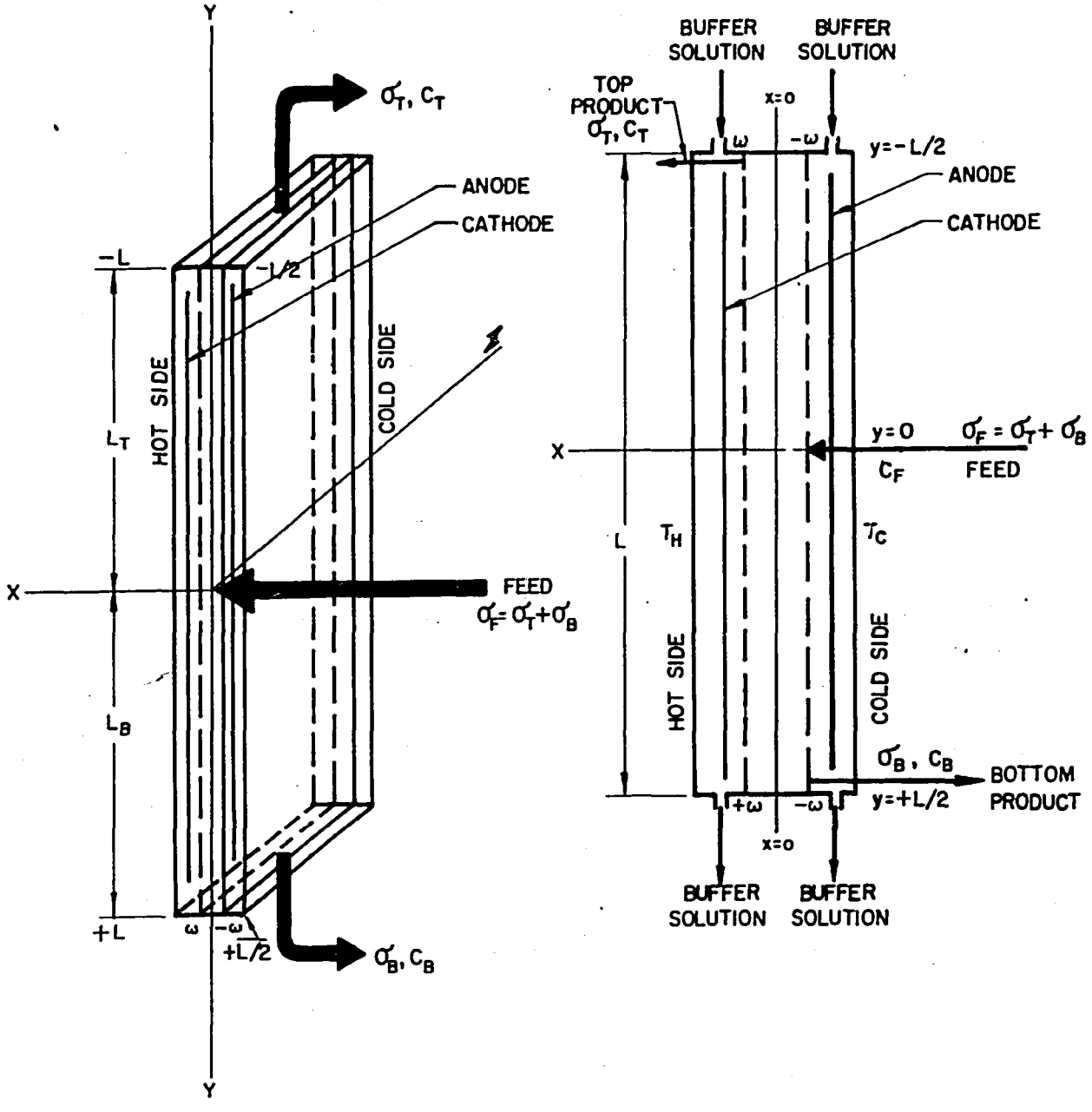
The application of the FJO procedure to multicomponent systems has been attempted for gaseous mixtures (81,82), but there appears to be no attempt to apply it to liquid mixtures in a general way. In electrophoresis one generally encounters a multicomponent system for separation and, therefore, one might reason the FJO procedure could not be used to describe the behavior of an electrophoresis column. This difficulty can, however, be surmounted, as explained later in this chapter, by reducing the multicomponent system to an appropriate two component system to which the FJO procedure can be applied. In order to accommodate the various column parameters, such as spacing, flow rate, temperature difference, field strength and to cover a variety of conditions of operation of the electrophoresis column, the FJO procedure will be exclusively followed for the mathematical description of the electrophoresis column.

Both steady state flow and transient batch operation will be considered in this thesis.

In this work a single protein in solution system has been used for experimental study of the performance of an electrophoresis column. However, the first steps of mathematical formulation are presented in a general context by considering initially a multicomponent system.

Consider a solution at a certain pH in which there are several components (for example proteins) P_1, P_2, P_3 . . . P_n having electric mobilities U_{1s}, U_{2s}, U_{3s} . . . U_{ns}. The solution is placed in batch thermoelectrogravitational column which has a direct current electric field and a temperature field. Figure 3-3 illustrates the co-ordinate system that is used for mathematical description of the electrophoresis column. The two membranes are separated by a distance equal to 2ω and the column is imagined to be separated into two sections vertically at the center line $x = 0$. The electrophoresis of proteins is generally carried out in alkaline solution. Under these conditions, they move toward the anode in the field, (with mobilities conventionally signed negative) the anode side being kept at a lower temperature. Communication of ions through the membranes maintains the pH of the solution.

The application of the electric field and the temperature gradient brings about two effects: (1) A flux of each mobile component of the solution relative to other components (one or many) will be brought about and



COORDINATE SYSTEM
FOR
CONTINUOUS-FLOW ELECTROPHORESIS COLUMN

FIGURE 3-3

(2) convection currents will be produced parallel to the membranes. These are caused by density differences arising in the solution because of the concentration change due to migration of the components at different velocities. The temperature gradient (if properly applied) will reinforce both the fluxes and the convection. Thermal diffusion fluxes in the horizontal direction will be very small compared to the electrodiffusion fluxes. At low field strengths and high temperature differences, convection reinforcement by the temperature gradient may, however, be comparable to that caused by the concentration gradient, because of the density differences between the hot wall and cold wall.

In contrast with thermal diffusion columns, there will occur in electrophoresis columns, a heat generation effect because of the electric current passing through the solution. The temperature profile will therefore be different. At higher temperature the proteins and most other biological systems may spoil and one would be required to avoid some maximum in the temperature profile within the solution space. This could be done by maintaining sufficiently high temperature differences between the two membranes, so that heat passes only in one direction from the hot membrane to the cold membrane.

In the electrophoresis process, it is also necessary to use sufficiently dilute solutions in order to have better movement of ions and the charged particles. The solvent acts as the supporting medium in which the particle moves.

Only dilute solutions will, therefore be considered in the formulation of the mathematical theory.

The mathematical formulation of the problem can now be made. In an ideal column, both the voltage and temperature gradients will exist in a direction normal to the membranes. The fluxes of component 1 due to the various diffusional forces in a solution containing other mobile components and a solvent (usually buffer solution) are given by (see Figure 3-4):

In the x direction:

Flux due to electrophoretic diffusion:

$$J_{x\text{-}ED} = \rho\left[-U_{1s}\left(\frac{dV}{dx}\right)\right]C_1C_s + \rho\left[-U_{12}\left(\frac{dV}{dx}\right)\right]C_1C_2 \ldots + \rho\left[-U_{1n}\left(\frac{dV}{dx}\right)\right]C_1C_n \qquad 3.1$$

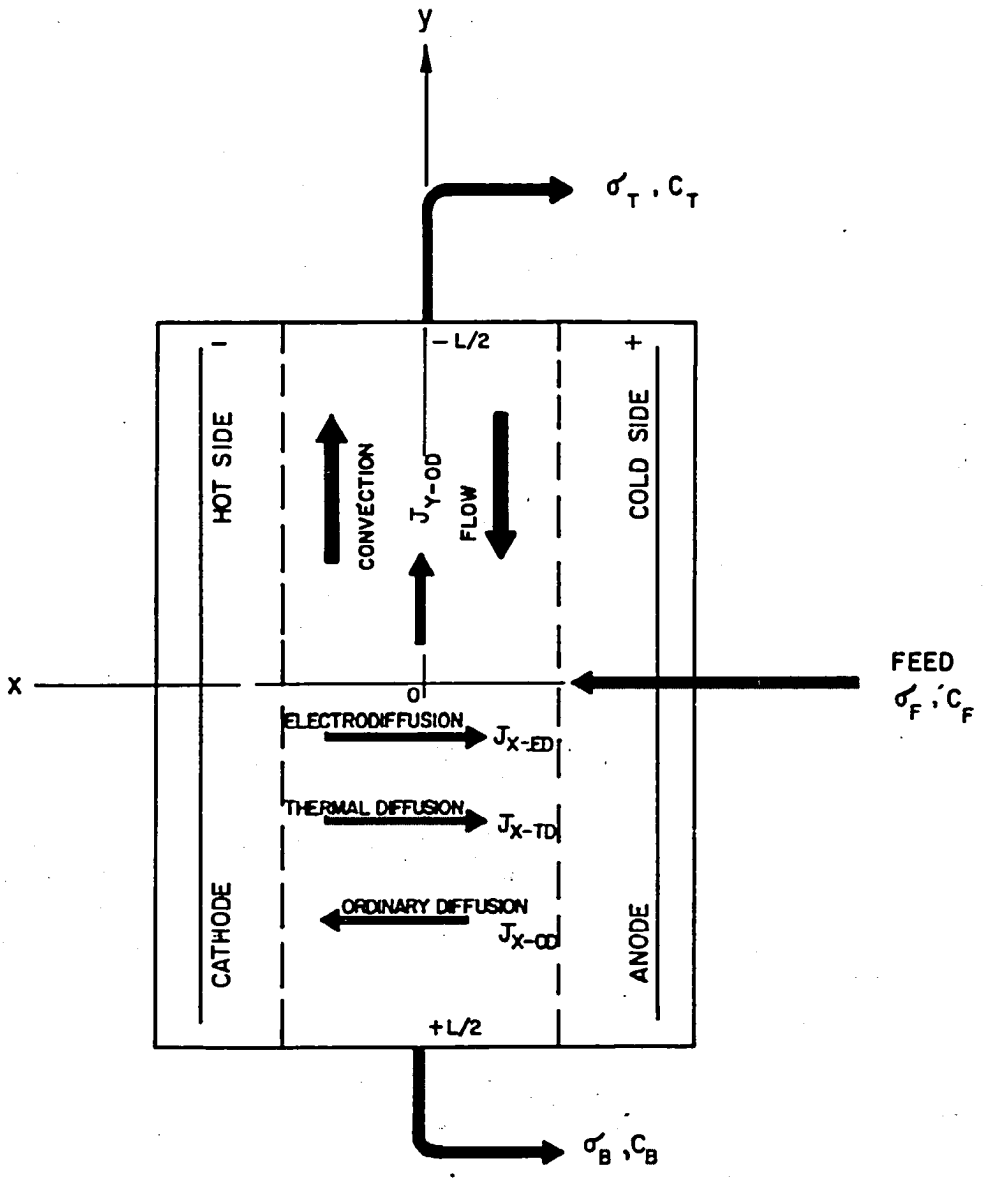
Flux due to thermal diffusion:

$$J_{x\text{-}TD} = -\rho\left[\frac{\alpha_{1s}D_{1s}}{\bar{T}}\right]C_1C_s\frac{dT}{dx} - \rho\left[\frac{\alpha_{11}D_{11}}{\bar{T}}\right]C_1C_2\frac{dT}{dx} + \ldots - \rho\left[\frac{\alpha_{1n}D_{1n}}{\bar{T}}\right]C_1C_n\frac{dT}{dx} \qquad 3.2$$

Flux due to ordinary diffusion:

$$J_{x\text{-}OD} = -\rho D_{1s}\frac{\partial C_1}{\partial x} - \rho D_{12}\frac{\partial C_1}{\partial x} - \rho D_{13}\frac{\partial C_1}{\partial x} \ldots - \rho D_{1n}\frac{\partial C_1}{\partial x} \qquad 3.3$$

In the y direction:



FLOWS AND FLUXES
IN
CONTINUOUS-FLOW THERMOELECTROGRAVITATIONAL
ELECTROPHORESIS COLUMN

FIGURE 3–4

Flux due to ordinary diffusion in the y direction:

$$J_{y\text{-}OD} = -\rho D_{1s} \frac{\partial C_1}{\partial y} - \rho D_{12} \frac{\partial C_1}{\partial y} - \rho D_{13} \frac{\partial C_1}{\partial y} \ldots - \rho D_{1n} \frac{\partial C_1}{\partial y} \qquad 3.4$$

1) It will be assumed that there are no gradients in the z direction.* This implies the assumption that the width is infinite in z direction therefore, edge effects are negligible. In above equations, U_{1s}, U_{2s}, U_{3s}, . . . U_{ns} are the electrical mobilities of the components, α_{1s}, α_{12} . . . α_{1n} are the binary thermal diffusion coefficients, D_{1s}, D_{12} . . . D_{1n} are binary ordinary diffusion coefficients, C_1, C_2 . . . C_n are mass fractions of the mobile components. The other symbols are defined:

V - Voltage at a point along the x direction

T - Absolute temperature which is a function of x

$\overline{T}$ - Average absolute temperature

x - The axis normal to the membranes

y - The axis parallel to the membranes

C_s - Mass fraction of the solvent in solution

The above equations have been written for one mobile component in a multicomponent system following conventional diffusion theory. However, in order to be able to apply the FJO procedure in a simple manner a multicomponent system should be reduced to a two component system consisting of

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*The assumptions which will be made in the development of the mathematical theory will all be numbered with numbers in half right brackets.

one mobile component and a solvent (the buffer solution) or two mobile components only. As mentioned earlier in this chapter, three main methods are followed for the electrophoretic separation of the proteins.

In the differential and reverse transport methods of electrophoretic separation, it is possible to reduce the multicomponent system to a two component system by treating the one desired species as one component and all other species together with the solvent as the other component. System properties such as the electrical mobility and ordinary diffusivity are to be calculated on the mass average basis. Even though the mobility and diffusivity of the solvent are zero, they should be taken into account when calculating the mass average properties of the hypothetical component in which the solvent is included.

In the isoelectric procedure of operation of the electrophoresis separation process, the reduction of the multicomponent system could be done in a similar manner. In this case, it would be done by considering as one component the isoelectric species together with the solvent (as both are immobile under the influence of electric field) and all the mobile species together as the other component, and using mass averaged properties for electrical mobility and an ordinary diffusivity for this hypothetical component.

The isoelectric procedure is by far the most efficient since it is capable of giving complete electrophoretic purity of at least one component, while only partial separation

could be achieved by the methods of differential transport or the reverse transport method. The isoelectric procedure would give a particular component in an electrophoretic purity in one or two steps of electrophoretic fractionation, while several steps would be required by the other two methods. Since the isoelectric procedure is preferentially followed wherever possible, in the present mathematical formulation, only the isoelectric procedure is considered and the equations will be derived for a two component system in which one hypothetical component is mobile while the other is immobile (solvent + isoelectric component).

Note here, that in writing the previous equations, it was assumed that the electrodiffusion flux of one component was a function not only of its own concentration but also of the concentration of the other components present in solution. Theoretically, such a postulate implies that the phenomenological theory of diffusion in electrophoresis can be developed using the binary electric mobilities of the components. The total electrodiffusion flux of a component is, therefore, given by the sum of all the binary electrodiffusion fluxes. In a solution where components 1,2,3 . . . n are in very small amount (dilute solution), the diffusion fluxes of a component with respect to the other mobile components will be negligible as compared to its diffusion with respect to the solvent, since the mass fractions of components C_1, C_2 . . . C_n will be small and their products such as C_1C_2, C_1C_3 . . . C_1C_n will be smaller

still. Similarly $U_{1s} >> U_{12}, U_{13}, \ldots U_{1n}$ and therefore all terms except the first in equation 3.1 can be neglected in each case. All other terms in the equation 3.1, 3.2, 3.3 except the first one will, therefore, be neglected and the total flux in each case will now be written as:

$$J_{x\text{-}ED} = \rho \left[-U_{1s} \left(\frac{dV}{dx} \right) \right] C_1 C_s \qquad 3.5$$

$$J_{x\text{-}TD} = \rho \left[- \frac{\alpha_{1s} D_{1s}}{\overline{T}} \left(\frac{dT}{dx} \right) \right] C_1 C_s \qquad 3.6$$

$$J_{x\text{-}OD} = -\rho D_{1s} \frac{\partial C_1}{\partial x} \qquad 3.7$$

$$J_{y\text{-}OD} = -\rho D_{1s} \frac{\partial C_1}{\partial y} \qquad 3.8$$

The equations 3.5 to 3.8 can be combined with a function that represents the convective velocity to obtain a partial differential equation which describes the concentration as function of the time and the two coordinates x and y. In order to simplify the resulting equations, the following additional assumptions will be made:

2) Temperature and voltage gradient occur only in the x direction.
3) The convective velocity is not a function of y and the end effects can be neglected.
4) In order to evaluate the properties of the solution in the column such as average viscosity and a mean average density, an integral average temperature $\overline{T}$ may be used.

5) The electric mobility, the thermal diffusion constant and the ordinary diffusivity are all constant at all positions in the column.

The first three assumptions should be reasonable for a carefully constructed column with a width (in the z direction) very much greater than the spacing between the membranes (in the x direction). The third, fourth and fifth assumptions seem justified if separations, electrical field strengths and temperature differences are not large. For example, Emery (14) has shown that ordinary diffusivity is constant for the range of temperature differences usually encountered in liquid thermal diffusion columns.

Theoretical Development

With these assumptions, and previously defined fluxes, a material balance around a differential element of the solution space over an infinitesimal time increment yields the partial non-linear differential equation having one term with a non-constant coefficient:

$$\frac{\partial C_1}{\partial t} = D_{1s}\left(\frac{\partial^2 C_1}{\partial x^2} + \frac{\partial^2 C_1}{\partial y^2}\right) + \left(\frac{\alpha_{1s} D_{1s}}{T}\frac{dT}{dx} + U_{1s}\frac{dV}{dx}\right)\frac{\partial C_1 C_s}{\partial x} - v(x)\frac{\partial C_1}{\partial y} \qquad 3.9$$

Implicit in the above derivation is the assumption 6) that the migratory components behave independently without any appreciable interaction with each other and therefore all processes are governed solely by the properties of the

individual component. Thus, the movement or transport of one component will not be hindered by other components and will be regulated by only the convective velocity and diffusion coefficients.

At the membrane walls, the net transfer of the mobile component due to ordinary, electric and thermal diffusion must be zero since the membrane is semipermeable and does not allow molecules of either the mobile component or the isoelectric component to pass through its pores. Therefore, any solution to Equation 3.9 should be subject to the boundary condition that:

$$-D_{ls}\frac{\partial C_l}{\partial x} + \left(U_{ls}\frac{dV}{dx} + \frac{\alpha_{ls}D_{ls}}{\bar{T}}\frac{dT}{dx} \right) = 0 \quad \text{at } x = +\omega, -\omega \qquad 3.10$$

(Hereafter the subscripts on D_{ls} and U_{ls} will be dropped for convenience since only one component is considered. However subscripts on α_{ls} will not be dropped to avoid confusion from α defined later in this chapter.) Moreover, the solution to equation 3.6 must also satisfy the material balance:

$$\tau = B\int_{-\omega}^{\omega} \rho C_l\, v(x)\, dx - B\int_{-\omega}^{\omega} \rho D\frac{\partial C_l}{\partial y}\, dx \qquad 3.11$$

where B is the column width, cm. (a constant)

ρ is the average density, in gm./cm.^{3} at the integrated average temperature $\bar{T}$

and τ is the net transport of component 1 down the column

The net transport τ is obviously the difference

between the transport resulting from vertical convection and that due to vertical ordinary diffusion. It will be assumed at this stage that:

7) A mean density of the solution can be used to convert volumetric flow rates to mass flow rates.

Equation 3.9, 3.10, 3.11 represent a mathematical description of the processes that take place within the thermoelectrogravitational electrophoresis column.

Because equation 3.9 is a nonlinear second order partial differential equation, a rigorous general solution would be extremely difficult to obtain. It has already been mentioned that for a thermogravitational thermodiffusion column, Furry and Jones and Onsager (18) have shown that equations of the type 3.9, 3.10, 3.11 can be reduced to an ordinary differential equation of first order in y with constant coefficients, which is known as the "Transport Equation". This procedure appears to be reasonable for electrophoresis columns as well because the concentration change in the x direction in such columns would be small compared with the total concentration difference in the y-direction. The basic development for a batch column was extended to flow columns by Jones and Furry (77) and later Powers (47) by making a slight modification in the method. A similar procedure has been followed in this work; the only difference is the superposition of one additional electrophoresis diffusion force upon the thermal diffusion field force across the solution in the horizontal direction.

Derivation of the Transport Equation for Batch and Continuous-Flow Thermoelectrogravitational Electrophoresis Column

In the previous section, a general non-linear partial differential equation describing the electrophoresis column operation has been obtained. The present section will describe the method of obtaining the transport equation to describe batch and continuous-flow operations of the thermoelectrogravitational electrophoresis column.

The form of equation 3.9 is simplified to considerable extent by neglecting vertical diffusion as compared to transport by vertical convection giving:

8) $$D \frac{\partial^2 C_1}{\partial y^2} << v(x) \frac{\partial C_1}{\partial y} ;$$

by considering only the steady state solution so that

9) $$\frac{\partial C_1}{\partial t} = 0 ;$$

and by assuming 10) laminar convective flow so that convective back mixing can be neglected.

The voltage gradient $\frac{dV}{dx}$ can be replaced by E, the electrical field strength by definition and 11) this will be assumed constant. Also 12) it will be assumed that $\frac{dT}{dx}$ can be replaced by an average value $\overline{\frac{dT}{dx}}$ to take into account the dependence of T on x.* When these assumptions are applied to the equation 3.9 one finds:

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*This assumption will be justified later in detail.

$$D \frac{\partial^2 C_1}{\partial x^2} + \left(UE + \frac{\alpha_{1s} D}{\overline{T}} \cdot \frac{\overline{dT}}{dx} \right) \frac{\partial C_1 C_s}{\partial x} - v(x) \frac{\partial C_1}{\partial y} = 0 \qquad 3.12$$

The boundary conditions of equation 3.10 are also modified to give

$$-D \frac{\partial C_1}{\partial x} + \left(UE + \frac{\alpha_{1s} D}{\overline{T}} \frac{\overline{dT}}{dx} \right) C_1 C_s = 0 \qquad \text{at } x = +\omega, -\omega \qquad 3.13$$

It may be mentioned here that the vertical diffusion term is retained in the material balance condition given by the equation 3.11 but is discarded in the partial differential equation 3.9.

At this point in their development, Furry, Jones and Onsager integrate the first term on the right of equation 3.11 by parts. This yields for the net transport of component 1, down the column:

$$\tau = B\rho \left[C_1 \int_{-\omega}^{\omega} v(x)\, dx \right]_{-\omega}^{\omega} - B\rho \int_{-\omega}^{\omega} \frac{\partial C_1}{\partial x} \left[\int_{\omega}^{x} v(x)\, dx \right] dx$$

$$- B \int_{-\omega}^{\omega} \rho D \frac{\partial C_1}{\partial y}\, dx \qquad 3.14$$

Batch Operation

The first term on the right of equation 3.14 vanishes at both limits for the batch case so equation 3.14 becomes:

$$\tau = -B\rho \int_{-\omega}^{\omega} \frac{\partial C_1}{\partial x} \left[\int_{-\omega}^{x} v(x)\, dx \right] dx - B \int_{-\omega}^{\omega} \rho D \frac{\partial C_1}{\partial y}\, dx \qquad 3.15$$

A partial integration of equation 3.12 with respect to x gives:

$$D\frac{\partial C_1}{\partial x} + \left(UE + \frac{\alpha_{1s} D}{\overline{T}} \frac{\overline{dT}}{dx} \right) C_1 C_s - \int_{-\omega}^{x} v(x) \frac{\partial C_1}{\partial y} dx = f(y) \qquad 3.16$$

But from the boundary condition at $x = -\omega$ (equation 3.13), the constant of integration, $f(y)$, is zero so that

$$D\frac{\partial C_1}{\partial x} + \left(UE + \frac{\alpha_{1s} D}{\overline{T}} \frac{\overline{dT}}{dx} \right) C_1 C_s - \int_{-\omega}^{x} v(x) \frac{\partial C_1}{\partial y} dx = 0 \qquad 3.17$$

or dividing throughout by D and rearranging:

$$\frac{\partial C_1}{\partial x} + \left(\frac{UE}{D} + \frac{\alpha_{1s}}{\overline{T}} \frac{\overline{dT}}{dx} \right) C_1 C_s = \frac{1}{D} \int_{-\omega}^{x} v(x) \frac{\partial C_1}{\partial y} dx \qquad 3.18$$

In order to satisfy the condition at $x = +\omega$, the following relation must hold:

$$\int_{-\omega}^{\omega} v(x) \frac{\partial C_1}{\partial y} dx = 0 \qquad 3.19$$

Moreover, if it is assumed that $\frac{\partial C_1}{\partial y}$ is independent of x, equation 3.19 can be satisfied only for conditions of no bulk flow or batch column operation.

Now substituting into equation 3.15, the expression for $\frac{\partial C_1}{\partial x}$ obtained in equation 3.18, one can get:

$$\tau = -B\rho \int_{-\omega}^{\omega} \left[- \left(\frac{UE}{D} + \frac{\alpha_{1s}}{\overline{T}} \frac{\overline{dT}}{dx} \right) C_1 C_s + \frac{1}{D} \int_{-\omega}^{x} v(x) \frac{\partial C_1}{\partial y} dx \right] \int_{-\omega}^{x} v(x)\, dx\, dx \qquad 3.20$$

Now assume:

13) that the product C_1C_s is essentially independent of x

14) that $\frac{\partial C_1}{\partial y}$ is independent of x so that all x dependence is retained within the integrals, and

15) that the mean concentration at x = 0 (center line) will be satisfactory in defining the y dependence of composition.

Equation 3.20 then becomes:

$$\tau = \overline{C_1C_s}\left\{\left[B\rho\left(\frac{UE}{D} + \frac{\alpha_{1s}}{\overline{T}}\frac{\overline{dT}}{dx}\right)\right]\int_{-\omega}^{\omega}\int_{-\omega}^{x} v(x)\,dx\,dx\right\} - \frac{d\overline{C_1}}{dy}\left\{\left[\frac{B\rho}{D}\int_{-\omega}^{\omega}\left(\int_{-\omega}^{x} v(x)\,dx\right)^2 dx\right]\right\} - \left\{B\rho D\int_{-\omega}^{\omega} dx\right\} \qquad 3.21$$

In equation 3.21, superbars indicate the mean value of C taken at x = 0.

It is observed here from equation 3.21 that all groups within brackets contain definite integrals. Thus, given a velocity distribution v(x), all expressions could be integrated to yield constants. Let the groupings be defined as:

$$H = B\rho\left(\frac{UE}{D} + \frac{\alpha_{1s}}{\overline{T}}\frac{\overline{dT}}{dx}\right)\int_{-\omega}^{\omega}\int_{-\omega}^{x} v(x)\,dx\,dx \qquad 3.22$$

$$K_c = \frac{B\rho}{D}\int_{-\omega}^{\omega}\left[\int_{-\omega}^{x} v(x)\,dx\right]^2 dx \qquad 3.23$$

$$K_d = B\rho D\int_{-\omega}^{\omega} dx \qquad 3.24$$

Further the K definitions can be combined into

$$K = K_c + K_d \qquad 3.25$$

The above expressions are similar in form to those obtained for the thermogravitational column, (18,88,48). The final expressions for the above constants will depend upon the nature of the velocity profile v(x), which will be discussed later to evaluate the integrals given by equation 3.22 and 3.23.

In equation 3.22, the term $\frac{UE}{D}$ represents the electrophoresis term while the term $\frac{\alpha_{1s}}{T}\frac{\overline{dT}}{dx}$ represents the thermal diffusion term. In the case of proteins, the thermal diffusion coefficient α_{1s} will be very small compared to the electric mobility of the protein and for all practical purposes 16) the thermal diffusion term may safely be ignored. The expression

$$H = B\rho\left(\frac{UE}{D}\right)\int_{-\omega}^{\omega}\int_{-\omega}^{x} v(x)\,dx\,dx \qquad 3.26$$

With these definitions of the column constants, the equation for the transport of the component 1 down the column will be given by the relation

$$\tau = H\,\overline{C_1 C_s} - K\frac{d\overline{C_1}}{dy} \qquad 3.27$$

in which $\frac{dC_1}{dy}$ is now an ordinary and not a partial derivative since it was assumed independent of x and, therefore, is a function of y alone. Note that equation 3.27 is simple in form but is still a nonlinear differential equation because

of the presence of the product term $\overline{C_l C_s}$.

This is the transport equation for a batch thermoelectrogravitational electrophoresis column. For steady state batchwise operation, the transport τ given by equation 3.27 is equal to zero.

The transport equation 3.27 has been derived for the case of a batch thermoelectrogravitational electrophoresis column with the assumption of a steady state batch operation. With certain modifications, it can also be used to describe the continuous-flow and transient modes of operation of the electrophoresis column. Its application to continuous-flow case is discussed in the section that follows. The use of the transport equation to describe the transient behavior of the column will be discussed later in this chapter.

Continuous-Flow Operation

At this point it is suggested (cf. Jones and Furry (80) and Powers (48)) because of the assumption that the velocity is independent of y, an additional linear transport may be impressed in the y direction in order to account for continuous removal of product. The values of H and K as defined by equations 3.26 and 3.23, 3.24 and 3.25 are applicable strictly to the batch case. However, for the flow case, they may be evaluated using batch velocity profiles if the net removal of product from the column is small compared to the internal convective flow (18,48). With this stipulation, when a linear transport σ_B gm./min. is impressed

in the y direction in the enriching (bottom) section, one obtains the equation:

$$\tau_B = H_B \overline{C_1 C_s} - K_B \frac{d\overline{C_1}}{dy} + \sigma_B \overline{C_1} \qquad 3.28$$

where σ_B is the impressed flow rate in gm. per unit time, τ_B is the new net transport down the column, and H_B and K_B are the column constants for the bottom section. Since the total amount removed from the bottom of the column must be the total net transport, equation 3.28 can be simplified by putting $\sigma_B \overline{C_B}$ for τ_B for the case of the steady state flow.

$$\sigma_B \overline{C_B} = \tau_B = H_B \overline{C_1 C_s} - K_B \frac{d\overline{C_1}}{dy} + \sigma_B \overline{C_1} \qquad 3.29$$

In these equations the subscript B indicates the bottom section of the column and $\overline{C_B}$ is the concentration of component 1 leaving the enriching (bottom) section. Rearrangement of the equation 3.29 for the bottom section of the column yields the equation:

$$\sigma_B (\overline{C_B} - \overline{C_1}) = H_B \overline{C_1 C_s} - K_B \cdot \frac{d\overline{C_1}}{dy} \qquad 3.30$$

Similar reasoning applied to the top section of the electrophoresis column gives:

$$-\sigma_T (\overline{C_T} - \overline{C_1}) = H_T \overline{C_1 C_s} - K_T \frac{d\overline{C_1}}{dy} \qquad 3.31$$

where $\overline{C_T}$ is the concentration of component 1 leaving the top section of the column. The equations 3.30 and 3.31 are the final forms of the transport equation for the continuous-flow

electrophoresis column.

It should be emphasized here, that the electrophoretic diffusion does not introduce much change in the form of the equations, but that increases in values of H and K are obtained. Solution of the transport equations will, therefore, follow the same pattern as in the case of thermodiffusion column. In the subsequent section of this chapter it will be shown how equations 3.30 and 3.31 can be utilized for several different cases of column operation. First, however, the evaluation of constants H and K will be considered.

Evaluation of the Constants H and K for the Batch Case

For the purposes of simplification in the interpretation of the column data it will be assumed that H and K hold the same values for both the top and bottom section of the column. An examination of the expressions for H and K reveals that their evaluation rests primarily on obtaining an expression for the velocity distribution $v(x)$. If it is assumed that the flow is Newtonian, then the Navier-Stokes equation for a Newtonian fluid is the starting point for the derivation of the desired velocity distribution. The Navier-Stokes equation can be written as:

$$\frac{\eta}{g_c}\frac{d^2v(x)}{dx^2} - \frac{dP}{dy} - \frac{\rho g_c}{g} = 0 \qquad 3.32$$

If one differentiates the above equation with respect to the variable x, one obtains:

$$\frac{d^3v(x)}{dx^3} - \frac{g}{\eta}\frac{d\rho}{dx} - \frac{g_c}{\eta}\frac{\partial}{\partial x}\left(\frac{dP}{dy}\right) = 0 \qquad 3.33$$

or

$$\frac{d^3v_1(x)}{dx^3} = \frac{g}{\eta}\left(\frac{d\rho}{dx}\right) \qquad 3.34$$

since $\frac{dP}{dy}$ is not a function of the variable x.

The derivative of ρ with respect to x will depend upon not only the temperature but also on the concentration distribution and, in general, will be given by the expression:

$$\frac{d\rho}{dx} = \left(\frac{\partial\rho}{\partial T}\right)\frac{dT}{dx} + \sum_{J=1}^{n}\left(\frac{\partial\rho}{\partial\bar{C}_J}\right)\left(\frac{\partial\bar{C}_J}{\partial x}\right) \qquad 3.35$$

Wherein n is the total number of components. The derivative $\frac{\partial\rho}{\partial\bar{C}_s}$ will be taken as zero since it will be assumed that the solvent concentration $\bar{C}_s$ will be nearly equal to 1 at all positions in the column.

The equation 3.35 reduces for the case of a single mobile component in a solution to:

$$\frac{d\rho}{dx} = \left(\frac{\partial\rho}{\partial T}\right)\frac{dT}{dx} + \frac{\partial\rho}{\partial\bar{C}_1}\cdot\frac{\partial\bar{C}_1}{\partial x} \qquad 3.36$$

The first term in equation 3.36 is a measure of the change in density because of a temperature gradient. In thermal diffusion the value of $\frac{dT}{dx}$ is obtained as $\frac{\Delta T}{2w}$ because of the linearity of the temperature distribution in x direction. In the case of electrophoresis, since the temperature distribution will be affected by the heat generated by the electrical current passing through the

solution, an integrated average value $\overline{\frac{dT}{dx}}$ of $\frac{dT}{dx}$ was used in the derivation of the material balance equation 3.9. Here, however, for the calculation of the velocity distribution, the effect of the heat generated in the column will be taken into consideration.

The second term is the measure of change in density caused by the concentration change and in thermal diffusion it is customarily called the "Forgotten Effect". When interpreting thermal diffusion column data, this effect is usually assumed negligible. This may be a poor approximation even in thermal diffusion as is pointed out by several authors (1,10,22,66) and it can lead to an appreciable error particularly in case of large plate spacings as is pointed out by de Groot (10). After de Groot et al. (10) obtained, by suitable approximations, an expression for $\overline{\frac{\partial C_1}{\partial x}}$ in the equation 3.36, the forgotten effect has been taken into consideration by Von Halle (66), Baldschweiler (1) and recently by Horne and Bearman (22). Electrophoretic field force is many times stronger than thermal diffusion field force and since its effect is reflected in the concentration difference that results, the concentration gradient term will not be negligible for an electrophoresis column. The present development of the velocity profile will therefore include the effect of heat generation and that of concentration gradient on the density gradient. Therefore in sections that follow immediately the evaluation of the two terms on the right hand side of equation 3.36 is considered first.

Evaluation of the Temperature Distribution

The temperature distribution will affect the velocity profile. The determination of the temperature distribution must include the heat generated by the passage of the electrical current. To obtain an energy balance on an incremental volume of solution space, several assumptions will be made. These are: 17) The steady state with respect to both the transfer of heat and the establishment of the voltage gradient is reached so that the temperature field is stabilized quickly, and 18) that although there is laminar convection in the column, heat transfer takes place only in the direction normal to the plane of membranes. 19) The thermal conductivity of the solution k is constant. Because of the passage of the direct current through the solution an amount of heat Q cal./cm.^{3} of solution space is generated during electrophoresis. This can be calculated if the electrical conductivity of the solution is known using the experimental values of the voltage and the current. 20) It will be assumed that for the dilute solution of the protein to be used in the present work the electrical conductivity is constant.

The resulting equation is:

$$\frac{d^2T}{dx^2} + \frac{Q}{k} = 0 \qquad 3.37$$

This equation must be solved subject to the following boundary conditions:

$$T = T_1 \text{ at } x = +\omega \qquad 3.38$$

$$T = T_2 \text{ at } x = -\omega \qquad 3.39$$

where T_1 - the temperature at the hot membrane

and T_2 - the temperature at the cold membrane.

Two successive integrations give the solution of equation 3.37:

$$T = -\frac{Q}{k}\frac{x^2}{2} + F'x + G' \qquad 3.40$$

The constants F' and G' are evaluated using the boundary conditions 3.38 and 3.39 and the final expression for the temperature is obtained as follows:

$$T = \frac{Q}{2k}(\omega^2 - x^2) - \frac{\Delta T}{2\omega}x + \frac{T_1 + T_2}{2} \qquad 3.41$$

where $$\Delta T \equiv T_1 - T_2 \qquad 3.42$$

It will be noticed from equation 3.41 that the temperature distribution is parabolic and not linear as in the case of the thermal diffusion column. When $Q = 0$, the equation 3.41 reduces to

$$T = -\frac{\Delta T}{2\omega}x + \frac{T_1 + T_2}{2} \qquad 3.43$$

which is linear.

On differentiating the equation 3.41, one obtains an expression for the temperature gradient which is:

$$\frac{dT}{dx} = -\frac{Q}{k}x - \frac{\Delta T}{2\omega} \qquad 3.44$$

and this is the expression to be used to obtain the velocity

distribution.

The average temperature at which the properties such as viscosity, density and diffusivity will be evaluated is obtained by integration of equation 3.41 and is given by:

$$\bar{T} = \frac{1}{2w} \int_{-w}^{w} T \, dx \qquad 3.45a$$

$$\text{or} \quad \bar{T} = \frac{1}{2w} \int_{-w}^{w} \left[\frac{Q}{k} (w^2 - x^2) - \frac{\Delta T}{2w} x + \frac{T_1 + T_2}{2} \right] dx \qquad 3.45b$$

When the integration is carried out and the constant of integration evaluated, one obtains:

$$\bar{T} = \frac{T_1 + T_2}{2} + \frac{Qw^2}{3k} \qquad 3.46$$

The equation 3.46 shows that the integral average temperature will be higher than the arithmetic average temperature by a value of $\frac{Qw^2}{3k}$ and will depend upon the value of Q, the heat generated, and the thermal conductivity of the solution. Q in turn depends upon the current and the electrical resistance of the solution and sets a limit on the value of the current strength that can be used so that the average temperature does not go beyond a certain point at which the protein in solution may get spoiled. In columns of small cross section, therefore, it may not be possible to use high current densities because of the difficulty involved in the removal of heat since small transfer area is available. This matter will appear again when discussing the nature of theoretical

results concerning the column behavior. As stated earlier the temperature gradient $\frac{dT}{dx}$ given by equation 3.44 is to be used in the first term of equation 3.36 for $\frac{\partial \rho}{\partial x}$. Given equation 3.44 for the temperature distribution, one still requires an expression for $\frac{\partial \bar{C}_1}{\partial x}$ in order to evaluate the velocity distribution. For the present it will be assumed that one can use some kind of average value of the concentration gradient $\frac{\partial \bar{C}_1}{\partial x}$ say $\overline{\frac{\partial \bar{C}_1}{\partial x}}$. An expression for its evaluation will be derived later.

Evaluation of the Velocity Distribution

With the use of the expression for $\frac{dT}{dx}$ given by equation 3.44 and the assumed value of $\frac{\partial \bar{C}_1}{\partial x}$ it is now possible to integrate the expression for the velocity distribution. The equation 3.34 is:

$$\frac{d^3v(x)}{dx^3} = \frac{g}{\eta} \cdot \frac{d\rho}{dx} \qquad 3.47$$

or

$$\frac{d^3v(x)}{dx^3} = \frac{g}{\eta} \left[\frac{\partial \rho}{\partial T} \cdot \frac{dT}{dx} + \frac{\partial \rho}{\partial \bar{C}_1} \cdot \frac{\partial \bar{C}_1}{\partial x} \right] \qquad 3.48$$

When the expression for $\frac{dT}{dx}$ given by the equation 3.44 is inserted in the equation 3.48, one obtains the equation:

$$\frac{d^3v(x)}{dx^3} = \frac{g}{\eta} \left[\frac{\partial \rho}{\partial T} \left(-\frac{Q}{k} x - \frac{\Delta T}{2\omega} \right) + \frac{\partial \rho}{\partial \bar{C}_1} \cdot \frac{\partial \bar{C}_1}{\partial x} \right] \qquad 3.49$$

Now if the following relations:

$$\frac{\partial \rho}{\partial T} = -\beta_T \qquad 3.50$$

$$\frac{\partial \rho}{\partial \bar{C}_1} = \beta_C \qquad 3.51$$

are employed in equation 3.49, one obtains the equation:

$$\frac{d^3 v(x)}{dx^3} = \frac{g}{\eta}\left[\frac{\beta_T \Delta T}{2\omega} + \frac{Q}{k}x + \beta_C \overline{\frac{\partial \bar{C}_1}{\partial x}}\right] \qquad 3.52$$

or
$$\frac{d^3 v(x)}{dx^3} = \frac{g}{\eta}\left[\frac{Q}{k}x + \left(\frac{\beta_T \Delta T}{2\omega} + \beta_C \overline{\frac{\partial \bar{C}_1}{\partial x}}\right)\right] \qquad 3.53$$

By assuming that 21) both β_T and β_C remain constant and after two successive integrations of the equation 3.53, one gets the expression for the convective velocity as:

$$v(x) = \frac{g}{\eta}\left\{\frac{\beta_T Q}{24k}x^4 + \frac{1}{6}\left(\frac{\beta_T \Delta T}{2\omega} + \beta_C \overline{\frac{d\bar{C}_1}{dx}}\right)x^3 + A'x^2 + B'x + C'\right\} \qquad 3.54$$

The constants A′, B′, and C′ in the equation 3.54 are to be evaluated by the following boundary conditions:

$$v(x) = 0 \text{ at } x = -\omega, \ \omega \qquad 3.55a$$

and by virtue of the batch operation of the column, the relation:

$$\int_{-\omega}^{\omega} v(x)\,dx = 0 \qquad 3.55b$$

when this is done, the expression for the velocity distribution v(x) results and is given by:

$$v(x) = \frac{g}{\eta}\left\{\frac{\beta_T Q}{24k}x^4 + \frac{1}{6}\left(\frac{\beta_T \Delta T}{2\omega} + \beta_C \overline{\frac{d\bar{C}_1}{dx}}\right)(x^3 - \omega^2 x) - \frac{\beta_T Q \omega^2}{20k}x^2 + \frac{\beta_T Q}{120k}\omega^4\right\} \qquad 3.56$$

Evaluation of $\overline{\frac{d\bar{C}_1}{dx}}$ for Use in Equation 3.56

The expression for $\frac{d\bar{C}_1}{dx}$ can be derived in the same manner as has been done by Von Halle (66). He used the case of batch operation ($\tau_B = \tau_T = 0$) and the same result will be used for the continuous-flow column 22) assuming that at low flow rates it can be applied without introducing much error. By definition:

$$\overline{\frac{\partial \bar{C}_1}{\partial x}} = \frac{1}{2\omega} \int_{-\omega}^{\omega} \frac{\partial \bar{C}_1}{\partial x} \, dx \qquad 3.57$$

If one introduces the expression for $\frac{d\bar{C}_1}{dx}$ from equation 3.18, one gets the following (neglecting thermal diffusion term):

$$\overline{\frac{\partial \bar{C}_1}{\partial x}} = \frac{1}{2\omega} \int_{-\omega}^{\omega} \left[- \frac{UE}{D} \overline{C_1 C_s} + \int_{-\omega}^{x} v(x) \frac{\partial \bar{C}_1}{\partial y} \, dx \right] dx \qquad 3.58$$

For the evaluation of integrals on the right hand side of equation 3.49, an expression for $\frac{\partial \bar{C}_1}{\partial y}$ is required. This expression is obtained from equation 3.21 for the batch case i.e. when $\tau = 0$ by assuming $\frac{\partial \bar{C}_1}{\partial y}$ to be independent of x and neglecting the vertical diffusion term $B\rho D \int_{-\omega}^{\omega} dx \cdot \frac{\partial \bar{C}_1}{\partial y} \cdot \frac{d\bar{C}_1}{dy}$ is then given by the expression:

$$\frac{d\bar{C}_1}{dy} = \frac{B\rho \int_{-\omega}^{\omega} \int_{-\omega}^{x} v(x) \, dx \, dx}{\frac{B\rho}{D} \int_{-\omega}^{\omega} \left[\int_{-\omega}^{x} v(x) \, dx \, dx \right]^2 dx} \qquad 3.59$$

Substitution of the expression for $\frac{d\overline{C}_1}{dy}$ given by equation 3.59 into equation 3.58 yields the equation for $\overline{\frac{\partial C_1}{\partial x}}$.

$$\overline{\frac{\partial C_1}{\partial x}} = \frac{UE}{D}\overline{C_1 C_s} - \frac{1}{2\omega}\int_{-\omega}^{\omega}\int_{-\omega}^{x} v(x)\,\frac{\int_{-\omega}^{\omega}\int_{-\omega}^{x} v(x)\,dx\,dx}{\int_{-\omega}^{\omega}\left[\int_{-\omega}^{x} v(x)\,dx\right]^2 dx}\,dx\,dx \qquad 3.60$$

When the velocity profile given by the equation 3.56 is used to evaluate the integrals in 3.60 and the small terms are neglected, one gets a value of $0.7\left(\frac{UE}{D}\right)\overline{C_1 C_s}$ for the second term in the equation 3.60. The resultant expression for $\overline{\frac{dC_1}{dx}}$ is therefore given by:

$$\overline{\frac{\partial C_1}{\partial x}} = \frac{3}{10}\frac{UE}{D}\overline{C_1 C_s} \qquad 3.61$$

The expression for $\overline{\frac{dC_1}{dx}}$ was first obtained in the above manner by Von Halle (66) for thermal diffusion. However, the expression obtained by de Groot and associates (10) also reduces to the above form after suitable approximation. In order to be able to use this expression explicitly to account for the forgotten effect, some average value of the product $\overline{C_1 C_s}$ is required. In the first place, $C_s \cong 1$ everywhere in the column. Therefore it is only necessary to find some average value for $\overline{C}_1$. Horne and Bearman (22) have shown that this shouldbe evaluated at $x = 0$ and $y = 0$ for a column with reservoirs in thermal diffusion. However, in the present work, the average value $(C_B + C_T)/2$ (concentration

of the component at the top and C_B that at bottom) will be considered satisfactory. Therefore, the expression for $\overline{\frac{\partial \bar{C}_1}{\partial x}}$ becomes:

$$\overline{\frac{\partial \bar{C}_1}{\partial x}} = 0.3 \left(\frac{UE}{D} \right) \left(\frac{C_B + C_T}{2} \right) \qquad 3.62$$

But $\frac{C_B + C_T}{2}$ is equal to C_o the concentration of the mobile component under consideration in the feed solution and therefore

$$\overline{\frac{\partial \bar{C}_1}{\partial x}} = 0.3 \left(\frac{UE}{D} \right) C_o \qquad 3.63$$

The equation 3.63 gives an integral average value of the concentration gradient. On the other hand, if the value of $\overline{\frac{\partial \bar{C}_1}{\partial x}}$ is evaluated at x = 0 and y = 0, a slightly different value results and $\frac{\partial \bar{C}_1}{\partial x}$ is given by:

$$\frac{\partial \bar{C}_1}{\partial x} = \frac{UE}{D} \overline{C_1 C_s} - \int_{-w}^{x} v(x) \frac{d\bar{C}_1}{dy} dx \qquad 3.64$$

For $\frac{\partial \bar{C}_1}{\partial x}$ evaluated at x = 0, the equation 3.64 yields:

$$\left(\frac{\partial \bar{C}_1}{\partial x} \right)_{oy} = \frac{UE}{D} (\overline{C_1 C_s})_{oy} - \left(\frac{d\bar{C}_1}{dy} \right)_{oy} \int_{-w}^{0} v(x)\, dx \qquad 3.65$$

Now again using the transport equation given by 3.21 neglecting vertical diffusion term and putting $\tau = 0$ for batch case, one obtains the value of $\left(\frac{d\bar{C}_1}{dy} \right)_{oy}$ and carries out the integration. The result is:

$$\left(\frac{d\bar{C}_1}{dy} \right)_{oy} = \frac{UE}{D} (\overline{C_1 C_s})_{oy} - \frac{11}{16} \left(\frac{UE}{D} \right) (\overline{C_1 C_s})_{oy} \qquad 3.66$$

$$\left(\frac{d\bar{C}_1}{dy}\right)_{oy} = \frac{5}{16}\left(\frac{UE}{D}\right)(\overline{C_1C_s})_{oy} \qquad 3.67$$

Now assume $(\overline{C_1C_s})_{oo} \cong \overline{C_1C_s}$ (original solution); the above relation becomes:

$$\left(\frac{\partial\bar{C}_1}{\partial x}\right)_{oo} = \frac{5}{16}\left(\frac{UE}{D}\right)C_o \qquad 3.68$$

It will be seen that the integral average of $\frac{\partial\bar{C}_1}{\partial x}$ is smaller than the local value of $\frac{\partial\bar{C}_1}{\partial x}$ at x = 0 and y = 0 (at the feed point in the column) by a value given by $\left(\frac{5}{16} - \frac{3}{10}\right) = 0.0125$ which is about 4.17% larger than that obtained by taking an average of the top and the bottom concentrations. In this work the integral average value given by equation 3.63 will be used in all calculations as an approximate value for the entire column.

When the integral average of $\frac{\partial\bar{C}_1}{\partial x}$ given by the equation 3.63 is used in the equation 3.56 for $\frac{\partial\bar{C}_1}{\partial x}$, the final form of the relation for v(x) results:

$$v(x) = \frac{g}{\eta}\left\{\frac{\beta_T Q}{24k}x^4 + \frac{1}{6}\left(\frac{\beta_T\Delta T}{2\omega} + \frac{\beta_C\ 0.3UEC_o}{D}\right)(x^3 - \omega^2 x)\right.$$

$$\left. - \frac{\beta_T Q\omega^2}{20k}x^2 + \frac{\beta_T Q}{120k}\omega^4\right\} \qquad 3.69$$

The evaluation of the constants H and K is now straightforward, although the integrals given by equations 3.26 and 3.23 involve tedious algebra. The values of the constants are:

$$H = B\rho \left(\frac{g\beta_T \Delta T}{2\omega\eta} \right) \left(\frac{UE}{D} \right) \frac{(2\omega)^4}{6!} + B\rho \left(\frac{g\beta_C \ 0.3UEC_o}{\eta D} \right) \left(\frac{UE}{D} \right) \frac{(2\omega)^5}{6!} \qquad 3.70$$

$$H = (H_T) + H_{TE} + H_E \qquad 3.71$$

wherein H_{TE} and H_E are the conveniently defined component constants of H so that:

H_{TE} - contribution to H due to the product of E and ΔT. This is an interaction term.

H_E - contribution to H due to E alone.

and H_T - contribution to H due to ΔT alone. This was already considered negligible, because of the very small thermal diffusion coefficient of the protein, when the thermal diffusion term $\frac{\alpha_{1s} \overline{dT}}{\overline{T}\ dx}$ was dropped from equation 3.22 to get equation 3.26.

Since H_T represents the thermal diffusion term in equation 3.71, which is neglected, the expression for H is given by:

$$H = H_{TE} + H_E \qquad 3.72$$

In the above equation therefore:

$$H_{TE} = B\rho \left(\frac{g\beta_T \Delta T}{\eta} \right) \left(\frac{UE}{D} \right) \frac{(2\omega)^4}{6!} \qquad 3.73a$$

and

$$H_E = B\rho \left(\frac{0.3UEg\beta_C C_o}{\eta D} \right) \left(\frac{UE}{D} \right) \frac{(2\omega)^5}{6!} \qquad 3.73b$$

Similarly the column constant K can be written in the following convenient form:

$$K = K_T + K_{TE} + K_E + K_d \qquad 3.74$$

with its component constants K_T, K_{TE}, K_E and K_d defined as follows:

K_T - contribution to K due to ΔT alone.

K_{TE} - contribution to K due to the product of ΔT and E.

K_E - contribution to K due to E alone.

and K_d - contribution to K due to ordinary diffusion.

The full expression for K has not been written here because it is excessively long. Evaluation of the integral for K gives the following:

$$K_T = \frac{B\rho}{D}\left(\frac{g\beta_T\Delta T}{\eta}\right)^2 \frac{(2\omega)^7}{9!} - \frac{63}{8}\left(\frac{B\rho}{D}\right)\left(\frac{g^2}{\eta^2}\right)\left(\frac{\beta_T^2\Delta TQ}{k}\right)\frac{(2\omega)^9}{9!}$$

$$- \frac{1511}{640}\left(\frac{B\rho}{D}\right)\left(\frac{g\beta_T Q}{\eta k}\right)^2 \frac{(2\omega)^{11}}{9!} \qquad 3.75a$$

$$K_{TE} = 2.0\left(\frac{B\rho}{D}\right)\left(\frac{g\beta_T\Delta T}{\eta}\right)\left(\frac{0.3g\beta_C C_o UE}{\eta D}\right)\frac{(2\omega)^8}{9!} \qquad 3.75b$$

$$K_E = -\frac{63}{8}\left(\frac{B\rho}{D}\right)\left(\frac{g^2}{\eta^2}\right)\left(\frac{\beta_T Q}{k}\right)\left(\frac{0.3UE\beta_C C_o}{D}\right)\frac{(2\omega)^{10}}{9!}$$

$$+ \frac{B\rho}{D}\left(\frac{g}{\eta}\cdot\frac{0.3UEC_o\beta_C}{D}\right)^2 \frac{(2\omega)^9}{9!} \qquad 3.75c$$

$$K_d = B\rho D\cdot(2\omega) \qquad \text{(due to vertical diffusion)} \qquad 3.75d$$

At small values of Q, the terms involving Q in above constants will be very small.

An examination of these equations and a comparison

with their thermal diffusion counterparts shows that the effect of the electric field is to give higher values of H and K in electrophoresis than those that are obtained when only temperature difference is operative in the column.

In order to obtain a theory for steady state continuous-flow column performance, the transport equations must now be solved.

Steady State Solution of the Transport Equation

In this section, the steady state solution of the transport equation will be presented. Repeated below is the transport equation for the top section:

$$-\sigma_T(\overline{C}_T - \overline{C}_1) = H_T\overline{C_1C_s} - K_T\frac{d\overline{C}_1}{dy} \qquad 3.76$$

and since $\overline{C}_s \cong 1$, the equation becomes

$$-\sigma_T(\overline{C}_T - \overline{C}_1) = K_T\overline{C}_1 - K_T\frac{d\overline{C}_1}{dy} \qquad 3.77$$

At this stage the notation will be simplified by dropping the superbars and the subscripts remembering always that the values of C are the values at x = 0. The final form is:

$$-\sigma_T(C_T - C) = H_TC - K_T\frac{dC}{dy} \qquad 3.78$$

The variables in the above equation are easily separable. The limits of integration over the top section are obvious upon inspection of the coordinate system in Figure 3-2. Thus, the integrals are:

$$\int_{C_M}^{C_T} \frac{dC}{(H_T - \sigma_T)C - \sigma_T C_T} = - \int_{0}^{C_T=-L/2} dy \qquad 3.79$$

Where the lower limit of the concentration is the concentration of component 1 at y = 0 for the steady state:

$$C = C_M \text{ at } y = 0 \qquad 3.80$$

When the integral is evaluated with this boundary condition, one gets the following expression for C:

$$C = - \frac{\sigma_T C_T}{H_T - \sigma_T} + \left(C_M + \frac{\sigma_T C_T}{H_T - \sigma_T} e^{\frac{-(H_T - \sigma_T)}{K} y} \right) \qquad 3.81$$

The expression relating C_T, the concentration at the top ($y = L_T = -L/2$), with C_M, the concentration at y = 0, is obtained by putting $y = L_T = -L/2$ in the equation 3.81 and then rearrangement gives:

$$\frac{C_M}{C_T} = \frac{e^{-\frac{H_T L}{2KT}\left(1 + \frac{\sigma_T}{H_T}\right)} - \frac{\sigma_T}{H_T}}{1 - \frac{\sigma_T}{H_T}} \qquad 3.82$$

The analogous treatment for the bottom section yields a relation between the bottom concentration C_B and C_M:

$$\frac{C_M}{C_B} = \frac{e^{-\frac{H_B L}{2K_B}\left(1 + \frac{\sigma_B}{H_B}\right)} + \frac{\sigma_B}{H_B}}{1 + \frac{\sigma_B}{H_B}} \qquad 3.83$$

In equations 3.82 and 3.83, let

$$\frac{H_T L}{2K_T} = A_T \text{ and } \frac{H_B L}{2K_B} = A_B \qquad 3.84$$

where L is the total separation length of the column.

The relationships developed in equation 3.82 and 3.83 are very general in character. However, since the experimental data were collected for a center-feed column and for reasons of further simplification in the method of comparison of theory and experiment, more restricted forms of equations 3.82 and 3.83 will be actually used in this work.

To obtain the restricted forms of the equations 3.82 and 3.83, the following further assumptions applicable to both the sections of the column are made:

$$\sigma_T = \sigma_B = \sigma \qquad 3.85a$$

$$H_B = H_T = H \qquad 3.85b$$

and $$K_T = K_B = K \qquad 3.85c$$

$$A_T = A_B = A \qquad 3.85d$$

and therefore $$A = \frac{HL}{2K} \qquad 3.85e$$

and the equations 3.82 and 3.83 reduce to:

$$\frac{C_M}{C_T} = \frac{e^{+A\left(1 - \frac{\sigma}{H}\right)} - \frac{\sigma}{H}}{1 - \frac{\sigma}{H}} \qquad 3.86$$

and

$$\frac{C_M}{C_B} = \frac{e^{-A\left(1-\frac{\sigma}{H}\right)} + \frac{\sigma}{H}}{1 + \frac{\sigma}{H}} \qquad 3.87$$

For dilute solutions, it is convenient to work with ratios of the concentrations, and the separation factor f is defined as the ratio of C_B/C_T. The separation factor $f = C_B/C_T$ can easily be obtained by dividing 3.87 into 3.86:

$$\text{Separation factor } f = \frac{C_B}{C_T} = \frac{\frac{C_M}{C_T}}{\frac{C_M}{C_B}}$$

$$= \frac{\left(e^{A\left(1-\frac{\sigma}{H}\right)} - \frac{\sigma}{H}\right)\left(1 + \frac{\sigma}{H}\right)}{\left(e^{-A\left(1+\frac{\sigma}{H}\right)} + \frac{\sigma}{H}\right)\left(1 - \frac{\sigma}{H}\right)} \qquad 3.88$$

In the present investigation, equation 3.88 will be the most useful form of describing steady state separation in a center-fed, continuous-flow thermoelectrogravitational electrophoresis column at low rates. The equation is applicable to solutions in which the concentration of the component to be separated is very small and provided that several other restrictions utilized in its derivation are closely satisfied.

Transient Behavior of Thermoelectrogravitational Electrophoresis Column

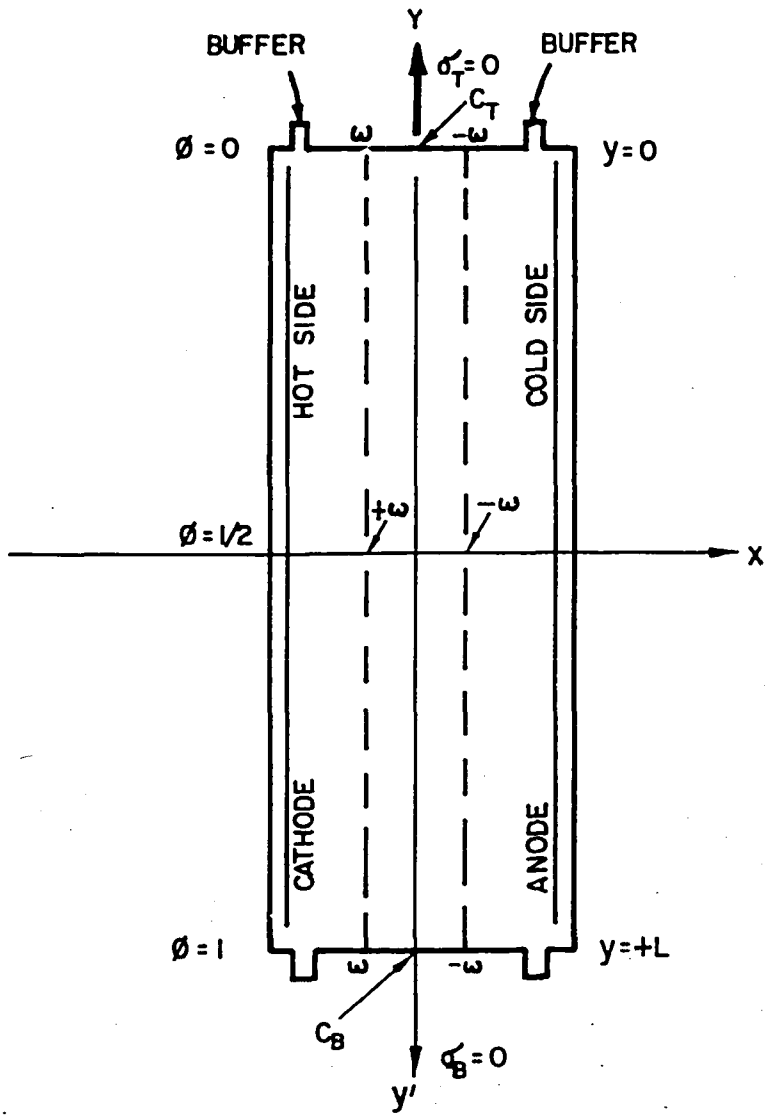
It is already mentioned that the transport equation

derived by considering batchwise operation can be utilized to develop mathematical expressions which could be used to predict and to interpret transient behavior of an electrophoresis column. The study of the transient behavior of the column not only helps to determine the time required to approach the steady state but provides an independent means to verify the validity of the theory developed. Analysis of batch transient operation of the column is therefore significantly important, and in the present section, the transport equation given by equation 3.27 is utilized to derive an expression for the transient separation factors.

Using the transport equation and taking a material balance on a differential volume element of the column, one obtains equation 3.89 which describes the transient behavior of the electrophoresis column:

$$m \frac{\partial C}{\partial t} = K \frac{\partial^2 C}{\partial y^2} - H \frac{\partial C}{\partial y} \qquad 3.89$$

where m is the mass of solution per unit length of the electrophoresis column and is considered constant. The solution of this equation is easily obtained by the use of Laplace transformation with the appropriate boundary conditions. (To state the boundary conditions, a modified coordinate system as presented in Figure 3-5 will be used.) The column in this case will not be divided into two top and bottom sections but y will be considered equal to zero at the top of the column and equal to L at the bottom. Thus:



MODIFIED COORDINATE SYSTEM
FOR
BATCH ELECTROPHORESIS COLUMN

FIGURE 3-5

$$HC - K\frac{\partial C}{\partial y} = 0 \quad \text{at } y = L \quad \text{all } t \qquad 3.90$$

$$HC - K\frac{\partial C}{\partial y} = 0 \quad \text{at } y = 0 \quad \text{all } t \qquad 3.91$$

$$C = C_o \quad \text{at } t = 0 \quad \text{all } y \qquad 3.92$$

The equations 3.89 to 3.92 are rewritten to obtain a more convenient form by substituting:

$$\varphi = \frac{y}{L} \qquad 3.93$$

The equations then are

$$\frac{\partial^2 C}{\partial \varphi^2} - 2A\frac{\partial C}{\partial \varphi} - \alpha\frac{\partial C}{\partial t} = 0 \qquad 3.94$$

$$\frac{\partial C}{\partial \varphi} - 2AC = 0 \quad \varphi = 0 \quad \text{all } t \qquad 3.95$$

$$\frac{\partial C}{\partial \varphi} - 2AC = 0 \quad \varphi = 1 \quad \text{all } t \qquad 3.96$$

$$C = C_o \quad t = 0 \quad \text{all } \varphi \qquad 3.97$$

where $$\alpha = \frac{mL^2}{K} \qquad 3.98$$

After taking the LaPlace transform with respect to time and defining $\dot{C}(\varphi,s)$ as the LaPlace transform of $C(\varphi,t)$ the equation becomes:

$$\frac{\partial^2 \dot{C}}{\partial \varphi^2} - 2A\frac{\partial \dot{C}}{\partial \varphi} - (\alpha s\dot{C} - C_o) = 0 \qquad 3.99$$

and the transformed boundary conditions become:

$$\frac{\partial \dot{C}}{\partial \varphi} - 2A\dot{C} = 0 \quad \varphi = 0 \quad \text{all } s \qquad 3.100$$

$$\frac{\partial \dot{C}}{\partial \varphi} - 2A\dot{C} = 0 \qquad \varphi = 1 \qquad \text{all } s \qquad 3.101$$

$$\dot{C} = \frac{C_o}{s} \qquad s = 0 \qquad \text{all } \varphi \qquad 3.102$$

The solution of the transformed ordinary second order differential equation 3.94 is given by:

$$\dot{C} = e^{-A\varphi}\left[B'' \cosh \sqrt{A^2 + \alpha s}\ \varphi + D'' \sinh \sqrt{A^2 + \alpha s}\ \varphi \right] \qquad 3.103$$

where B" and D" are two constants. The constants are evaluated by using 3.100 and 3.101:

$$B'' = \frac{2AC_o}{\alpha s^2} \frac{\left[\cosh \sqrt{A^2 + \alpha s} - \dfrac{A \sinh \sqrt{A^2 + \alpha s}}{\sqrt{A^2 + \alpha s}} - e^{-A}\right]}{\dfrac{\sinh \sqrt{A^2 + \alpha s}}{\sqrt{A^2 + \alpha s}}} \qquad 3.104$$

$$D'' = \frac{2A^2 C_o}{s^2} \frac{\left[e^{-A} - \cosh \sqrt{A^2 + \alpha s} + \dfrac{\sqrt{A^2 + \alpha s}}{A} \sinh \sqrt{A^2 + \alpha s} \right]}{\sinh \sqrt{A^2 + \alpha s}} \qquad 3.105$$

The transform $\dot{C}$ of C is therefore given by:

$$\dot{C} = \frac{C_o}{s}$$

$$+ 2AC_o e^{-A\varphi}\left[\frac{\cosh \sqrt{A^2+\alpha s} - A \sinh \sqrt{A^2+\alpha s} - e^{-A}}{\alpha s^2 \dfrac{\sinh \sqrt{A^2+\alpha s}}{\sqrt{A^2+\alpha s}}} \right] \cosh \sqrt{A^2+\alpha s}\ \varphi$$

$$+ \frac{2A^2 C_o e^{-A\varphi}}{\alpha s^2}\left[\frac{e^{-A} - \cosh \sqrt{A^2+\alpha s} + \dfrac{\sqrt{A^2+\alpha s}}{A} \sinh \sqrt{A^2+\alpha s}}{\sinh \sqrt{A^2+\alpha s}}\right] \sinh \sqrt{A^2+\alpha s}\ \varphi$$

3.106

The steady state part of the solution of the equation 3.106 is obtained by finding the residue at the double pole s = 0 and is given by:

$$C(\varphi,\infty) = \frac{2AC_o e^{2A\varphi}}{e^{2A} - 1} \qquad 3.107$$

The transient part of the solution is obtained by finding the residues at the simple poles given by:

$$\sinh \sqrt{A^2 + \alpha s} = 0 \qquad 3.108$$

Zeros of this are given by the relation:

$$\sqrt{A^2 + \alpha s} = in\pi \qquad 3.109$$

and the transient part of the solution then becomes:

$$C(\varphi,t) = 4AC_o e^{-A\varphi} \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(n^2\pi^2 + A^2)^2} \left[1 + (-1)^{n+1} e^{-A}\right] e^{-\frac{A^2+n^2\pi^2}{\alpha}t}$$

$$x \left(\cos n\pi\varphi + \frac{A}{n\pi} \sin n\pi\varphi\right) \qquad 3.110$$

The total solution is therefore given by:

$$C(\varphi,t) = \frac{2AC_o e^{A\varphi}}{e^{2A} - 1} + 4AC_o e^{-A\varphi} \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(n^2\pi^2+A^2)^2} \left[1 + (-1)^{n+1} e^{-A}\right]$$

$$x\ e^{-\frac{n^2\pi^2 + A^2}{\alpha} t} \left(\cos n\pi\varphi + \frac{A}{n\pi} \sin n\pi\varphi\right) \qquad 3.111$$

Concentration at $\varphi = 1$ (bottom) and $\varphi = 0$ (top) are obtained from the equation by substitution and are:

$$C(1,t) = \frac{2AC_o e^{2A}}{e^{2A} - 1}$$

$$+ 4AC_o e^{A} \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2 + n^2\pi^2)^2} \left[1 + (-1)^{n+1} e^{-A}\right] (-1)^n e^{-\frac{A^2+n^2\pi^2}{\alpha}t}$$

3.112

$$C(0,t) = \frac{2AC_o}{e^{2A} - 1} + 4AC_o \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2 + n^2\pi^2)^2} \left[1 + (-1)^{n+1} e^{-A}\right] e^{-\frac{A^2+n^2\pi^2}{\alpha}t}$$

3.113

This solution was first obtained in thermal diffusion by Bardeen (2) by using the method of separation of variables, but later Von Halle (66) obtained it by the Laplace Transform. Therefore, the separation factor f at time t is given by:

$$f = \frac{C(1,t)}{C(0,t)} = \frac{C_B}{C_T}$$

$$= \frac{e^{2A} + 2e^{A}(e^{2A} - 1) \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(n^2\pi^2 + A^2)^2} \left[1 + (-1)^{n+1} e^{-A}\right] (-1)^n e^{-\frac{A^2+n^2\pi^2}{\alpha}t}}{1 + 2(e^{2A} - 1) \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2 + n^2\pi^2)^2} \left[1 + (-1)^{n+1} e^{-A}\right] e^{-\frac{A^2+n^2\pi^2}{\alpha}t}}$$

3.114

It was mentioned before that equation 3.88 can be used to predict and to interpret continuous-flow column data. Similarly equation 3.114 describes the transient column behavior and can be used to predict and to interpret the transient experimental data obtained by batch operation of the column. These equations have been derived by taking into

consideration the factors such as flow rate, mobility, temperature difference, field strength and viscosity of solution. The next section discusses the computational results of this development in order to indicate the trends expected for the operation of a center-fed continuous-flow electrophoresis column.

Discussion of Theoretical Results

To demonstrate the implications of the mathematical theory developed so far, for the case of continuous-flow electrophoresis column, theoretical calculations were made using a 1410 Fortran computer. Theoretical velocity profiles, temperature distributions and separation factors as functions of column flow rate were calculated at various conditions approximating those used later in the experimental work. Calculations were done both with and without the heat generation term. The values of the column parameters used in these computations are given in Table 3.1.

Velocity Distribution, v(x)

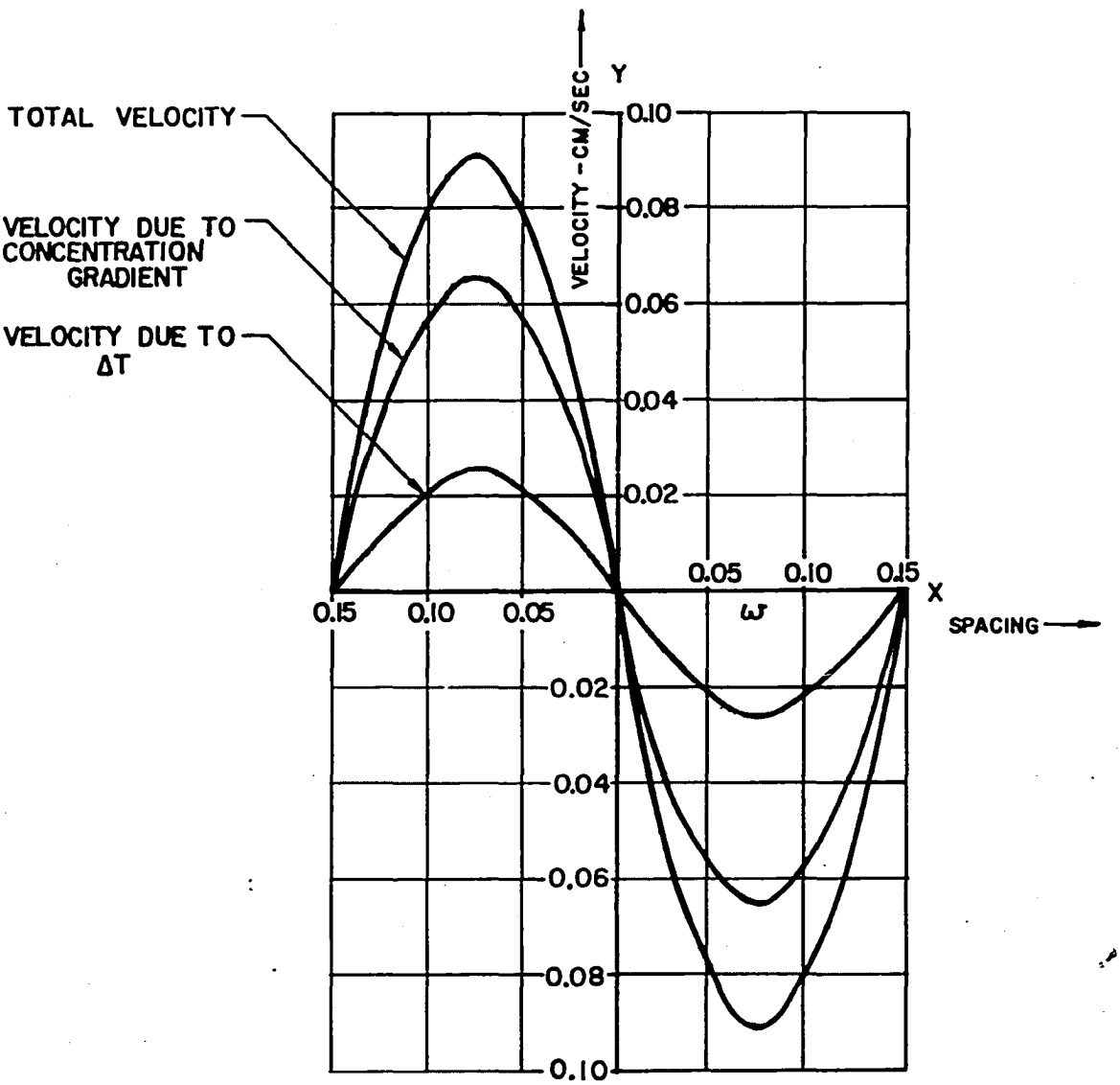
The velocity profiles were calculated at various values of E, ΔT and membrane spacing. Figures 3-6 and 3-7 show calculated velocity distributions at two E values of 0.0423 and 0.423 volts/cm., respectively at constant ΔT of 8.5°C and a constant membrane spacing of 0.3018 cm. When heat generation is taken into consideration, the velocity at $x = 0$ is not zero but slightly different. The velocity profile curve is therefore shifted from the origin, but this

TABLE 3.1

Column Parameters Used for Theoretical Computations

ΔT °C	E volt/cm.	pH	2ω cm.	Theoretical Set No.
8.5	0.0423	8.6	0.3018	T1
8.5	0.141	8.6	0.3018	T2
8.5	0.282	8.6	0.3018	T3
8.5	0.423	8.6	0.3018	T4
16	0.282	8.6	0.3018	T5
16	0.423	8.6	0.3018	T6
0	0.282	8.6	0.3018	T7
0	0.423	8.6	0.3018	T8
8.5	0.282	8.6	0.1354	T9
0	0.282	8.6	0.1354	T10
8.5	0.98	6.0	0.1354	T11

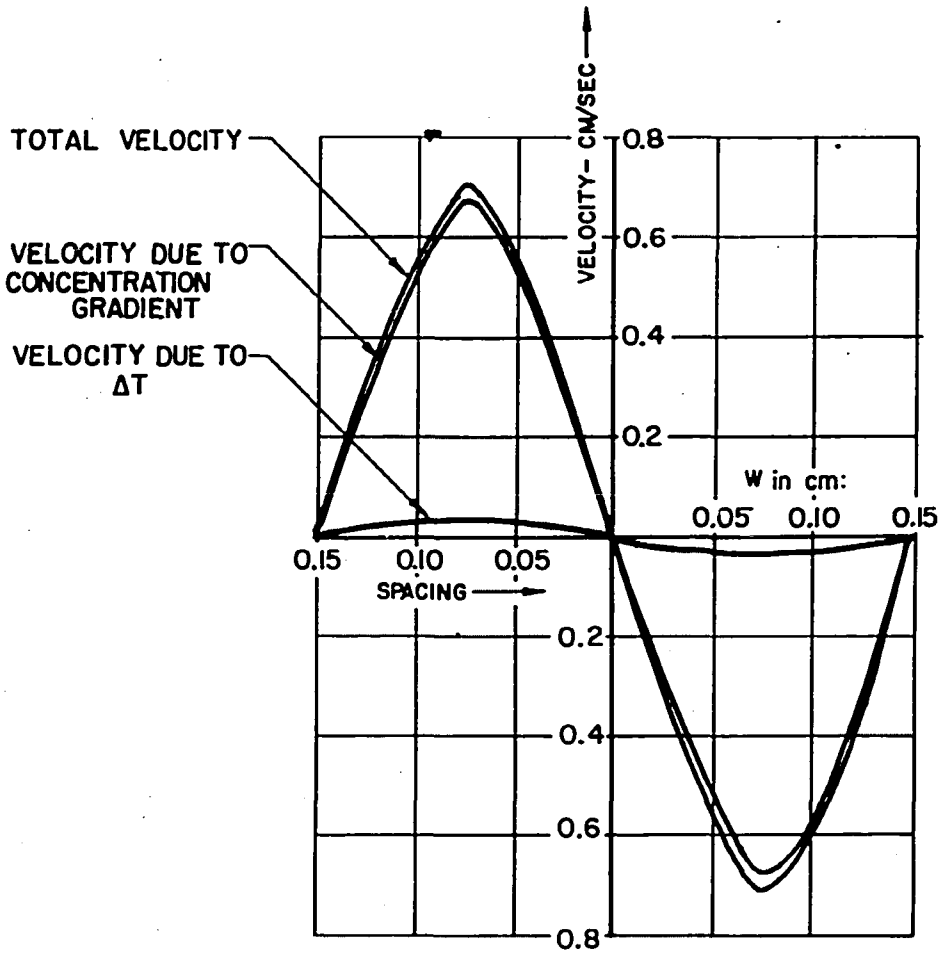
THEORETICAL VELOCITY DISTRIBUTION CURVE



VELOCITY PROFILES AT $\Delta T = 8.5°C$ and $E = 0.0423$ volt/cm

FIGURE 3-6

THEORETICAL VELOCITY DISTRIBUTION CURVE



VELOCITY PROFILES AT $\Delta T = 8.5°C$ and $E = 0.423$ volt/cm
(HEAT GENERATION NEGLECTED)

FIGURE 3-7

cannot be seen in Figures 3-6 and 3-7 since the change in velocity is very small [v(x) at x = 0 changes by 10^{-5} cm./sec. at E = 0.423 volts/cm.).

At much higher values of Q, corresponding to very small column volumes operating at rather high E (say 50 volts/cm. field strength), the effect would be more pronounced.

Temperature Distribution as a Function of x

The equation 3.41 states that T is quadratic in x. For the small Q, appropriate to these calculations, the effect on T was found to be negligible (less than 0.001°C) and the temperature distribution was linear.

Column Constants and Separation Factors

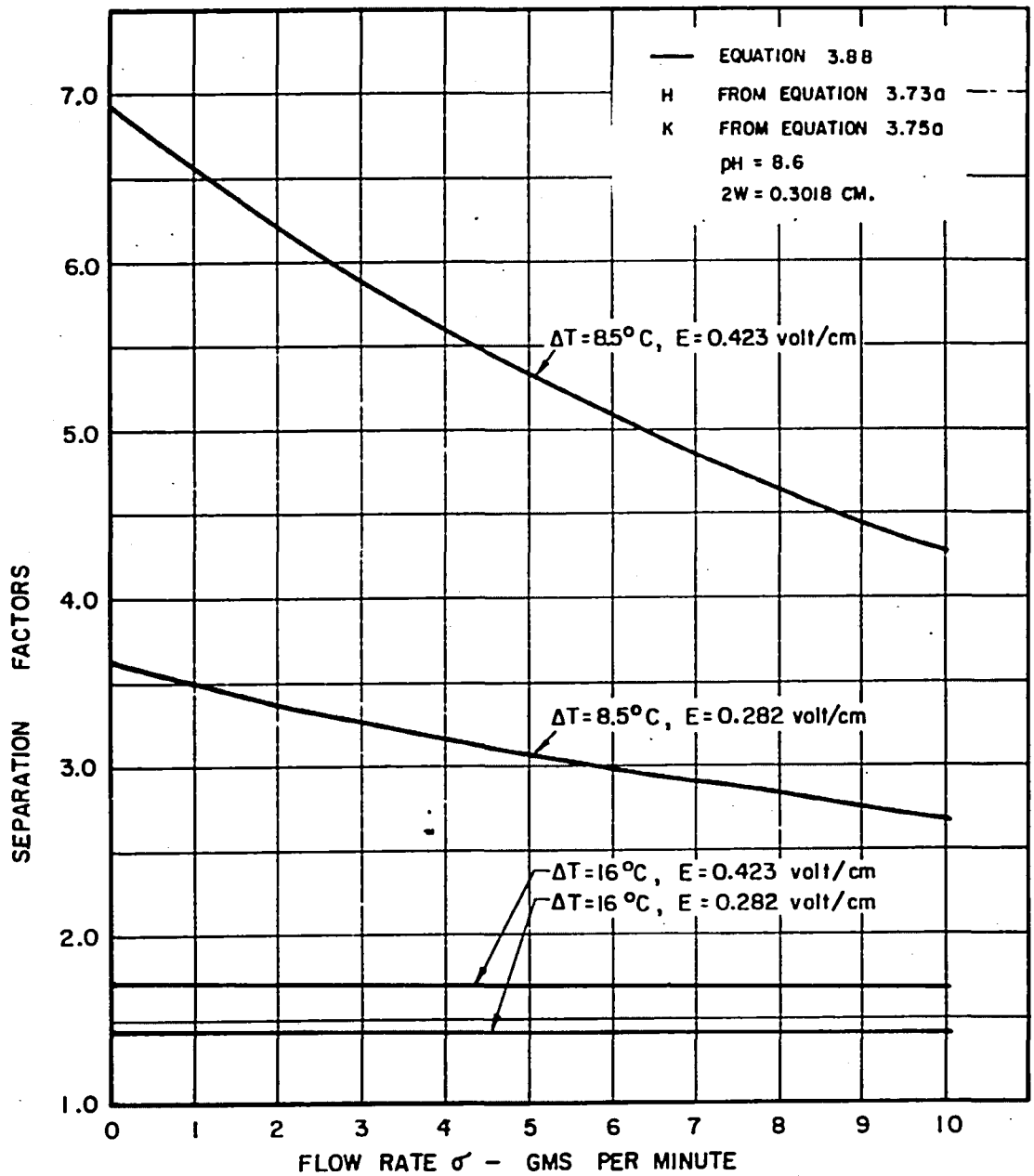
The values of constants H and K and their component parts calculated from the theory are listed in Table 3.2. From Table 3.2 it will be seen that at the lower value of field strength (0.0423 volt/cm.), H_{TE}, the contribution to total H because of both ΔT and E, is 1.6812 gm./min. while H_E, the contribution due to E alone, is 4.23 gm./min. Thus H_{TE} amounts to about 28 percent of the total H. On the other hand, the contribution to K of $K_T + K_{TE}$, the combined effect of both ΔT and E, is about 40 percent (Table 3.2).

Using these H and K values, curves were prepared for different conditions of column operation and are illustrated by Figures 3-8 and 3-9. These figures show the expected trend of results obtainable from an electrophoresis column.

TABLE 3.2

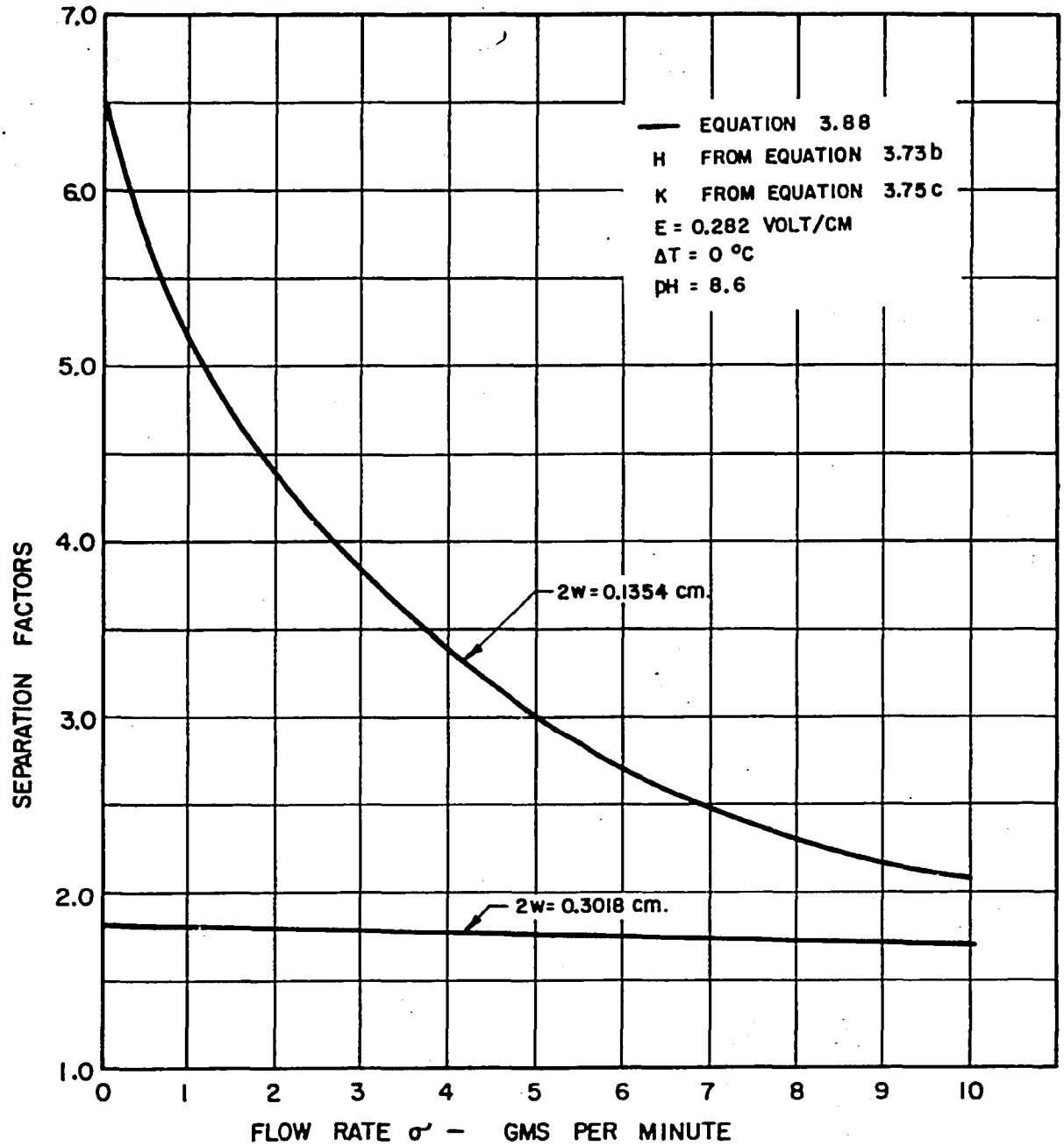
Theoretical Values of Various K Constants and H Constants

Set No.	H_{TE} gm. min.	H_E gm. min.	H gm. min.	K_{QT} gm.-cm. min.	K_{QE} gm.-cm. min.	K_T gm.-cm. min.	K gm.-cm. min.	K_{TE} gm.-cm. min.	K_E gm.-cm. min.	K gm.-cm. min.
T1	1.681	4.229	5.91	small	-0.005 -0.0058	1259.3 1259	-0.0126	6335	7967	14300
T2	5.604	46.986	52.59	small	-0.0058	1259.3	-0.468	21110	88520	109600
T3	11.211	187.94	199.15	small	-0.2233	1259.3	-3.750	42230	354000	396300
T4	16.811	422.87	439.70	small	-0.5025	1259.3	-12.63	63350	796700	860000
T5	36.491	165.211	201.70	small	-1.482	14986	-6.39	135700	307100	442800
T6	54.74	371.81	426.50	small	-3.177	14984	-21.57	203500	691100	894700
T7	0	219.11	219.66	small	---	---	---	---	420211	420211
T8	0	494.2	494.20	small	---	---	---	---	945469	945469
T9	0.454	3.416	3.870	small	---	4.62	-0.0055	69.31	260.7	330.00
T10	0	3.992	3.992	small	---	---	---	---	309.43	309.43
T11	0.0532	0.0464	0.0997	small	---	4.62	-0.7×10^{-5}	8.056	3.5111	11.56



SEPARATION FACTORS AS FUNCTIONS OF FLOW RATE

FIGURE 3-8

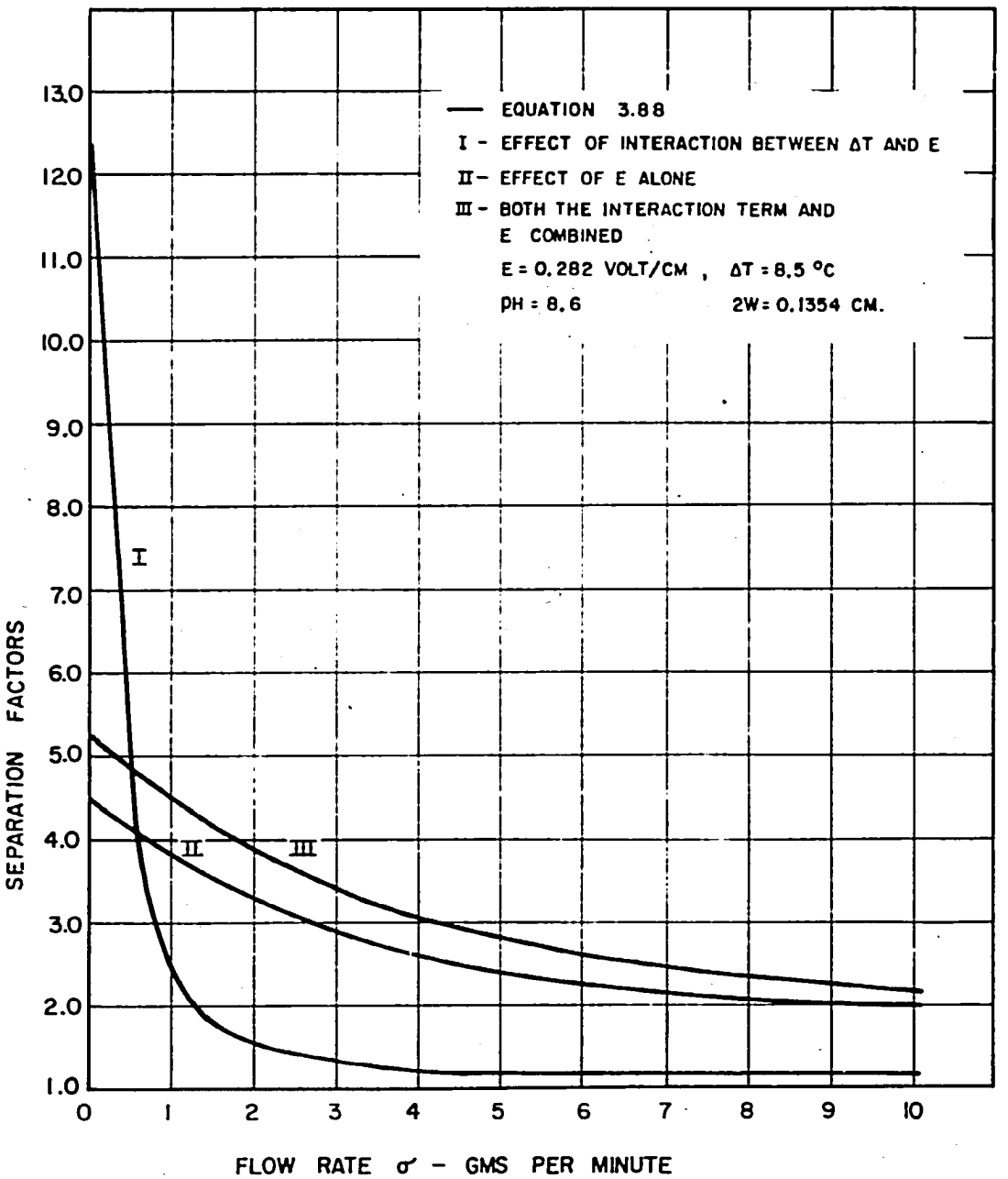


SEPARATION FACTORS AS A FUNCTION OF FLOW RATE AT TWO DIFFERENT MEMBRANE SPACINGS

FIGURE 3-9

As in thermal diffusion, it will be seen that separation decreases with increasing flow rate and membrane spacing, and increases with an increase in the field strength. However, in the present case, the separation decreases with increasing temperature difference. In electrophoresis, the H is theoretically proportional to ΔT while K is proportional to ΔT^2. For a given field strength therefore, $A = \left(\frac{HL}{2K} \right)$ is inversely proportional to ΔT, i.e. it decreases with an increase in ΔT. Since A occurs exponentially in the expression for the separation factors, decreased value of A with increase in ΔT gives lower values of separation factors.

In Figure 3-10, calculated separation factors as function of flow rate are presented to show the predicted trend with regard to the relative effectiveness of the field strength and the temperature difference. The curve labelled I represents the effect of the product of ΔT and E (the interaction term); the curve labelled II represents the effect of E alone while the curve labelled III shows the combined effect of both E and the interaction term. In Figure 3-10, the curves I and II are not added arithmetically to get the curve III representing the combined effect of ΔT and E. This is because although the column constants H and K are obtained by addition of the individual separate contributions, they affect the separation factor values through exponential function such as $\overline{C}_B/\overline{C}_T = e^{HL/2K}$ for steady state batch operation where in the total H and K appear as an exponent of e. A comparison of these separation



EFFECT OF FIELD STRENGTH AND ITS INTERACTION WITH ΔT

FIGURE 3-10

factors shows that the effect of interaction term is considerable at very low rates and drops off rapidly with increase in flow rate. The effect of the field strength is more uniform over a wide range of flow rates. By a comparison of H values in Table 3.2, it is seen that at low values of E the interaction term is about 28% of the total H values. It therefore seems that the thermal field would be of help at small field strengths and flow rates. At high E and flow rate values the contribution due to small temperature differences appears to be insignificant.

Summary

In this Chapter, the method developed by Furry, Jones and Onsager (18) to explain the behavior of a conventional thermogravitational thermal diffusion column has been applied to describe phenomenologically the operation of a center-fed thermoelectrogravitational electrophoresis column. Equations have been established for a system in which one component is mobile and the other is at its isoelectric point.

The effect of both the temperature difference and the concentration gradient (brought about by horizontal migration) on the convective flow pattern have been considered. It has been shown theoretically that for low values of Q, the velocity and temperature distribution are mostly unaffected by the generation of heat due to the passage of electrical current through the solution.

Separation factors for the steady state operation of

a center-fed electrophoresis column with equal product flow rates from the top and the bottom are given by the relation:

$$\frac{C_B}{C_T} = \frac{\left(e^{A\left(1-\frac{\sigma}{H}\right)} - \frac{\sigma}{H} \right)\left(1 + \frac{\sigma}{H} \right)}{\left(e^{-A\left(1+\frac{\sigma}{H}\right)} + \frac{\sigma}{H} \right)\left(1 - \frac{\sigma}{H} \right)} \qquad 3.88$$

which can be used to predict column behavior and to interpret column data. The solution for the transient behavior of the column without flow was also obtained and is given by:

$$f = \frac{C_B}{C_T}$$

$$= \frac{\frac{2AC_o e^{2A}}{e^{2A}-1} + 4AC_o e^{A} \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2+n^2\pi^2)^2} (-1)^n \left[1 + (-1)^{n+1} e^{-A} \right] e^{-\frac{A^2+n^2\pi^2}{\alpha} t}}{\frac{2AC_o}{e^{2A}-1} + 4AC_o \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2+n^2\pi^2)^2} \left[1 + (-1)^{n+1} e^{-A} \right] e^{-\frac{A^2+n^2\pi^2}{\alpha} t}} \qquad 3.114$$

which can be used to predict transient behavior of the column and also to interpret the data obtained from a batch column.

Theoretical calculations for the velocity profiles, temperature distribution, and separation factors have been made and presented as functions of flow rate with ΔT, E, 2ω, U as variables and the expected trend of results given.

CHAPTER IV

EXPERIMENTAL INVESTIGATION

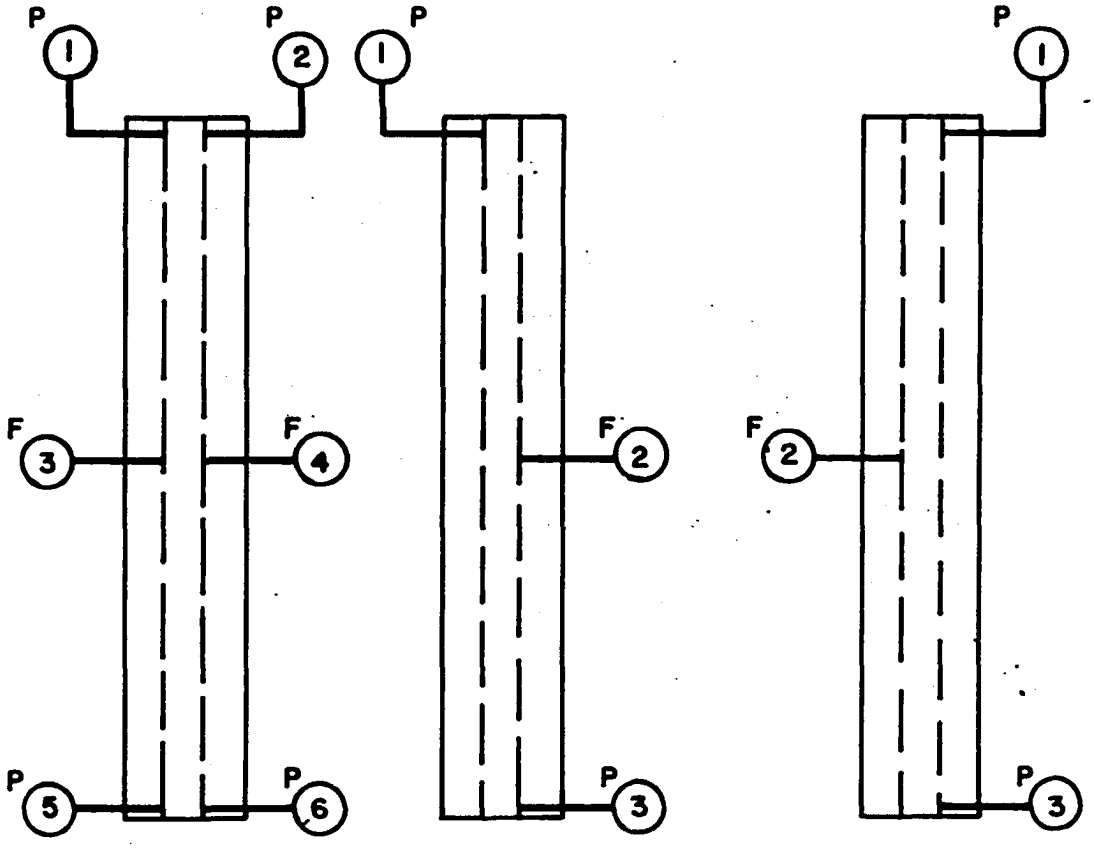
Continuous-Flow Thermoelectrogravitational Electrophoresis Column

In Chapter III, a theory was developed using the Furry, Jones and Onsager (18) procedure to describe the performance of continuous-flow electrophoresis column. It was also shown there how the transport equation obtained for batch case could be utilized to obtain a mathematical relationship to describe the transient behavior of the electrophoresis column which enables the column operation to be studied under conditions of no flow. The present chapter is devoted to the detailed description of a continuous-flow electrophoresis column and auxiliary equipment used to obtain the experimental data necessary to test this theory. Before details of the column construction are described, some qualitative aspects of the column operation and the problems in its construction will be considered.

Since the column is to be operated continuously, some provision for continuous feed and product removal must be made. The location of feed and product streams depends upon the nature of the components or more precisely, their

mobilities. Observation of Figure 4-1 makes it clear that there are four positions at the ends of the column, at which the products can be withdrawn. The feed location should in principle be such that the feed is introduced into the column at a point where the concentration of the feed is approximately the same as that of the solution in the column at this point. This is more or less impractical in the case of dilute solutions because the column concentration is not symmetrical (Figure 4-1) about the midpoint of the column. The point at which the solution concentration equals that of the feed will not be at $y = 0$ (center of column) but at some other point in the column which cannot be precisely prelocated. (This point can roughly be located theoretically. However, its exactness cannot be guaranteed because of the approximate character of the theoretical development.) Therefore, the column is fed at the center even though this is not exactly correct procedure because it facilitates the column construction.

The actual location of the feed points and the product points will be determined by the mobility behavior of the components to be separated. Consider a system in which two components A and B are to be separated. Let U_A and U_B be their mobilities. Depending upon the magnitude of U_A and U_B either component will be proportionately more in concentration at one end and the other at the other end. The side of the column on which the feed will be introduced should be chosen to obtain maximum separation. Different cases will have to



P – Location for product withdrawal
F – Location for feed into the column

POSSIBLE FEED AND PRODUCT STREAM LOCATIONS

FIGURE 4–1

be considered.

Case 1: Assume that $U_A >> U_B$ and that U_A and U_B are of the same sign such that both A and B move towards the anode. Now if A is the component desired to be separated, then it passes toward the anode (chosen as the cold wall) and sinks to the bottom. Since U_B is very small compared with U_A, component B will not concentrate appreciably towards the anode (cold wall). The material diluted in A will move upward in the cathode or hot section of the column while the material concentrated in A moves downwards in the anode or cold section. Therefore, it will be advantageous to feed fresh solution on the anode or cold side while removing the products on the anode or cold side at the bottom (right) and on the cathode or hot side at the top (left) as shown in Figure 4-1b.

Case 2: Assume that $U_A >> U_B$ but that the special case when $U_B = 0$ exists. The same reasoning as in case 1 applies and therefore the same arrangement of feed and product withdrawal would be appropriate.

Case 3: Assume U_A and U_B are of opposite sign. Suppose A moves towards the anode or cold wall and B towards the cathode or hot wall. In this case a judicious choice of feed point will depend upon the component which is most desired after separation. If A were required, the feed point should be on the cathode side so that A moves towards the anode while B moves

to the cathode side and the composition of A on the anode side is not disturbed by the feed. If, on the other hand, it is B that is desired then the feed should be on the anode side so that the composition of B on the cathode side is not affected (Figure 4-1c).

In practice, therefore, the feed will be introduced into the column at the center, and either on the anode or cathode side depending upon the relative mobilities of the components.

In addition to the location of the feed and product positions in the column, there are engineering problems of construction of the column. The various aspects that need be considered are: (1) Materials of construction, (2) Electrode reactions, (3) Convective back-mixing, and (4) Heat generation and its effects.

Materials of Construction

Since an electric field is to be applied, the cell itself and the components in the vicinity of the solution to be fractionated and the background buffer solutions should be good insulators. The Plexiglass is a good material for this purpose since it is a good electrical insulator, has suitable strength, is inert to action by many chemicals and can be easily machined.

The porous membranes should offer at least two properties: semipermeability and sufficient mechanical strength. The semipermeable membrane must be such that it

allows only small bulk flow through its pores, gives low electrostatic effects and low voltage drop. It should have a minimum electro-osmotic effect. In the initial stages of the present experimental work, several types of cellophane membranes were tried, but only the cellulose dialysis membrane supplied by Viscose Rayon Corporation, Chicago, proved to be useful. Flexolith filters were used as strong background support for the membranes.

Electrode Reactions

During an electrophoresis experiment, electrolysis is also taking place at the electrode. If this is excessive and the gases formed are not removed they will accumulate and transmit their influence through the porous membranes, thereby disturbing the laminar convection in the solution separation space of the column. Therefore, provision must be made to remove the electrolysis products efficiently. The generation of gas within the solution space will be higher if the buffers in the electrical compartment are static. In the apparatus to be described, the effect of gas generation within the solution space was negligible. However gas generated in the buffer compartments was removed by suitable venting arrangement.

The electrodes themselves are very important because their frequent replacement after corrosion would be cumbersome. Although alloys such as Hastelloy C and Terrelloy are satisfactory as anode material, platinized titanium available

from Englehard Limited, Newark, N.J. is more suitable because it is corrosion resistant and can stand electrode reactions very well.

Convective Flow in the Cell

In order to obtain as large a separation as possible the fluid flow in the cell should be laminar, and the effects of back-mixing should be avoided. However, increased separations have been reported in thermal diffusion (84) under conditions of turbulance.

Heat Generation

During the process of electrophoresis, heat is generated in the cell, and this will increase the temperature of the solution. If the temperature rises too high, some substances such as proteins may spoil. The heat generated would cause uneven temperature gradients and disturb the laminar convection. The heat must be removed by maintaining the sides of the cell at sufficiently low temperatures. This would allow heat to be transferred out of the cell, thus stabilizing the laminar convection in the cell and keeping the temperature of the solution anywhere in the separation space of the column low enough to avoid the spoilage of the protein material.

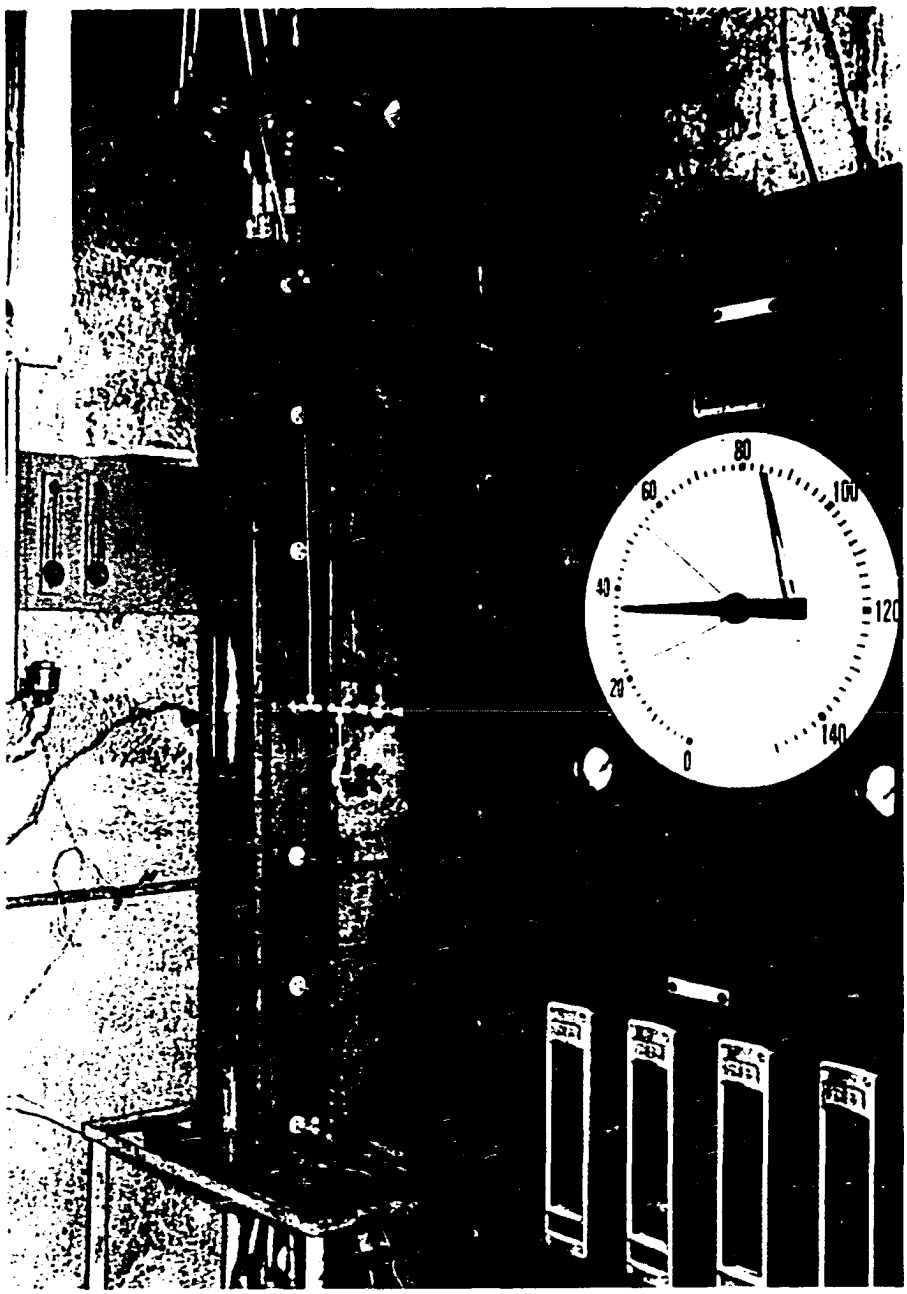
On the basis of this qualitative information, the thermoelectrogravitational electrophoresis column used in the present investigation will now be described.

The electrophoresis column was a parallel membrane

device (Figure 4-2). The working space between the membranes was about 4 inches wide and 58 inches long. Because the product and sample ports were not quite at the ends of the working space of the column, the effective length was actually less than 58 inches and measured 145 cm. between the top product sample port and the bottom product sample port. The spacings of 0.3018 cm. and 0.1354 cm. were obtained by using two different plastic spacers of appropriate thickness between the two membranes.

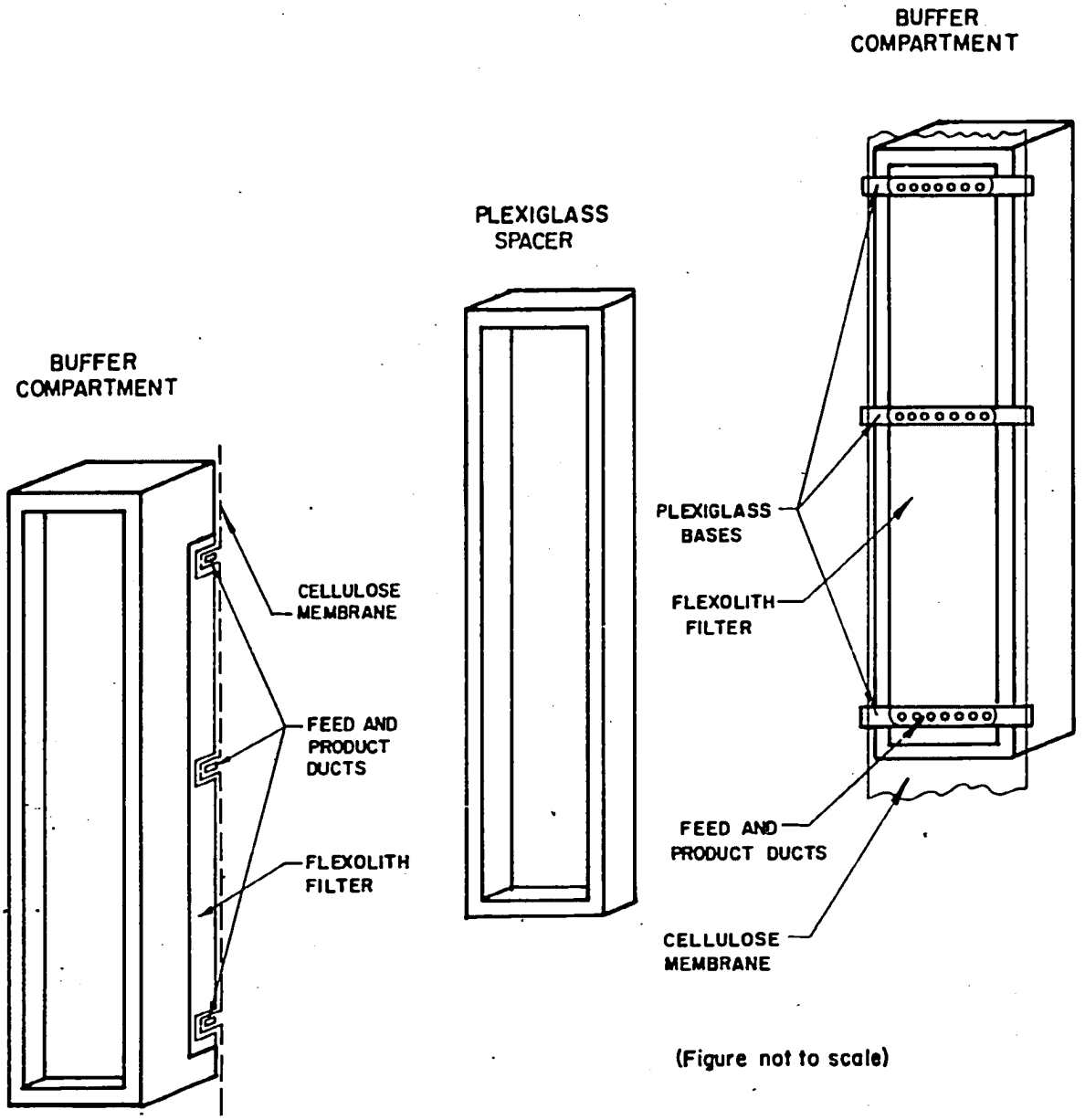
The working space between the membranes is illustrated in Figure 4-3. The membrane material was not available in plane sheet form of required size and therefore, the membranes were prepared by cutting Visking Cellulose dialysis tubing and spreading it in rectangular sheet fashion. The original tubing was obtained from Viscose Corporation, Chicago, Illinois and was 3 1/4 inches in diameter. Overall dimensions of these membranes were kept at 8 inches by 63 inches, somewhat larger than required, in order to fix them more easily into the cell. The function of these membranes is to prevent the protein component from passing through them and to reduce the bulk flow between the separation space of the cell and the buffer compartments behind the membranes. Their pore size must allow only buffer salt ions to pass through, so that the pH in the separation space is maintained constant by flow of ions through the membranes, as well as the action of the buffering compound itself.

Since the membranes are not particularly mechanically



THE THERMOELECTROGRAVITATIONAL ELECTROPHORESIS COLUMN

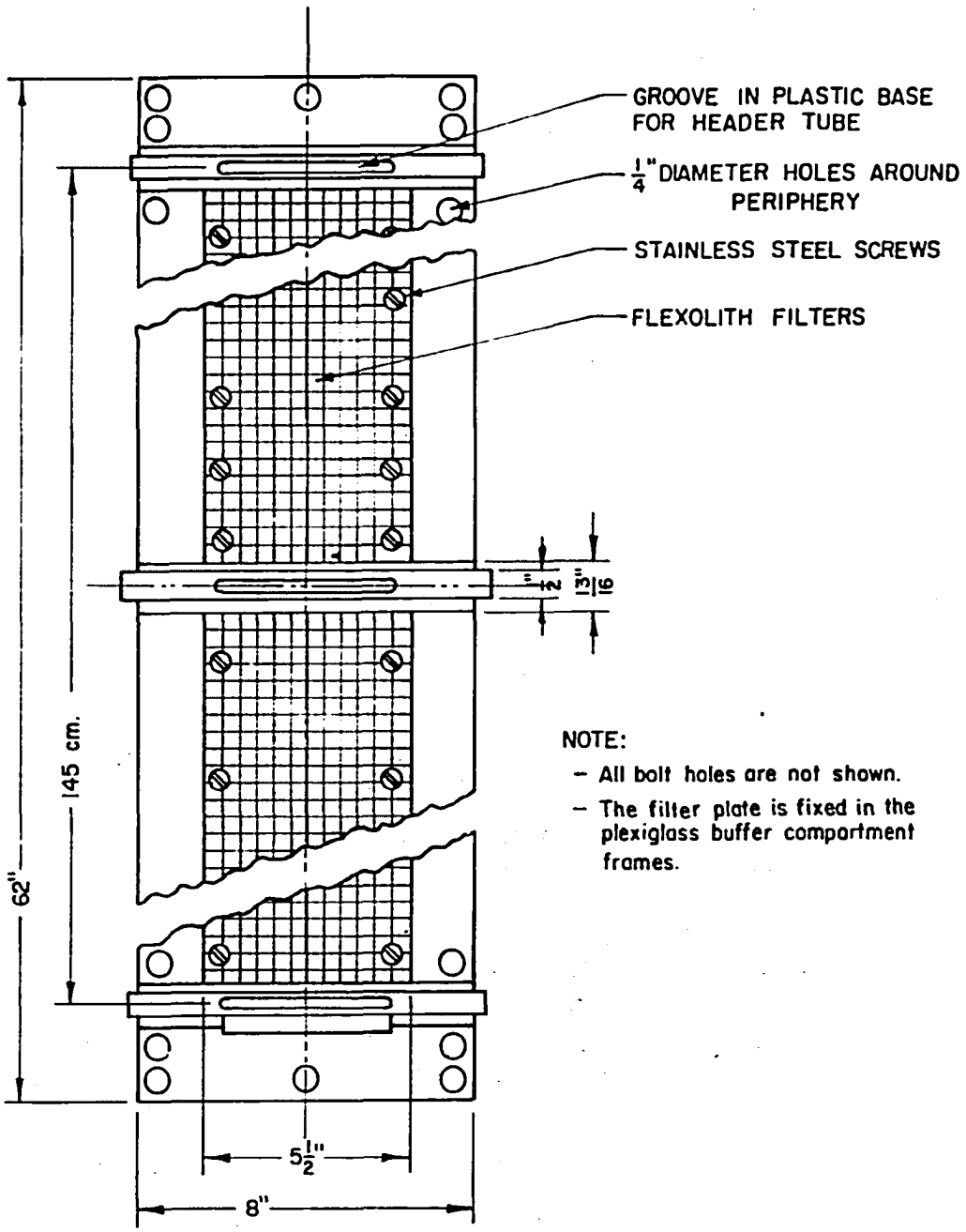
FIGURE 4-2



EXPLODED VIEW OF WORKING SPACE

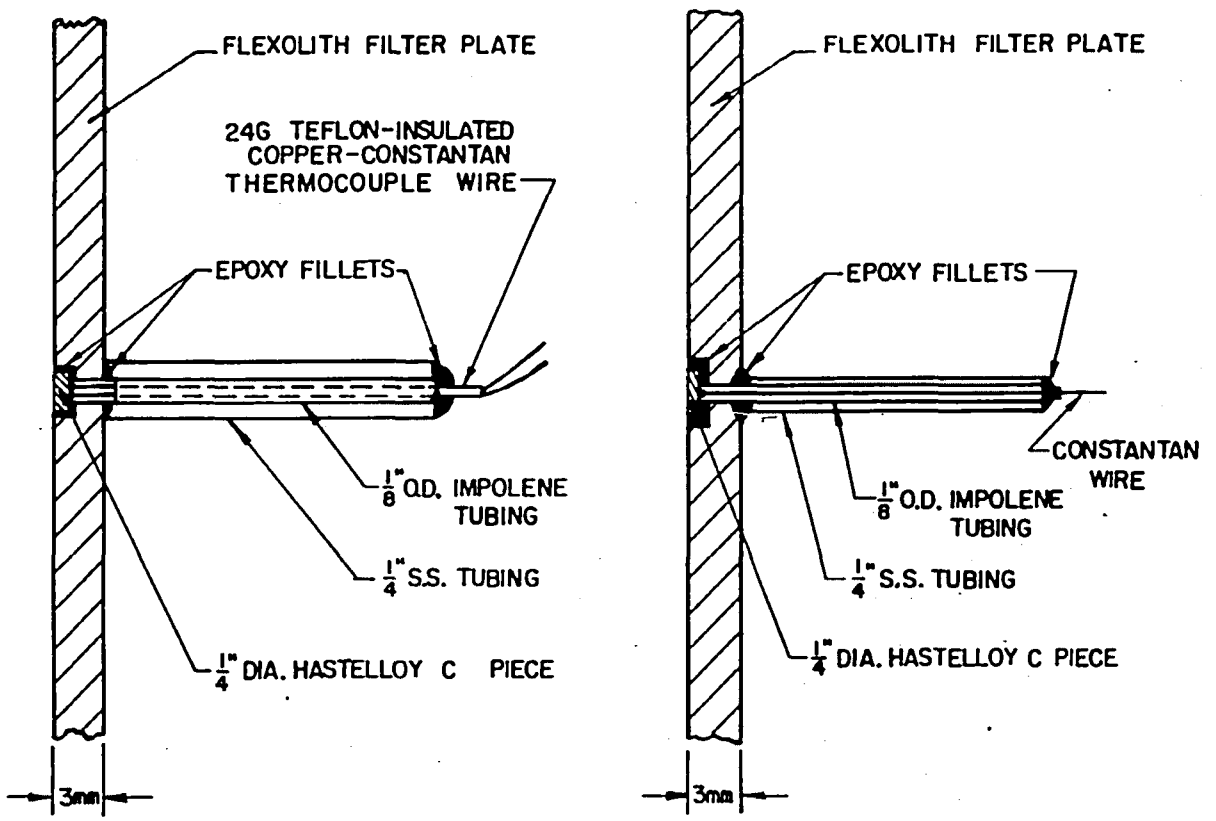
FIGURE 4—3

strong and quite large flow rates of cooling buffers might be required, these membranes were supported by mechanically strong Flexolith filter plates obtained from Wilhem Schuler, Filtertechnik, Germany. The pores of these were of macro size and buffer solution as well as proteins could flow through them. Therefore they themselves could not be employed as semipermeable membranes. As a result the cellulose membranes were always in contact with the background buffer. The Flexolith filter plates were each 3 mm. in thickness, 20 inches long and 6 inches wide in size. They were fixed to the plexiglass frame of the buffer compartment using screws so that they could be removed easily if necessary (see Figure 4-4). There was small open space between individual pieces of Flexolith so that air trapped between the membrane and Flexolith plate would be removed when the cell was first filled with solution. Two copper-constantan thermocouples were fixed on the face of each of the Flexolith plates behind the cellulose membrane (Figure 4-5a) midway at a distance of about 60 cm. from the center of the column. Two bare chromel wires were also fixed to the Flexolith at two points also midway but at a distance of 30 cm. from the center of the column on each side to experimentally measure the electric potential (Figure 4-5b). Thermocouple voltages were measured on a Leeds and Northrup Portable potentiometer (No 8662) and the voltages converted to temperatures through the use of standard conversion tables. The potentiometer could be read to 0.001 millivolts, which corresponds to approximately



INSTALLATION OF FLEXOLITH FILTERS

FIGURE 4-4



a) Installation of thermocouple

b). Installation of voltage probe

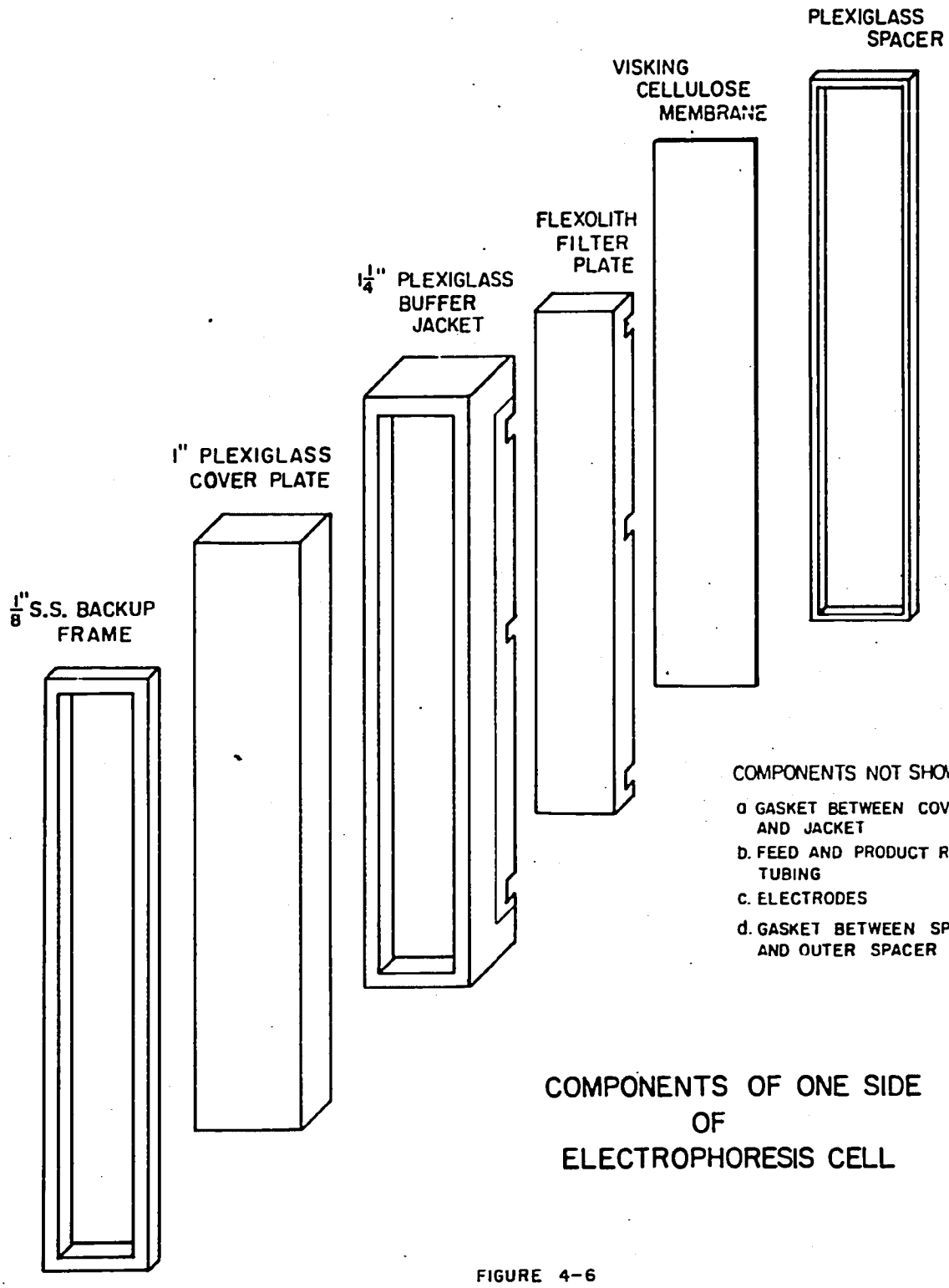
DETAILS OF THERMOCOUPLE AND VOLTAGE PROBE INSTALLATION IN FILTER PLATE

FIGURE 4-5

± 0.4°F. The reference junction was maintained constant at 32°F by use of crushed ice and water in a thermos bottle.

Voltage and temperature differences at various experimental conditions were first measured without spacers so that the two membranes were in contact with each other. Small differences were found, but they were subtracted from the values obtained during the experiments in order to obtain the voltage and temperature differences across just the solution space when spacers were inserted and there was solution in between the membranes. The attachment of the thermocouples and voltage probes to the Flexolith filters is illustrated by Figure 4-5.

A temperature gradient across the separation space was maintained by allowing buffer solution at two different temperatures to flow past the Flexolith filters. The membranes were fixed to the plexiglass buffer compartments in front of the Flexolith plates. The Flexolith forms one side of each buffer flow compartment while the other side was formed by the Plexiglass cover plate. Each buffer compartment was one and a half inches wide, while the cover plate was one inch thick. The components of one half of the column are shown in Figure 4-6. The entire assembly was held together by 1/4 inch diameter by 8 1/2 inch long stainless steel bolts, 32 National Fine threads spaced every three inches around the periphery of the column. Each cover plate was supported by a 1/8 inch thick stainless steel frame to give sufficient rigidity to the cell assembly. These bolts are omitted in



COMPONENTS OF ONE SIDE
OF
ELECTROPHORESIS CELL

FIGURE 4-6

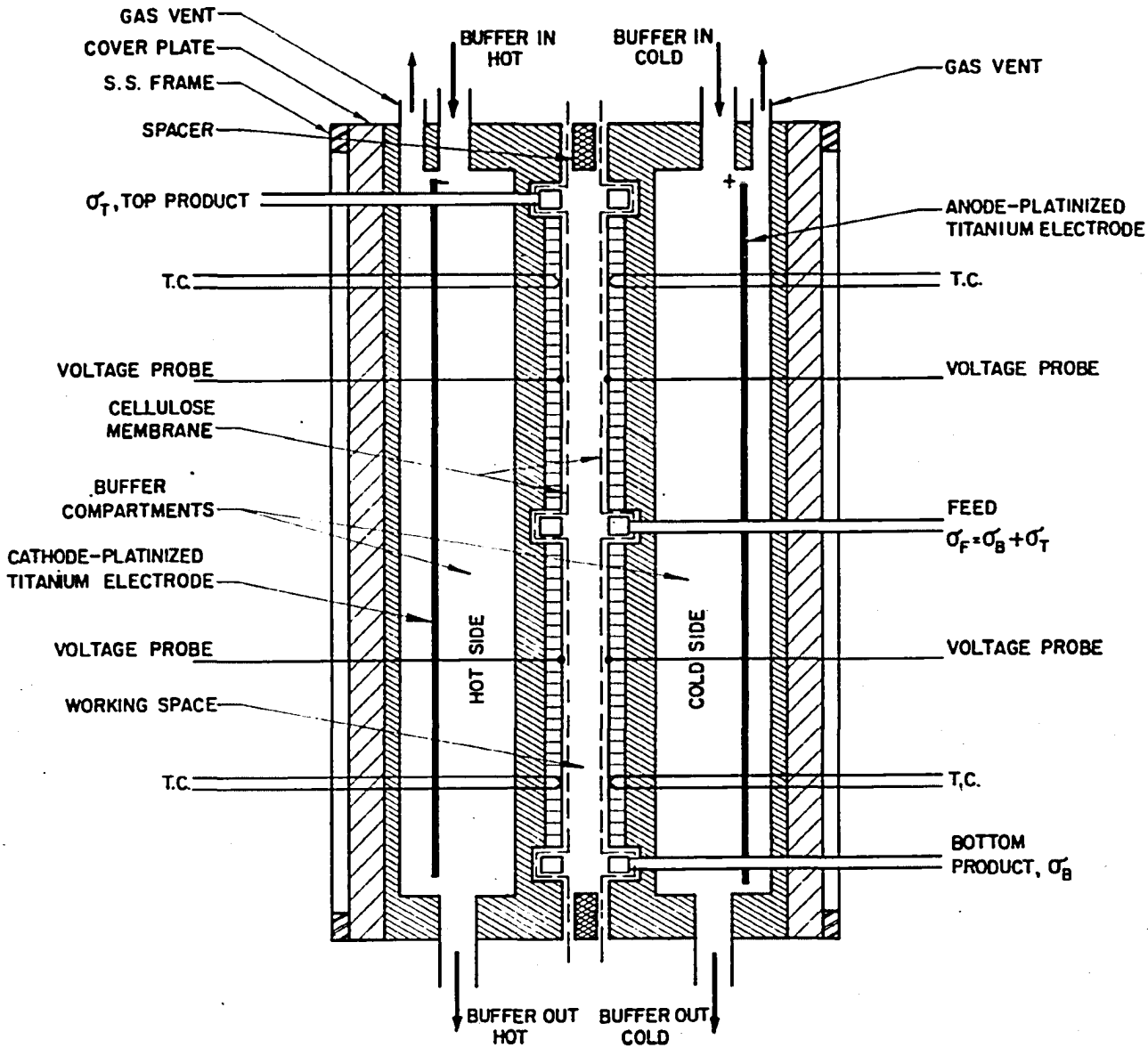
all diagrams for clarity, but are clearly visible in Figure 4-2. The cover plate was sealed to the buffer compartment along the periphery by a nylon-filled neoprene gasket, 0.015 inch thick supplied by Industrial Gaskets, Oklahoma City.

A cross section of the assembled electrophoresis column is shown in Figure 4-7. The column was supported by a metal stand and was fixed to the wall in a vertical position. A view of the electrophoresis column placed in position is shown in Figure 4-2 already referred to.

The essential layout of the gravity flow and product draw off system is presented in Figure 4-8. All material in contact with the working fluid (protein in buffered solution) or the buffer solution was either cellulose, stainless steel insulated with an acrilon spray, Impolene, Teflon, Plexiglass, polyethylene or Penton (poly-propylene).

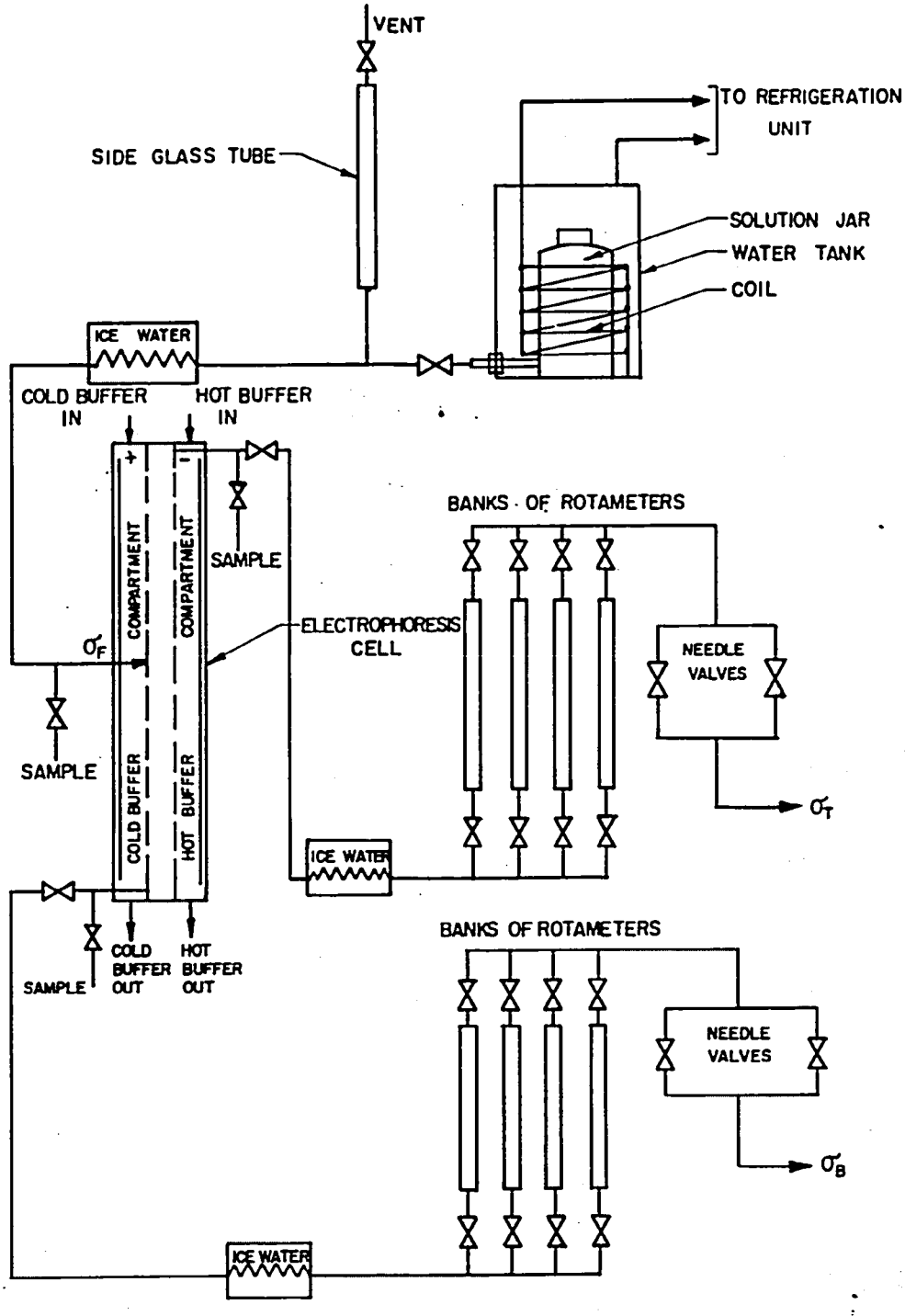
The feed was stored in a 5 gallon capacity polyethylene jar about 13 feet above floor level. A sight glass indicated the liquid level in the jar. This jar was placed in a rectangular tank filled with water at about 4°C and cooled by a small Freon refrigeration unit. The solution had to be cooled in order to avoid its denaturation on standing. The jar was connected to an outlet valve through the wall of the Plexiglass tank. Feed solution was added to the jar manually whenever required. 5 to 10 runs at low flow rates (up to 5 to 6 gm./min.) could be made without refilling the feed storage jar.

Since it was desirable to admit the feed to the cell



A CROSS SECTION OF A CONTINUOUS-FLOW THERMOELECTROGRAVITATIONAL COLUMN

FIGURE 4-7



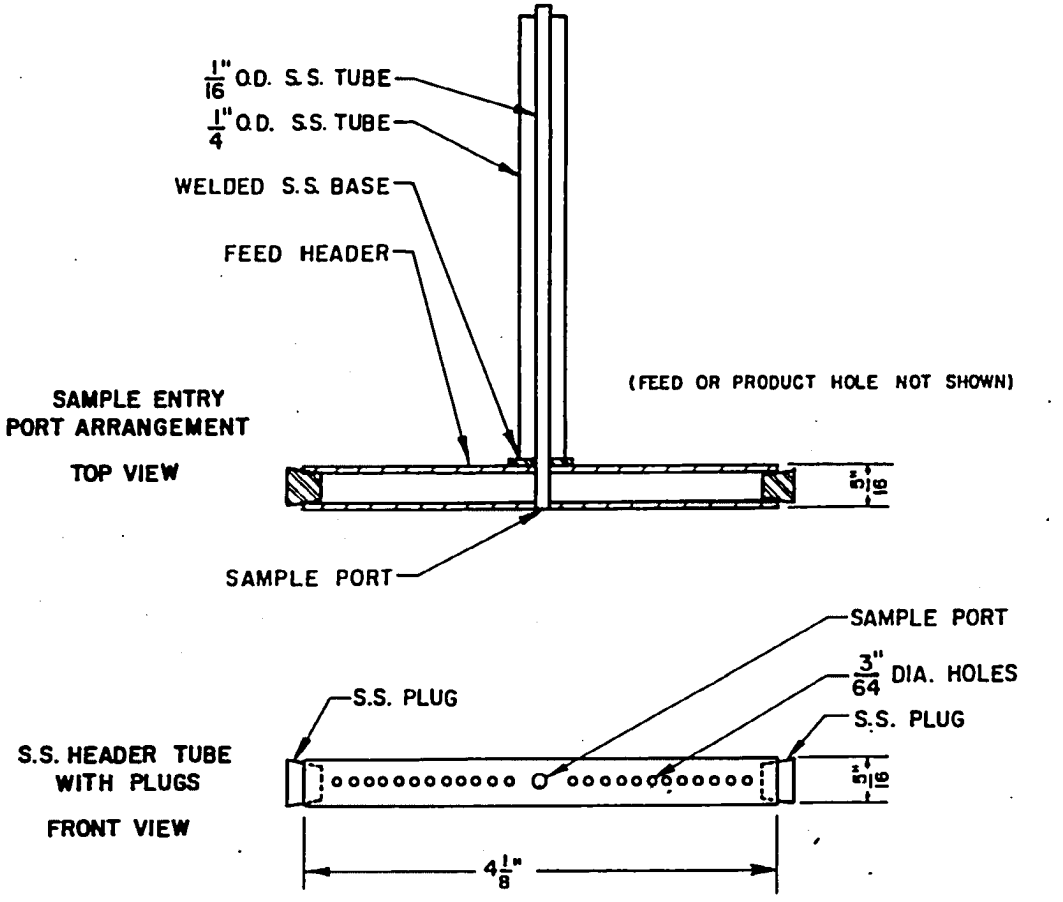
GRAVITY FLOW FEED SYSTEM

FIGURE 4-8

at the cold membrane, the feed which gets warmed up while flowing through tubing was again precooled to about the same temperature by passing it through a small stainless steel coil immersed in an ice bath before it entered the column proper.

Each feed entry and product removal connection was of special construction and supported by a Plexiglass base. The connections were a header made of rectangular 5/16 inches x 1/16 inch stainless steel tubing welded to feed and product lines of 1/4 inch diameter stainless steel tubing. A sketch of a feed or product connection is given in Figure 4-9. Each header had about 32 holes, each 3/64 inch in diameter, drilled horizontally on one side of the rectangular tubing fixed in a Plexiglass base. These holes allowed uniform flow of feed or products over the entire width of the electrophoresis column. The products collected through the holes traveled through the 1/4 inch diameter stainless steel tubing to rotameters. This arrangement is shown in Figure 4-8 presented a little earlier in this section. The rectangular tubing as well as their connecting tubing were sprayed with acrilon insulating material in 3 or more layers to insulate them electrically.

The method of attaching the Flexolith plates and the membranes to the face of the buffer compartment frame has already been shown in Figure 4-4. The pieces of Flexolith were fixed to the Plexiglass compartment by stainless steel screws in between the three positions for the feed and the

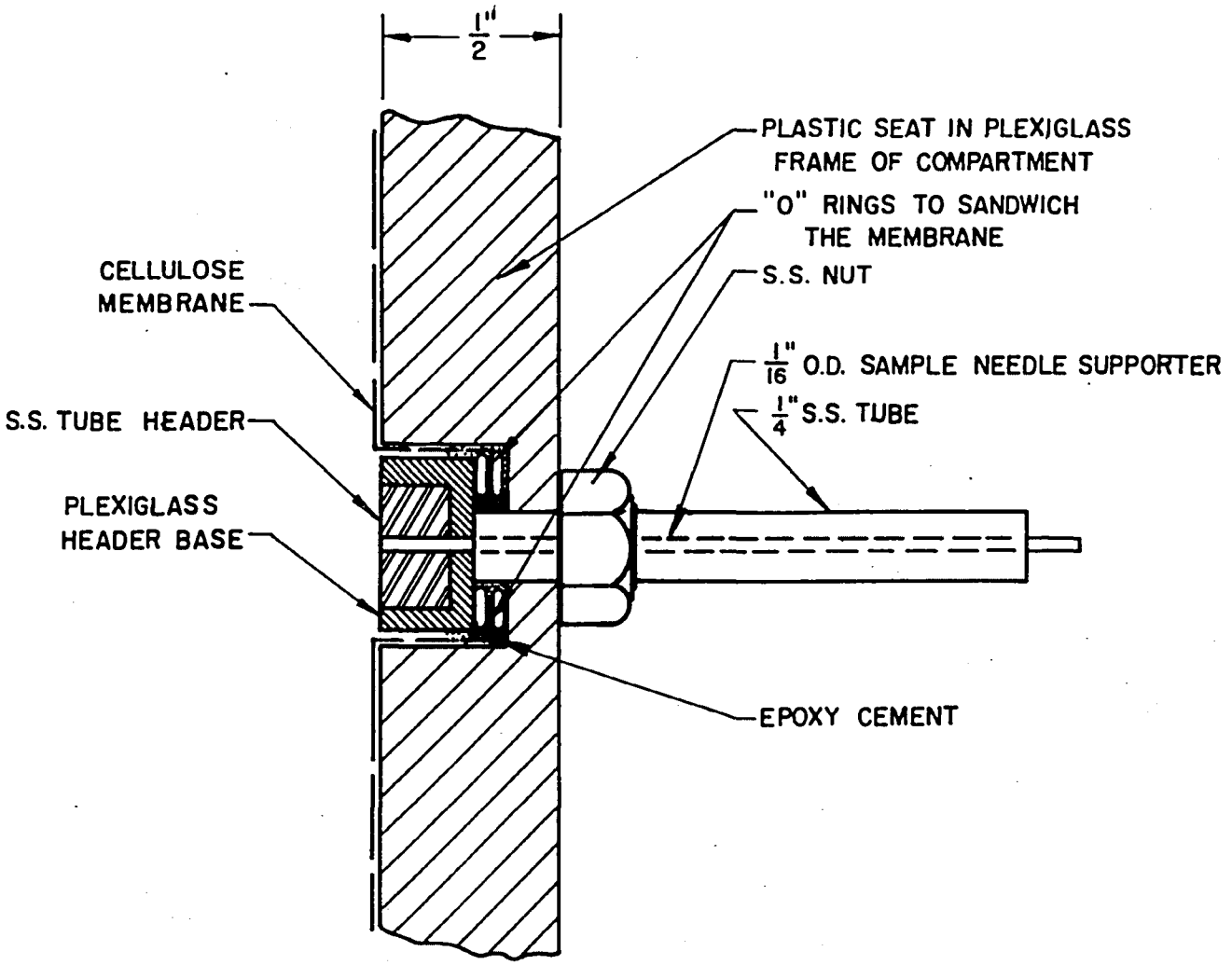


FEED AND PRODUCT HEADER CONSTRUCTION

FIGURE 4-9

product collectors. The Flexolith extended into the Plexiglass face of the compartment about 3/4 inch around the inside periphery of the buffer compartment. The remaining 1 1/4 inch width around the face of the compartment contained the outer Plexiglass spacer and a sponge neoprene gasket about 1/4 inch in width.

The attachment of the membranes to the buffer compartment frame together with the feed or product connections is shown in Figure 4-10. The rectangular stainless steel header was fixed into a groove in a plastic base. Behind the plastic base around the 1/4 inch diameter connector tube was an "O" ring of 1/4 inch diameter. The membrane was laid over the Flexolith and the required openings for the connector tubes located by using spare plastic bases and then the holes were punched. The connector tubes were inserted through the holes and then another "O" ring was slid around the connector tube behind the membrane. The plastic base with its connector tube was then fitted into the plastic seat on the buffer gasket frame. A small amount of epoxy resin mixed with an appropriate quantity of activator (obtained from Armstrong Co., Warsaw, Indiana), was applied around the "O" rings. About 2 1/2 inches of the rear of connector tube was threaded (1/4 inch N.F.) and a 1/4 inch nut tightened from behind the plastic seat. This arrangement gave a very good seal and solved earlier difficulties encountered in attaching the membrane to the frame of the buffer compartment.

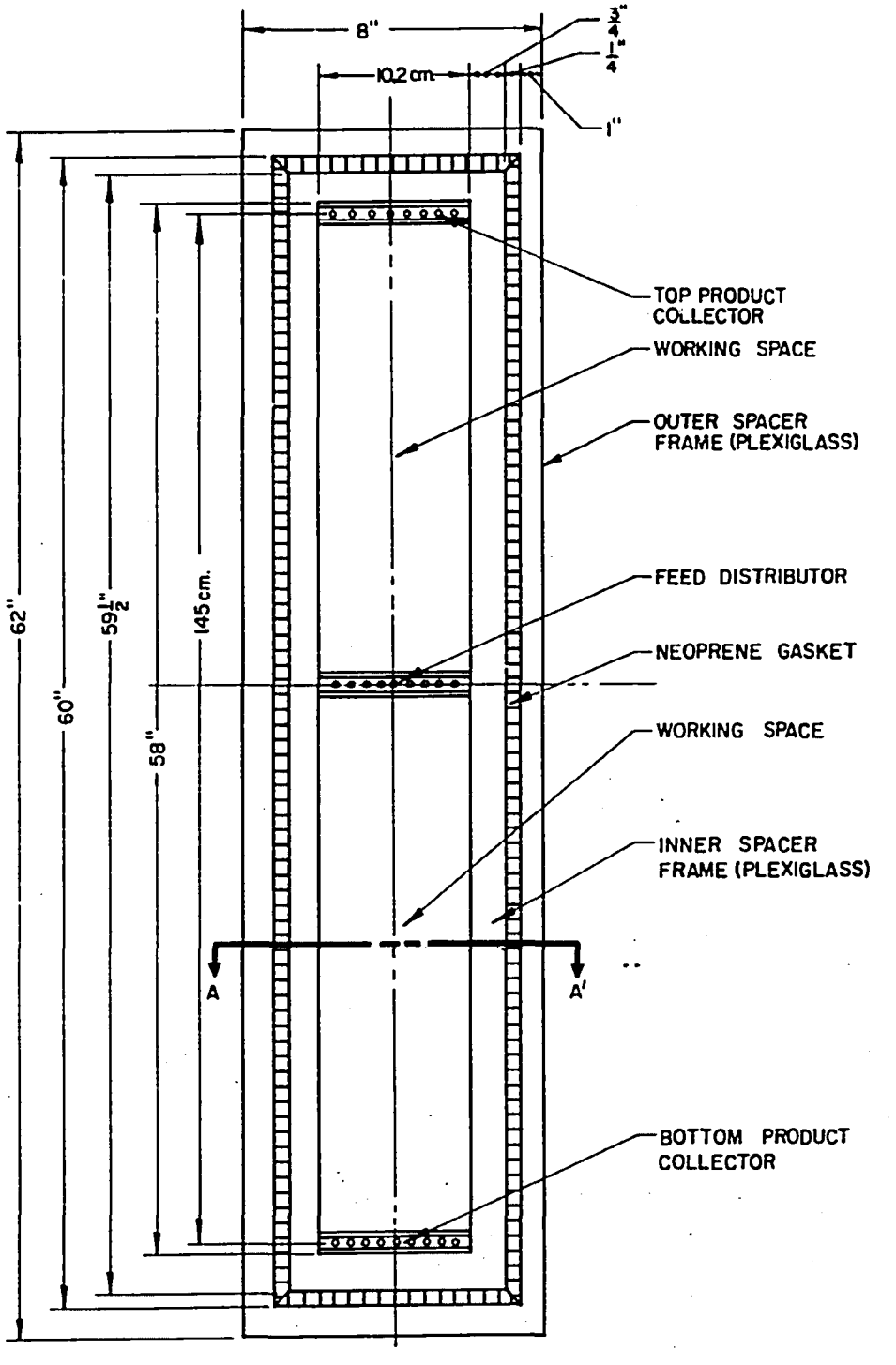


FIXATION OF MEMBRANE IN THE PLASTIC SUPPORT

FIGURE 4-10

Once the two buffer compartments were ready, a spacer and another plastic frame defining the working space in the cell were placed on the membrane. The sponge gasket slightly less than 1/4 inch in width and about 5 times as thick as the plastic spacer to be used was then placed between the spacer and the plastic frame defining the working space. This arrangement is shown in Figure 4-11. The inner dimensions of the gasket were cut approximately two percent smaller than the outer dimensions of the spacer frame in order to insure a good fit around the framework. When cut and ready for installation, the gasket was approximately 1/4 inch in width and, when placed in position, fitted in between the inner spacer frame and another outer spacer placed around the bolts with a little clearance space on the side of bolts. The gasket material acquired a permanent set when compressed between the membranes for a period of time. As a result, it was necessary to cut a new gasket each time the column was assembled.

The above method of sealing the working volume and defining the plate spacing was utilized for all the experimental runs. This method of using the gasket as stated by Fleming (17) avoids the disadvantage of "gasket creep". Reproducing the spacing between the membranes was not, therefore, a problem. The other compartment was then inverted and placed on the spacer and the holes for the stainless steel bolts carefully aligned. The two parts of the column were then tightened together. Further alignment of the two



SPACER FRAMES AND GASKET ARRANGEMENT

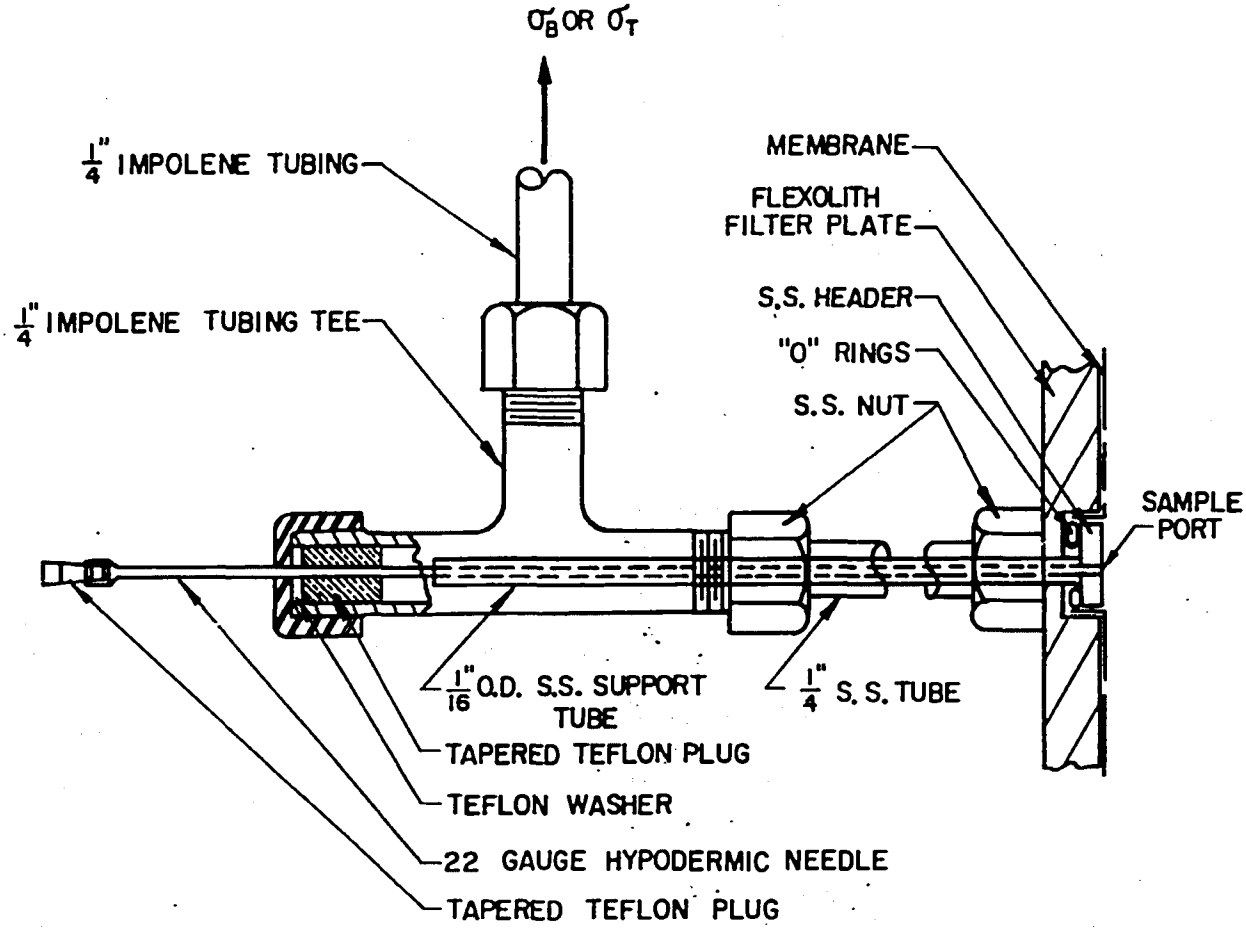
FIGURE 4-11

parts was necessary during the tightening process. The Neoprene sponge gasket material was very satisfactory for preventing leaks at the periphery of the cell. However, some leakage could occur initially around the feed and product lines. This was eliminated by adding epoxy at those positions when the column was assembled.

The inner dimensions of the plastic spacer frame defined the working volume and were held constant during eight sets of data at a width of 10.2 cm., a length of 145 cm. and a thickness (membrane spacing) of 0.3018 cm. In the other three sets of data the dimensions were width 10.2 cm., length 145 cm. and thickness 0.1354 cm. (Figure 4-11).

The products from the column flowed through small stainless steel coils cooled by ice water. During the experimental runs samples were withdrawn from the apparatus by 5 inch long 22 gage stainless steel hypodermic needles which could slide through a 1/16 inch diameter stainless steel protecting tube fixed to the front side of the rectangular tubing through the connecting tube (Figure 4-9). The sampling needle could be removed or inserted at will for cleaning when not experimenting but remained in position during the experiment. The needles extended all the way into the column so that sampling lag was eliminated and the samples were available directly from the working volume. This permitted transient batch studies of the column behavior. A sketch of the sampling arrangement is shown in Figure 4-12.

All the leads for thermocouples, power supply and



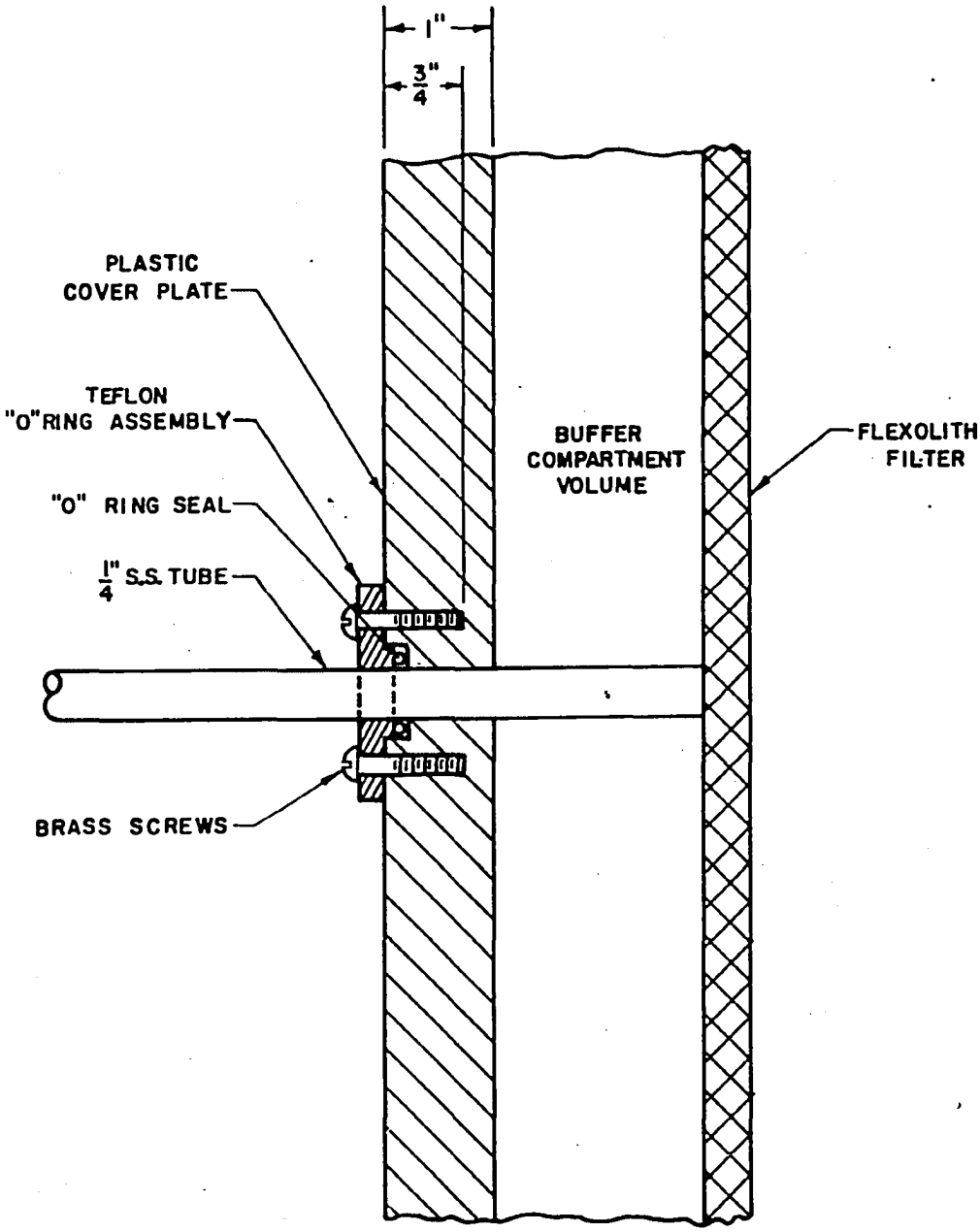
SAMPLE TAP CONSTRUCTION

FIGURE 4-12

voltage probes as well as the sample taps were taken out through the buffer compartment cover plate and required sealing. The arrangement for this is shown in Figure 4-13.

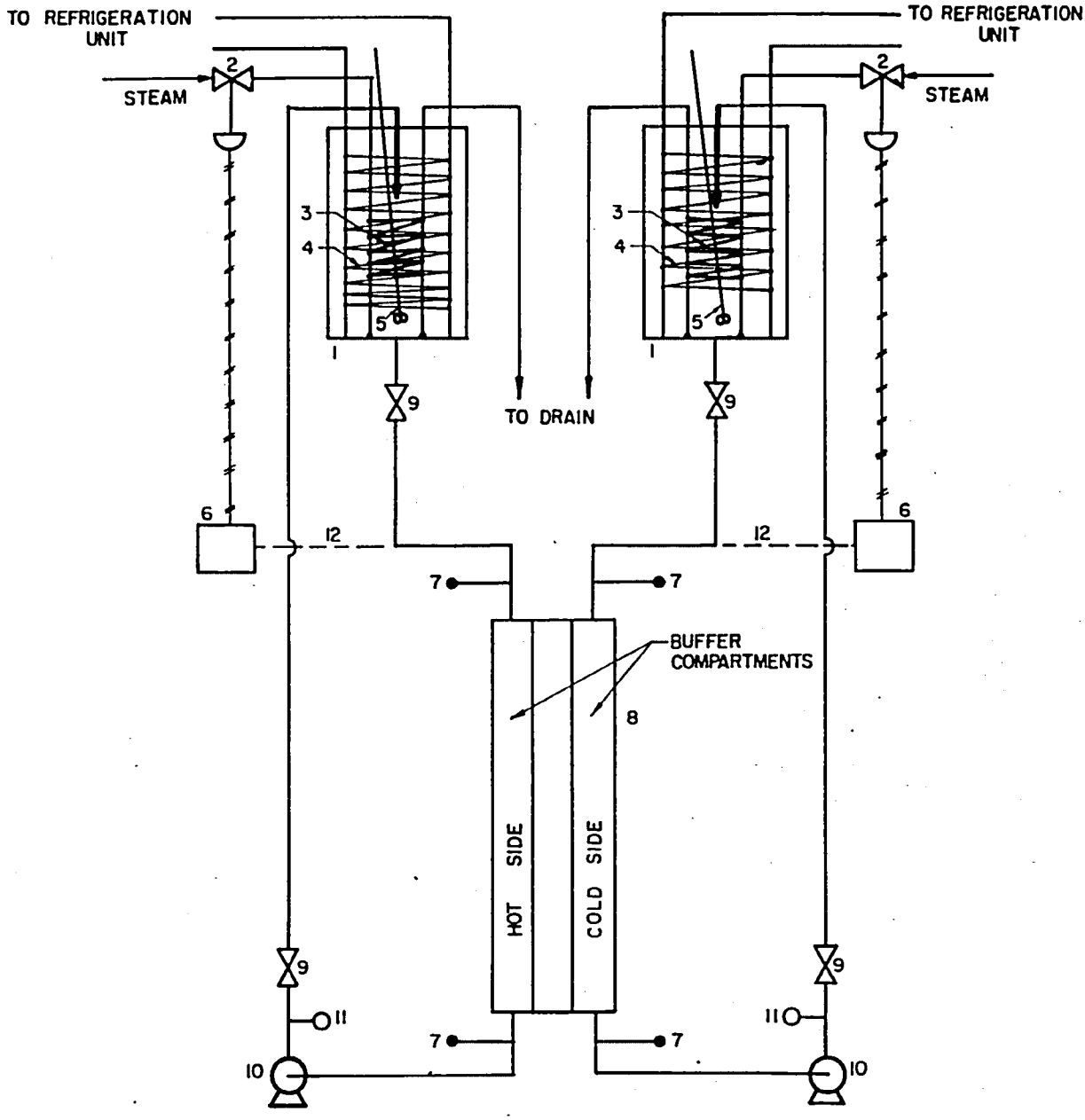
The product flow rates were indicated on two banks of Fischer and Porter Flowrators with sapphire ball floats and stainless steel fittings. The range covered by these rotameters was about 0.02 to 200 gm. per minute. The product flow rates were controlled at high rates (greater than one gm. per minute) by 1/4 inch needle valves (Avaco series 1050). At low flow rates (less than one gm. per minute) the needle valves did not control the flow accurately. Various lengths of 1/16 inch diameter stainless steel tubing were placed in series with the product line to obtain steady flow at low rates (see Boyer (7)).

The flow diagram for the hot and cold circulating buffer solution systems which hold pH constant maintained a temperature difference across the thermoelectrogravitational electrophoresis column is shown in Figure 4-14. Hot and cold buffer flow rates from 1 to 2 gallons per minute were maintained for all tests. Such high rates of buffer past the membranes were possible because of the supporting Flexolith plates. These high rates of buffer maintained constant pH for four experiments (about four days) with the same solutions. When there was appreciable change in pH of the solutions, fresh buffer solutions were prepared before taking the next run. These flows also limited the vertical temperature change of either the hot or cold buffer through



LEAKAGE SEALING AT COVER PLATE

FIGURE 4-13



1 – 55 Gallon polyethylene tanks
2 – Air operated diaphram valves
3 – S.S. steam coils
4 – S.S. cooling coils
5 – S.S. stirrers
6 – Temperature controllers recorders
7 – Thermometers
8 – Electrophoresis column
9 – Penton globe valves
10 – Penton centrifugal pumps
11 – S.S. pressure guages
12 – Thermocouples

BUFFER CIRCULATION SYSTEM

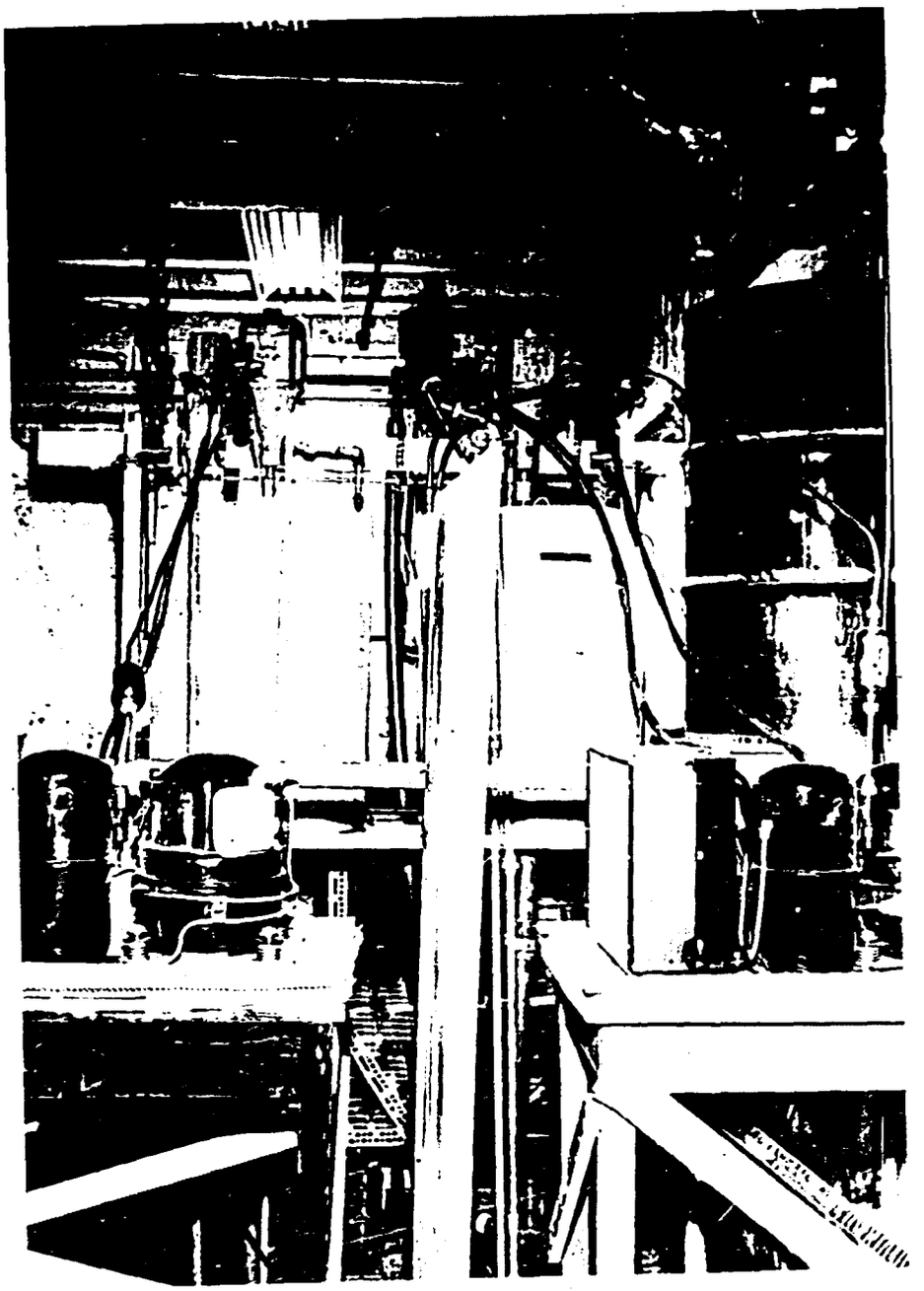
FIGURE 4 – 14

the buffer compartments to less than 1°F. The column was operated below room temperature to prevent the protein system from spoiling.

Elevated cold buffer solution storage tanks were uninsulated 55 gallon polyethylene tanks supplied by Cole Parmer Co., Chicago. The solutions in the surge tanks were stirred constantly by 1/4 H.P. stainless steel stirrers obtained from Patterson Foundry Co., East Liverpool, Ohio. Each tank was provided with a refrigeration coil of stainless steel with approximately 15 sq. ft. of surface area and another stainless steel heating coil of about 5 sq. ft. area. Saturated steam passed into the heating coils. The refrigeration units were 1 ton capacity for each tank. A view of hot and cold circulating buffer tanks together with the refrigeration units is shown in Figure 4-15. The hot and cold buffers were circulated through the system by means of 1/2 Horse power Penton pumps (Chemtrol Corporation Co., California).

The steam passing into the steam coil was regulated by Honeywell air operated diaphragm valves. These valves were actuated by Brown circular chart Electronnik temperature recorder controllers. Thermocouples (Brown type T, copper-constantan) located in the pump section supplied the input signals to the controllers. Temperature variations were always less than 0.5°F.

The buffer flow was controlled by Penton bypass valves (Vanton Pump Co., Hillside, N.J.). All the main lines

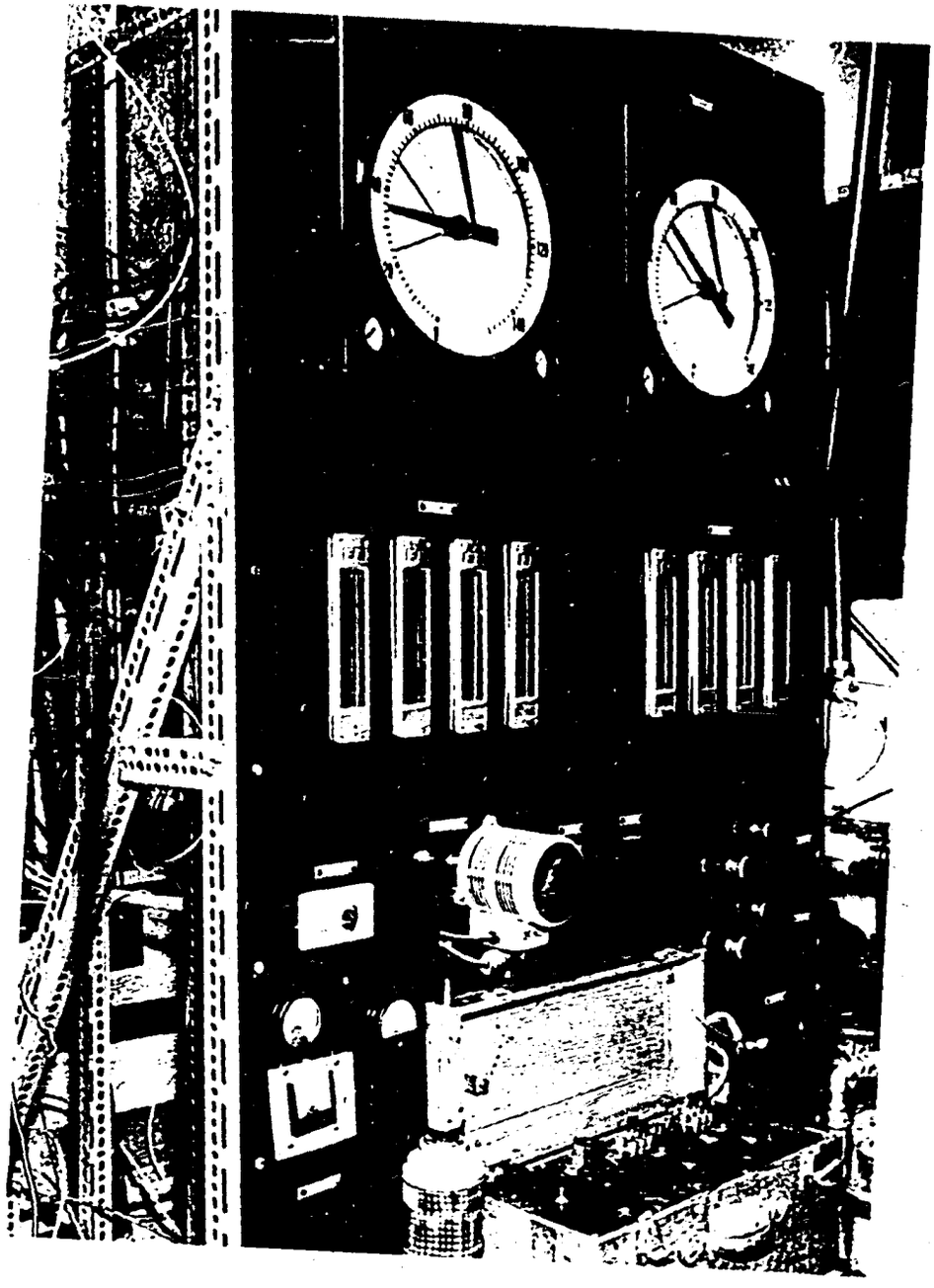


A VIEW
OF
SOME AUXILIARY EQUIPMENT

FIGURE 4-15

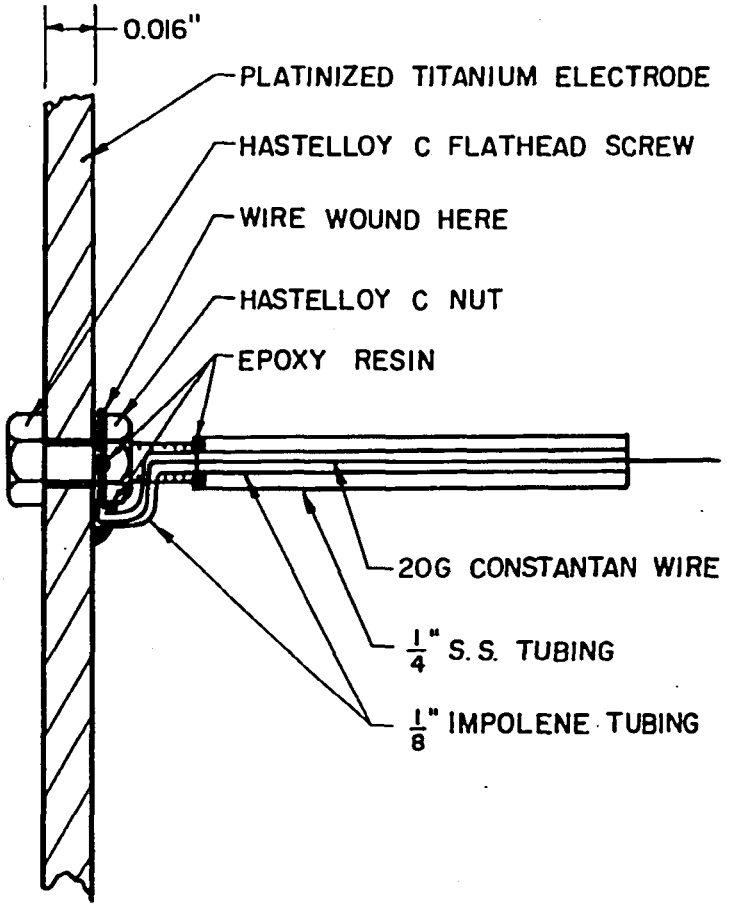
in the buffer systems were of nominal 1 1/2 inch diameter, extra heavy polyvinyl pipe and threaded fittings were employed. The lines were not covered with insulation. The piping was connected to the column through semiflexible impolene·plastic tubing to avoid any vibrational effect on the column itself. Thermometers installed in all buffer solution lines entering and leaving the column were used to check the temperature shown by the controllers. The buffer storage tanks were connected directly to the buffer compartments of the cell and the solutions were pumped from the cell to the tank to avoid pump pressure on the membranes. The level of the buffer solution in each tank was maintained to the same height so that equal hydrostatic pressure was exerted on the two membranes. The feed jar was at a higher level than the storage tanks so that the membranes were pressed towards their supporting Flexolith plates. Where possible, instruments were centralized on a control panel shown in Figure 4-16.

The direct current field was applied across the membranes by means of two platinized titanium plates each of 0.016 inch thickness situated in the buffer jackets coextensive to the membranes. The lead wires of copper were attached as shown in Figure 4-17. The direct current was supplied by an electronic D.C. power supply procured from Harison Laboratories, Berkley Heights, N.J. capable of producing 36 volts at 5 amperes. Low voltages had to be used in these experiments because at currents more than about 2.5 amperes,



CONTROL PANEL

FIGURE 4-16



INSTALLATION OF LEAD WIRE
FOR
POWER SUPPLY

FIGURE 4-17

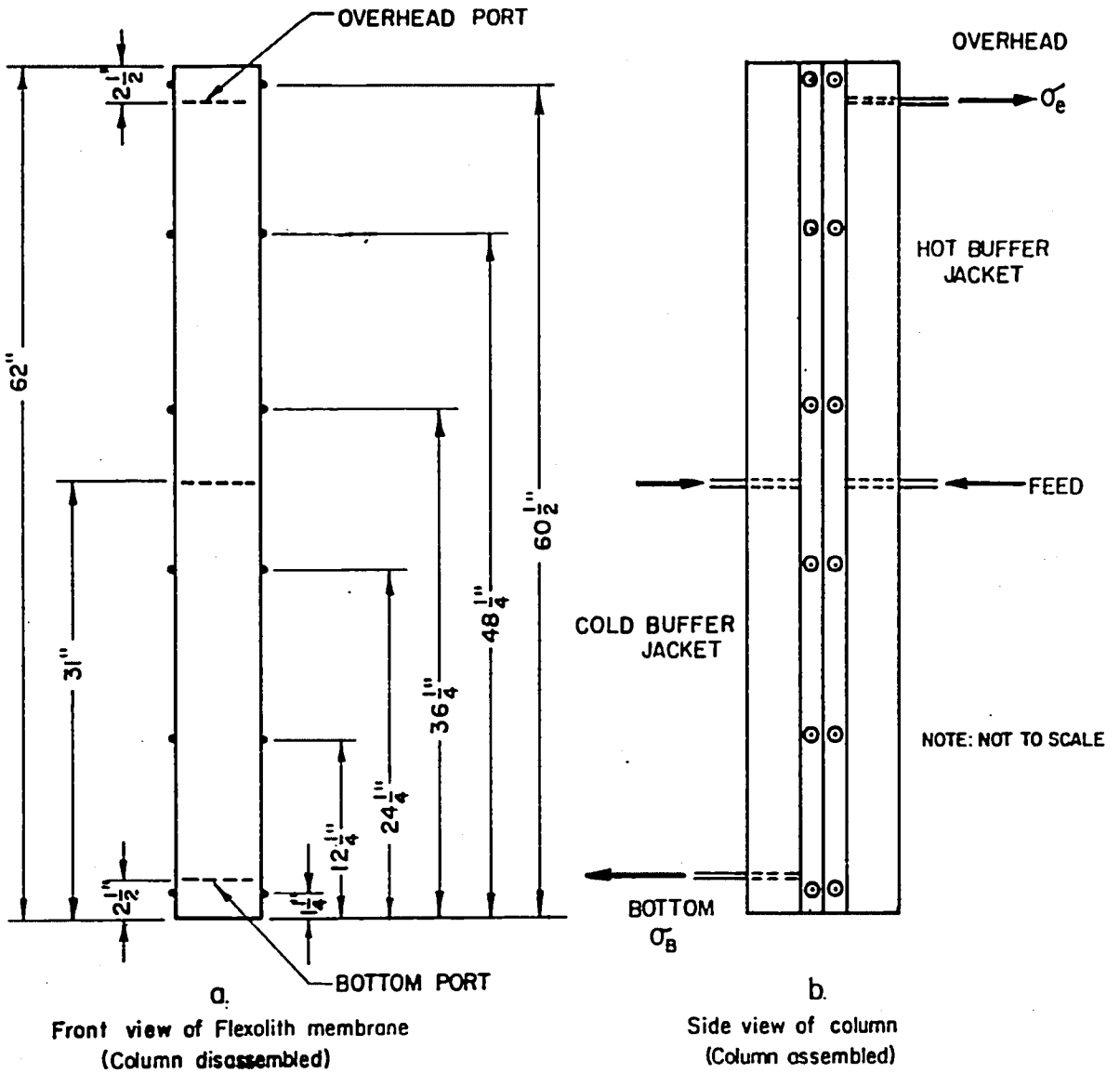
there was excessive foaming. The current through the circuit was measured by a calibrated ammeter while the voltage across the membranes was indicated by voltmeters and measured by the precision potentiometer, (Leeds Northrup).

Experimental Procedure

In experimental runs with the continuous flow thermogravitational electrophoresis column, the steady state separation factor was obtained as a function of flow rate. The temperature difference, membrane spacing, pH of the solution and electric field strength were treated as parameters. A set of runs consisted of product compositions measured as a function of flow rate for a fixed grouping of the various parameters. New membranes had to be installed each time the column was taken apart. The buffer compartments were always kept full of buffer solutions so that the membranes remained wet. A small amount (about 3 to 5 gm.) of benzoic acid was added to the buffer solution (50 gallons) to prevent bacteria from attacking the cellulose membranes. Membrane spacing was adjusted by means of the Plexiglass spacers of different thicknesses. Very low spacing could not be employed because Plexiglass material in very low thicknesses was not available and metallic material such as stainless steel was considered unsuitable for spacer. The membrane spacing was measured by the special technique developed by Boyer (7) for thermal diffusion studies. For this purpose pairs of aluminum pegs were attached adjacent

to each other on the edge of the hot and cold buffer compartments as shown in Figure 4-18. The column was bolted together without spacers in place and the distance between a pair of pegs was determined by a micrometer, reading to one thousandth of an inch. The column was then taken apart and reassembled with a spacer in place and another reading taken between the same pair of pegs. The membrane spacing at that point was the difference between the pairs of readings. Twelve pairs of pegs were spaced around the edge of the column, and the average of all measured points was reported as the membrane spacing for a run. This method of membrane spacing determination could not be checked with the volumetric method described by Powers (48) because of the porous membranes required for this column.

Prior to taking a set of data, the column was assembled with whatever spacer (approximate membrane spacing) was to be investigated using a torque wrench to tighten all bolts to 150 lb. torque. The assembled column was placed in position and connected to the pipe lines. It was filled with distilled water and allowed to stand for at least a day to wash residual glycerine from the membranes. It was then emptied through the bottom and filled with buffer solution, and the hot and cold buffer systems started and brought to their selected temperatures. The mean column temperature level was taken as the integrated average temperature (Equation 3-44). This was not much different from the mean arithmetic temperature obtained from the temperatures



LOCATION OF ALUMINUM PEGS UTILIZED FOR MEASURING MEMBRANE SPACING

FIGURE 4—18

indicated by the hot and cold buffer temperature controller recorders.

The cold buffer temperature in all experiments was maintained at approximately 4°C while the hot buffer temperatures were maintained at about 4°C, 12.5°C and 16°C as desired. No higher temperatures could be employed because of the instability of the protein solution at or above room temperature.

Laboratory Procedure

The first test of any set of separation factors as a function of flow rate was a transient batch test in order to study the transient behavior of the column and incidentally to determine the time required to reach the steady state concentrations at the top and the bottom of the column. For the transient run, the product lines were purged and the feed flow started. After about 15 minutes, the flows of hot and cold buffers were started and the outlet valves on the product lines were closed stopping the feed. About two hours were allowed for the temperature field to establish and samples from both top and bottom were collected. (The samples taken at the beginning of each transient experiment never showed any separation.) After first samples were taken, the power supply was turned on and adjusted to the desired value of E. Samples were then collected from the bottom and top of the column at regular time intervals (about an hour long). A particular transient run was stopped after a time equal to

that required to reach steady state in a long transient run carried out previously at low field strength. In subsequent transient runs, each run was continued sufficiently longer than their estimated time, to insure that steady state was reached. The time required to reach steady state in a transient run was then utilized as a guiding factor in deciding the time for withdrawal of the first sample in a flow run.

When a flow run was to be taken, the feed and product flow lines were first purged and product flow rates set at the desired values as indicated by rotameters. Actual flow rates were calculated by weighing samples of about 5 to 25 grams taken over a measured time interval in the case of each sample. Flow rate sample bottles were immersed in ice water during collection of the samples to reduce the evaporation loss of solvent water. Two product samples, one from each end of the column and each about 0.75 to 1 ml. in volume were collected for the concentration determination. Before collecting these samples, the sampling needle was purged by allowing some solution to flow out of it.

The first sample of a flow run was collected after a time interval equal to that required for the transient run to reach a steady state separation. Another sample was collected one-half hour later and concentrations reported are averages of these two readings. This procedure was necessary because the colorimetric methods used to analyze the solutions quantitatively were not rapid enough to check for the steady

state condition while the test was in progress.

Reagents and Analysis

A single component system, that of bovine albumin in two different suitable buffer solutions, was employed to obtain experimental data on the separation behavior of the electrophoresis column. No isoelectric component was added in order to simplify analysis of the samples from the apparatus. Bovine albumin was selected for study because it was available in sufficient purity and quantity from Nutritional and Biochemical Corporation and also because its properties such as mobility and diffusivity were available from the literature (85). It has a wide range of mobilities and is therefore suitable for the study of the effect of pH of the solution upon the transport. It was also comparatively easy to analyze by a colorimetric method.

The bovine albumin material obtained was of 95% purity and was further purified before its use in experimental work. A solution of bovine albumin of concentration about 2 to 2.5 gm./100 ml. was prepared and centrifuged to remove insoluble or dissolved material. It was then electrodialysed at 4°C overnight and filtered again. The filtered material contained a little hemoglobin but this was in negligible amount (less than 0.05%). This solution was then stored at 4°C.

Boric acid and borax buffer was used to obtain a solution at pH 8.6 and phosphate buffer was used to prepare

a solution of pH 6.0. In order to prepare the final buffered solution of required pH for use in experiments, the following procedure was followed.

The concentration of albumin in the unbuffered solution was determined by estimation of its nitrogen content by Kjeldahl procedure. The final buffered solutions to be used in experiments were prepared by mixing together a required amount of the unbuffered solution and the required amounts of buffer compounds and then making up the predetermined volume by addition of water. Solutions of the same concentration containing 1 gm. per 100 ml. protein were used in all experiments.

To avoid denaturation of the protein in solution, the pH was kept in the alkaline range. In addition, a small quantity of sodium merthiolate was used to prevent bacterial attack and subsequent spoilage of the solution. In order to avoid formation of foam during electrophoresis and during the preparation stage of the solution small amounts of antifoam agent Silicone A were sprayed on the solution.

Buffering salts used in preparation of required solution were of reagent quality. Physical properties such as specific gravity and viscosity of solution were measured by using a specific gravity bottle and Oswald viscometer respectively. The electrical conductivity was obtained by using a conductivity apparatus. All these data are listed in Appendix C. The density coefficients $\left(\frac{\partial \rho}{\partial T} \right)$ used in the calculations were those of water, and were calculated from

the density data for the appropriate temperature levels. The values of diffusivities and electrophoretic mobilities were obtained from the literature (85) and were corrected for the temperature and viscosity. These values are reported in Appendix C.

The determination of bovine albumin in a sample was done colorimetrically by the biuret method described by Robinson et al. (53). A measured quantity (0.5 ml. of the sample was placed into a 15 ml. graduated centrifuge tube and about 0.2 ml. of 10% trichloroacetic acid was added to the solution to precipitate the protein. Normally this solution would be centrifuged and decanted, but this procedure could not be followed here because of the small amount of solution. The precipitate was allowed to stand overnight, and then dissolved in sodium hydroxide solution. The volume of the solution was then increased to about 8.5 ml. and the solution allowed to stand about two hours to dissolve any protein spikes formed. Then, 0.2 ml. of 20 per cent copper sulfate was added to develop the biuret pink color. The volume was then brought carefully to 10 ml. by adding sodium hydroxide solution and the solution was gently shaken to insure thorough mixing and allowed to stand about 2 hours to allow the color to develop fully. The solution was then filtered through Whatman 42 filter paper, the first few drops being discarded. The transmitivity and the absorption of the solution were then determined using a Beckman Du spectrophotometer at 560 μ, the wave length of maximum

absorption. The concentration of the sample was then obtained by comparing the absorption obtained with that given by a solution of known concentration. The concentration was calculated by using the formula (3):

$$C_{SA} = \frac{R_{SA}}{R_S} \cdot m_s \cdot \frac{100}{V} \qquad 4.1$$

where C_{SA} = gm. of bovine albumin in sample solution in gm./100 ml.

m_s = concentration of bovine albumin in standard solution, gm./100 ml.

V = volume of sample solution actually used in biuret color production, ml.

R_S = optical density of the standard solution

R_{SA} = 2 - log t' where t' is the transmittance reading of the unknown solution

Equal volumes of the unknown and the standard solutions were taken for color production and both were diluted to the same final volume. It was possible to determine the concentrations by the method employed here within an accuracy of about 1.5 per cent.

Results

The primary data resulting were concentrations at the top and bottom of the column, or separation factors, as functions of flow rates for a given temperature difference, membrane spacing, electric field strength and solution pH.

These data are summarized in Appendix B. Transient data were also obtained corresponding to each one of the flow data sets and the results of these experiments are also presented in Appendix B.

The other data obtained were the temperature of the membranes, voltage difference, temperatures of the cold and hot buffer solutions at entrance and exit of the cell, temperatures indicated by recorder-controllers, the room temperature and the ice bath reference temperature. These secondary data are not listed in this thesis.

CHAPTER V

DISCUSSION OF EXPERIMENTAL RESULTS

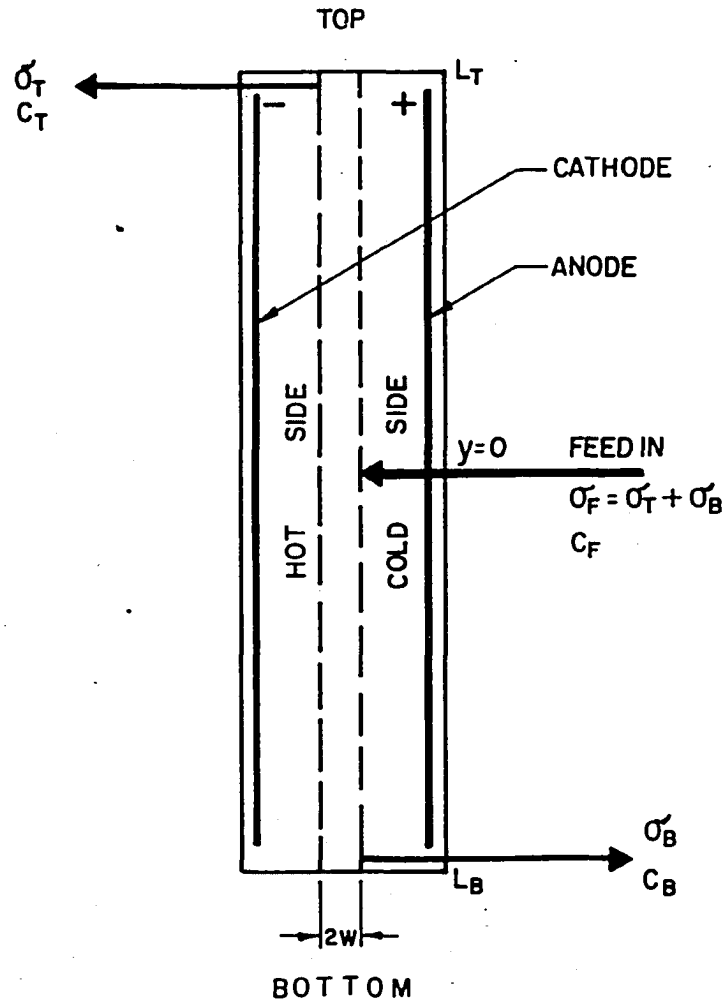
In Chapter III, the theoretical treatment of batch thermogravitational columns originally proposed by Furry, Jones and Onsager (18) and later applied to continuous-flow thermogravitational columns by Jones and Furry (81) and later by Powers (48) was extended to include the case of both batch and continuous flow thermoelectrogravitational electrophoresis column operation. Data obtained using the equipment and procedure described in Chapter IV provide a means to test the range of application of the theory proposed, and, if possible, to explain deviations from the theory.

The solution investigated in the continuous-flow electrophoresis column was a single mobile component dilute solution of bovine albumin. This single component system was chosen for several reasons: (1) There are no data as yet available on such systems using center-fed continuous-flow electrophoresis column. (2) Considerable information is available in literature on bovine albumin. (3) The solution to the transport equation is of a straightforward form. Experimental conditions were adjusted to satisfy the assumptions under which the transport equation was derived.

As mentioned in Chapter IV, first a transient run was taken for a group of the values of the parameters and then a set of flow runs was carried out. An experimental "set" of data was normally a series of steady state separation factors (C_B/C_T) obtained as a function of flow rate (σ) data taken with the center-feed electrophoresis column, treating as parameters the field strength (E), the temperature difference (ΔT), the membrane spacing (2ω) and the pH of the solution. The experimental results are presented as (1) steady state flow data and (2) transient and steady state batch data. It was not possible to keep the temperature level $\overline{T}$ the same in all experiments owing to the limited temperature range available for the operation of the column. Column length and physical system were never varied.

Detailed data for all steady state continuous flow runs are presented in Tables D.1 to D.11 of Appendix B. The column parameters and the conditions of the experiment are listed for each of the data sets. The flow pattern in all the sets of runs is diagrammed on Figure 5-1.

Sets 1,2,3 and 4 were taken to study the effect of varying the field strength with other variables kept constant ($\Delta T = 8.5°C$, pH = 8.6, $2\omega = 0.3018$ cm.). In these sets the effects of both ΔT and E were operative. Data sets 5 and 6 were taken at two different E values but at $\Delta T = 16°C$ to study the effect of ΔT on the column operation. In the data sets 1 to 8, the spacing between the membranes was held constant (0.3018 cm.). The data sets 9 and 10 were taken at



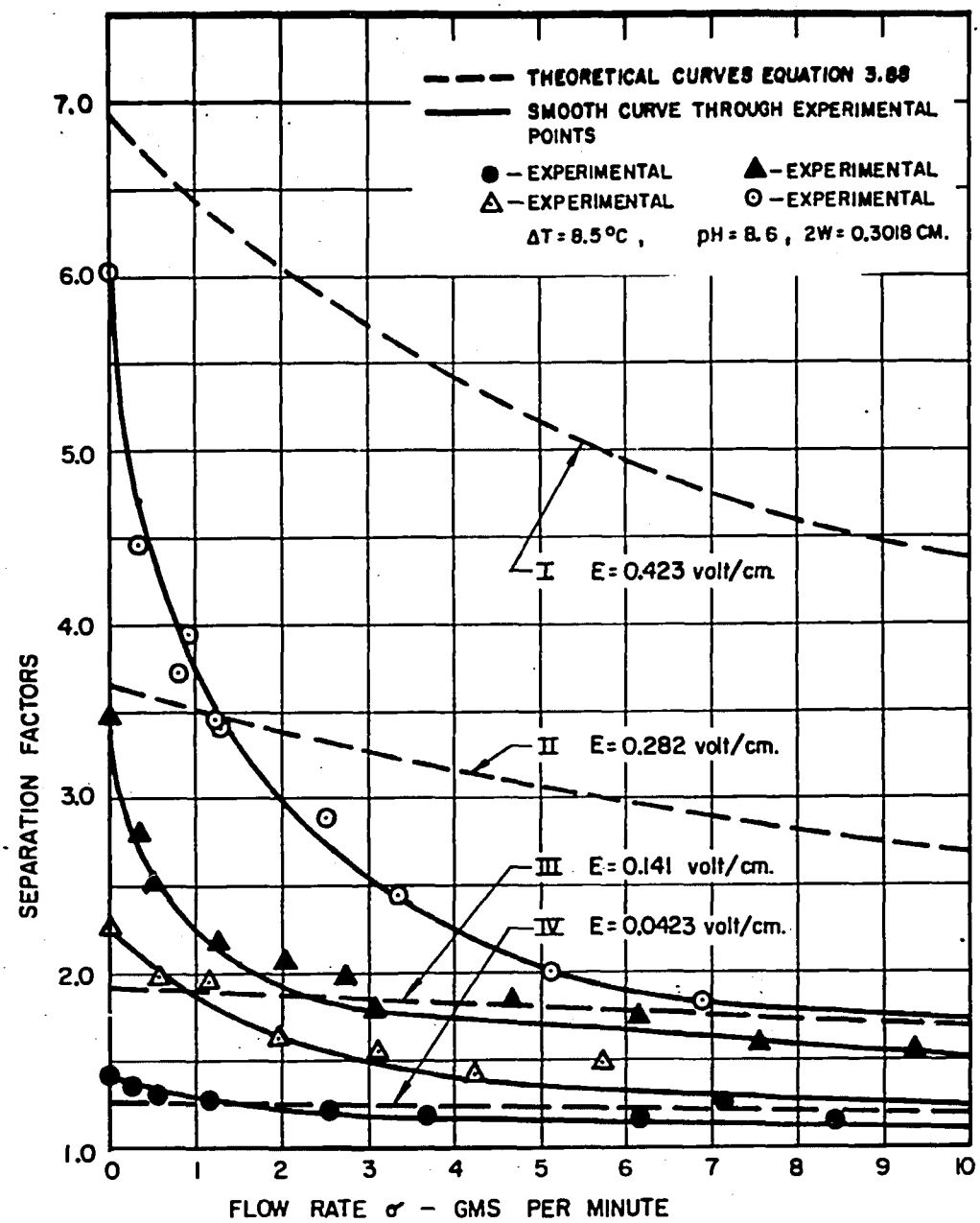
FLOW PATTERN USED IN EXPERIMENTAL SETS I-II

FIGURE 5-1

a different membrane spacing (0.1354 cm.) to study the effect of variation in this parameter. pH in all the runs of data sets D.1 to D.10 and D.1A to D.10A was maintained at 8.6. Data set 11 was taken at the second spacing of 0.1354 cm. but at a lower pH of 6.0 to have a lower mobility value.

In the case of each data set, a transient run as described in Chapter IV was taken. The results of these transient runs for all of the data sets are listed in Tables D.1A to D.11A of Appendix B.

In Figure 5-2 are presented some steady state separation factor versus flow rate data to illustrate the variations noted with different values of E. The sets of data were all taken at the same membrane spacing and temperature difference. It is observed that there is no quantitative correspondence at all between theory and experiment. However, there appears to be a functional similarity between the experimental data and the theory as seen from the similar shape of the curves. Therefore, it seems possible to correlate the experimental data by introducing correction factors as was done by many investigators for thermal diffusion columns. In what follows therefore, the effect of varying the parameters such as flow rate, temperature difference, membrane spacing and the mobility of the component on the values of separation factors realized in an experiment is discussed from two aspects, namely qualitative functional correspondence and quantitative correspondence.



COMPARISON
OF
THEORETICAL AND EXPERIMENTAL SEPARATION FACTORS

FIGURE 5–2

Qualitative Aspects of the Theory

The several assumptions that were utilized in the derivation of the equation 3.88 are summarized in Appendix E. Many of these assumptions are reasonably valid when laminar conditions exist in the column and when the temperature difference, field strength and concentration change are not very large. Experimental conditions were chosen to satisfy these and other restrictions as far as possible. The validity of the assumptions can only be evaluated by comparing the theory with the results of experiments. In no case would complete correspondence be expected unless conditions such as low flow rate, low membrane spacing, low temperature difference and low field strength are satisfied quite accurately and unless the effects for parasitic remixing are properly evaluated. In view of these inherent difficulties in the interpretation of the experimental data, they were first used to test the general qualitative aspects of the theoretical development. The quantitative aspects of the agreement between theory and experiment are discussed later in the chapter.

Procedure for Comparison

As seen from Figure 5-2, it is evident that there is no complete correspondence between theoretical separation factors and the experimental values. At lower E values, the difference between the two is small, but the difference between theory and experiment is larger at larger values of

the field strength. In general, the experimental values of the separation factors are lower than the theoretical, although there appears to be a qualitative functional correspondence. The equation for the separation factors developed in Chapter III,

$$\frac{C_B}{C_T} = \frac{\left(e^{\frac{HL}{2K}\left(1 - \frac{\sigma}{H}\right)} - \frac{\sigma}{H}\right)\left(1 + \frac{\sigma}{H}\right)}{\left(e^{-\frac{HL}{2K}\left(1 + \frac{\sigma}{H}\right)} + \frac{\sigma}{H}\right)\left(1 - \frac{\sigma}{H}\right)} \qquad 3.88$$

can be used to compare the experimental data with the theory. The above equation shows that for a given column length L, the separation factor versus flow rate data should be correlated by the two column constants, H and K. The experimental data can therefore be studied by retaining the above theoretical functional form of the equation and determining the H and K values empirically to best fit the set of separation factor versus flow rate data. The behavior of these empirically determined H and K values can be compared as functions of the various parameters to their theoretical expressions.

For the continuous-flow thermal diffusion column, Powers (48) devised a new method of fitting separation versus flow rate data to determine empirically H and K at low flow rates. A modification of this procedure will be used to best fit the experimental data on separation factors as functions

of flow rate to determine H and K in this work.

The steady state batch separation factor (i.e. at $\sigma = 0$, $t \to \infty$) is given by the equation

$$\left(\frac{C_B}{C_T}\right)_{\substack{t\to\infty \\ \sigma=0}} = e^{2A} \qquad 5.1$$

where $$A = \frac{HL}{2K} \qquad 5.2$$

If this is divided into equation 3.88, the result is an equation of the form:

$$\frac{\left(\frac{C_B}{C_T}\right)_\sigma}{\left(\frac{C_B}{C_T}\right)_{\sigma=0}} = \frac{\left(e^{A\left(1-\frac{\sigma}{H}\right)} - \frac{\sigma}{H}\right)\left(1-\frac{\sigma}{H}\right)}{e^{2A}\left(e^{-A\left(1+\frac{\sigma}{H}\right)} + \frac{\sigma}{H}\right)\left(1+\frac{\sigma}{H}\right)} \qquad 5.3$$

where $$A = \frac{HL}{2K} \qquad 3.85e$$

The above equation represents the steady state flow separation factor normalized with respect to the maximum batch separation factor. Equation 5.3 was used to obtain the values of H and K for a particular set of flow rate data. For a given flow rate, a value of A as calculated from the batch separation factor was taken and a value of H was assumed. The ratio on the left hand side in equation 5.3 was then calculated for the assumed value of H and compared with the experimental value. The actual value of H chosen was

then found by repeated trial and error procedure. To fit the data of the entire set, an arithmetic average of H values for the individual flow rates in the data set was used. The value of K_{total} was then calculated from the relation

$$K = \frac{HL}{2A} \qquad 5.4$$

Such empirical values of H and K for all the flow-data sets, and calculated in the manner described before are presented in Table 5.1. The procedure described above of finding the values of H and K was used for all the flow rate data sets. Sample calculations are given in Appendix D.

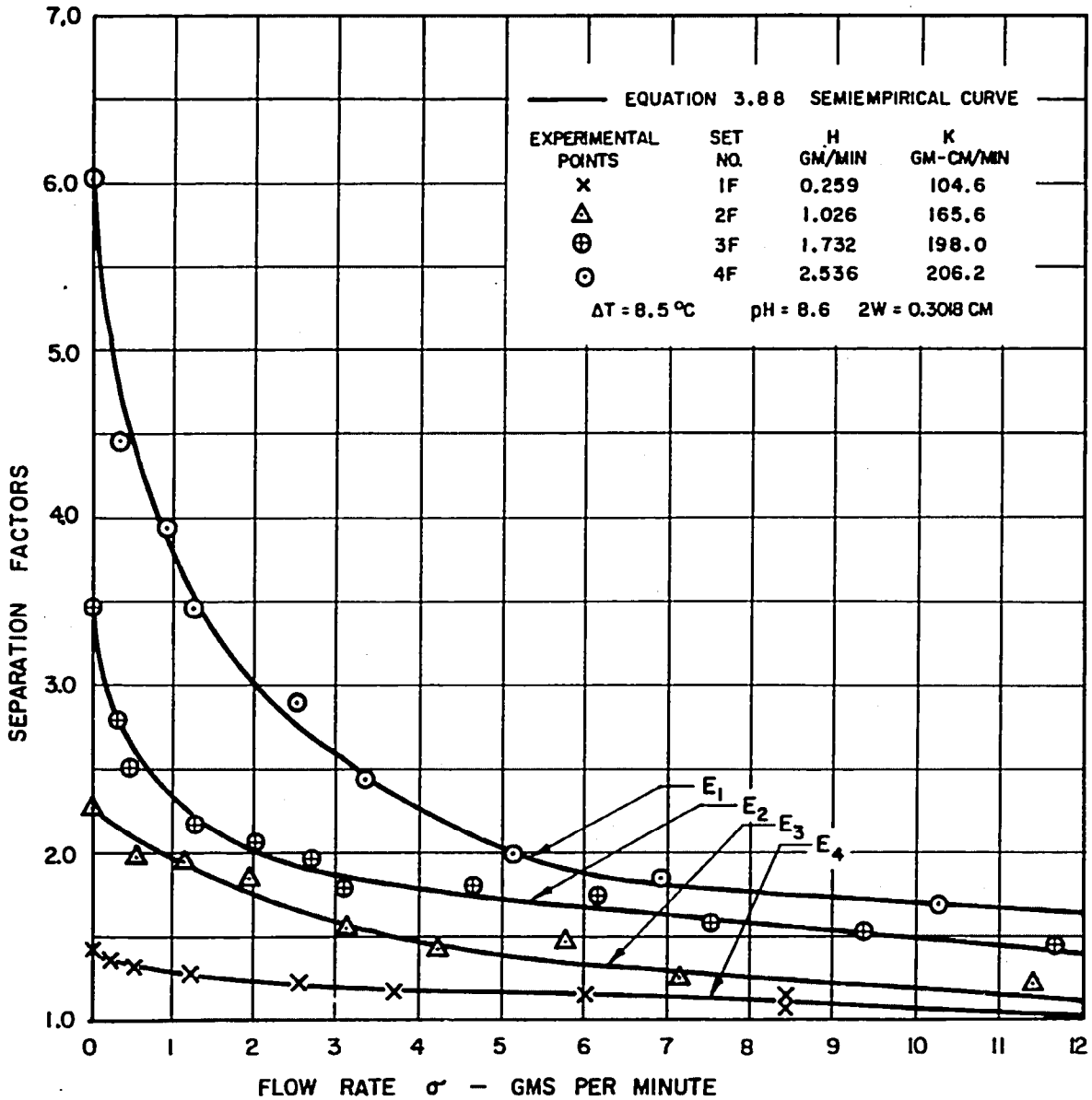
All experimental flow data show that the functionality of equation 3.88 is correct and therefore separation factors can be calculated by using the same equation if empirical values of H and K are used.

Experimental separation factors are presented as a function of flow rate for various field strengths and temperature differences, on Figures 5-3, 5-4 and 5-5. The solid lines represent the curves obtained from equation 3.88 using the empirical values of H and K. The fits obtained are very good. The shape of the semi-empirical curves obtained and the excellent fits of the experimental data show that FJO theory is at least qualitatively applicable to the case of continuous-flow electrophoresis columns. The data do not show appreciable deviations from the empirical curves even at moderately high flow rates of up to 10 gm. per minute. It is seen that Figures 5-3, 5-4 and 5-5 definitely show that

TABLE 5.1

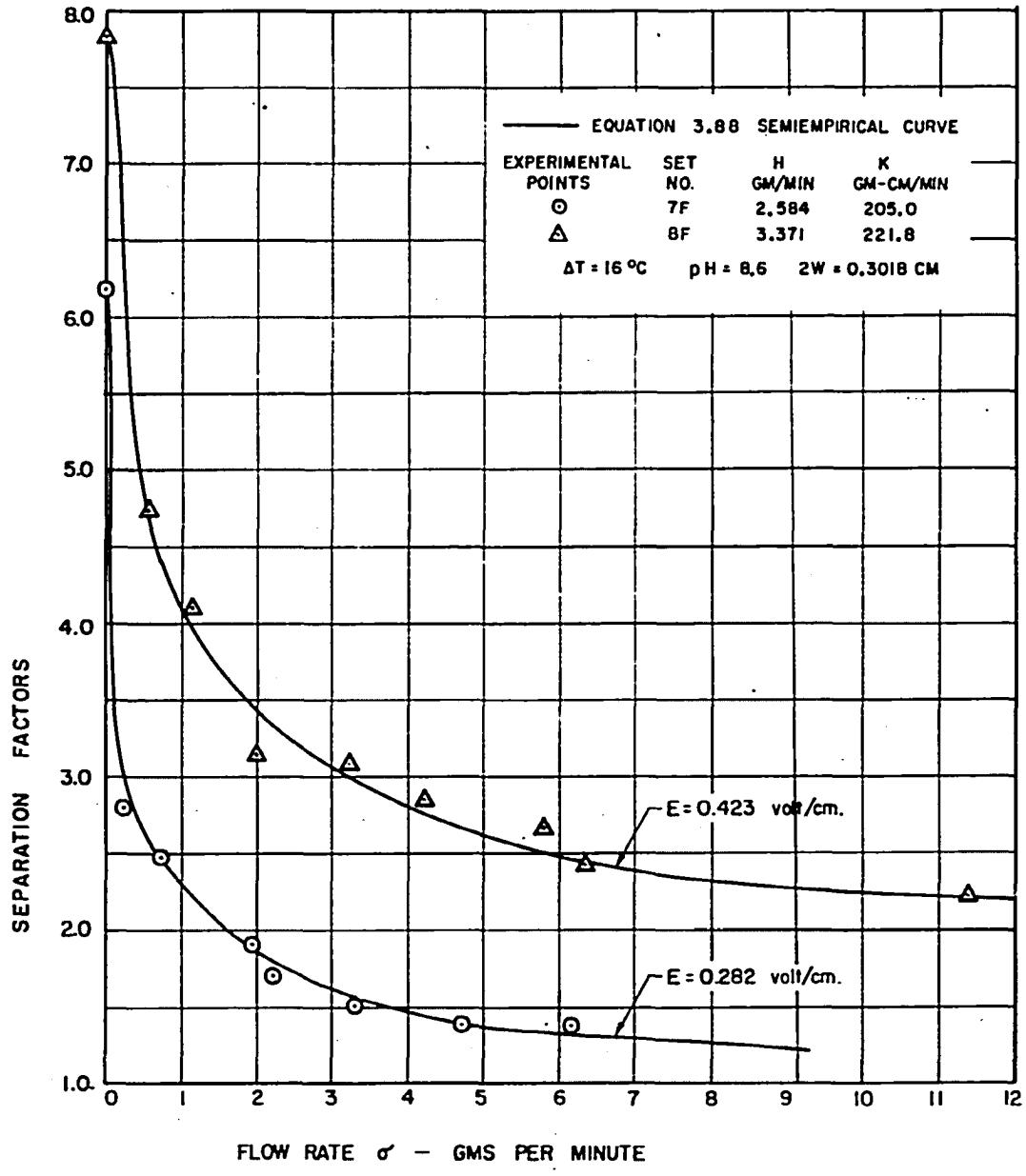
Empirical Values of Column Constants

Set No.	2ω cm.	ΔT °C	E Volts/cm.	pH	H gm./min.	K gm.-cm./min.	A HL/2K
1	.3018	8.5	0.0423	8.6	0.259	104.6	0.18
2	.3015	8.5	0.141	8.6	1.026	165.6	0.406
3	.3015	8.5	0.282	8.6	1.7320	198.0	0.634
4	.3015	8.5	0.423	8.6	2.5360	206.2	0.915
5	.3018	0	0.282	8.6	0.646	49.5	0.947
6	.3018	0	0.423	8.6	1.460	92.8	1.143
7	.3018	16	0.282	8.6	2.584	205.0	0.915
8	.3018	16	0.423	8.6	3.371	221.8	1.104
9	.1354	8.5	0.282	8.6	2.54	175.4	1.050
10	.1354	0	0.282	8.6	1.448	87.5	1.104
11	.1354	8.5	0.282	6.0	0.148	54.2	0.198



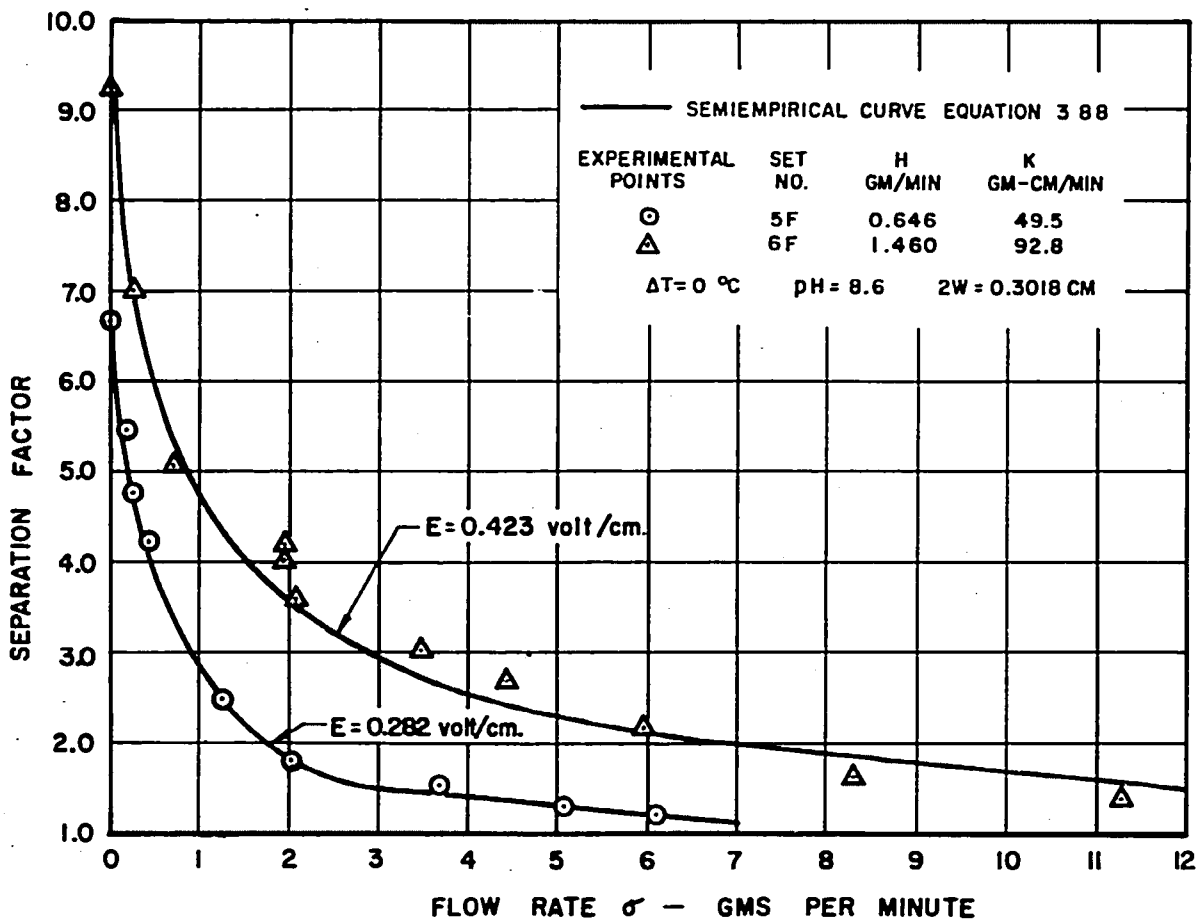
COMPARISON OF EXPERIMENTAL AND EMPIRICAL SEPARATION FACTORS AT FOUR DIFFERENT FIELD STRENGTHS

FIGURE 5-3



SEPARATION FACTORS AS A FUNCTION OF FLOW RATES AT $\Delta T = 16°C$

FIGURE 5—4



COMPARISON OF EMPIRICAL AND EXPERIMENTAL SEPARATION FACTOR AT $\Delta T = 0$ FOR 2 VALUES OF FIELD STRENGTHS

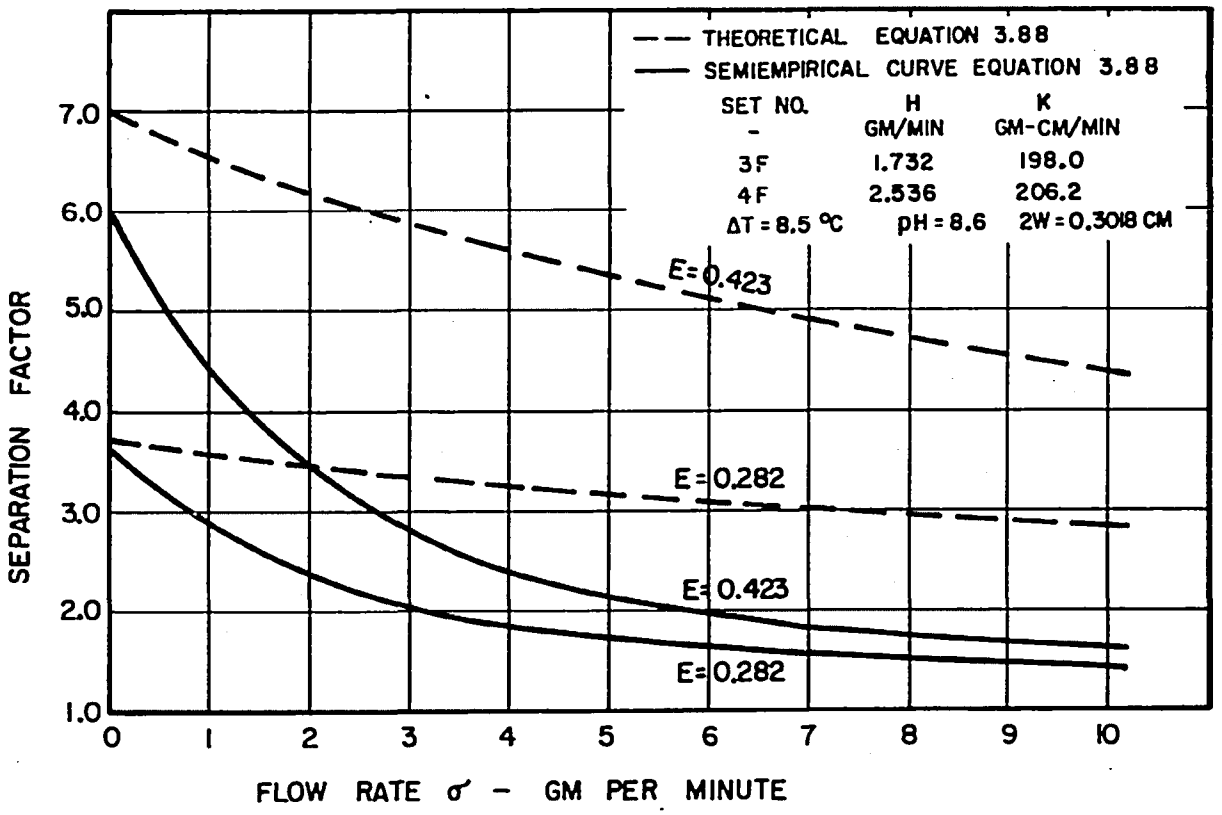
FIGURE 5-5

the form of the equation 3.88 is at least qualitatively correct for almost all flow rates.

In no case however do the separation factor values calculated by using semi-empirical values of H and K agree with those calculated by using theoretical values of H and K as shown in Figure 5-6. This is true for the moderately low flow rates (except at flow rate $\sigma = 0$ where agreement is very close) and for all values of the field strengths used in the present investigation. In all the sets, separation factors decrease with increase in flow rate. However, at low values of E, the empirical values and the theoretical values are closer to each other. The empirical values of H and K obtained for the data set D.1F at several flow rates are listed in Table 5.2. An examination of these values of H and K shows that both H and K appear to be independent of the flow rates within experimental error. This is in agreement with the theory which does not envisage a dependence of the column constants H and K on flow rate. This experimental observation leads to the conclusion that the use of H and K calculated on the basis of the batch velocity profiles is justified.

Functional Dependence of Column Constants

The question now arises whether the empirical values of H and K obtained show the functional dependence upon the parameters of operation (such as field strength, temperature difference, membrane spacing, and mobility).



SEPARATION FACTOR AS A FUNCTION OF FLOW RATE

EMPIRICAL AND THEORETICAL VALUES

FIGURE 5-6

TABLE 5.2

Comparison of Empirical Column Constants H and K

at Various Flow Rates

Data Set D-1

Flow Rate gm./min.	H gm./min.	K gm.-cm./min.
0.289	0.259	104.9
0.521	0.257	104.2
1.187	0.257	104.2
2.536	0.259	104.8
3.704	0.260	105.3
6.187	0.260	105.6
8.418	0.260	105.7
Average Value	0.259	104.9

*For conditions of the experiment and figure, see Figure 5-2.

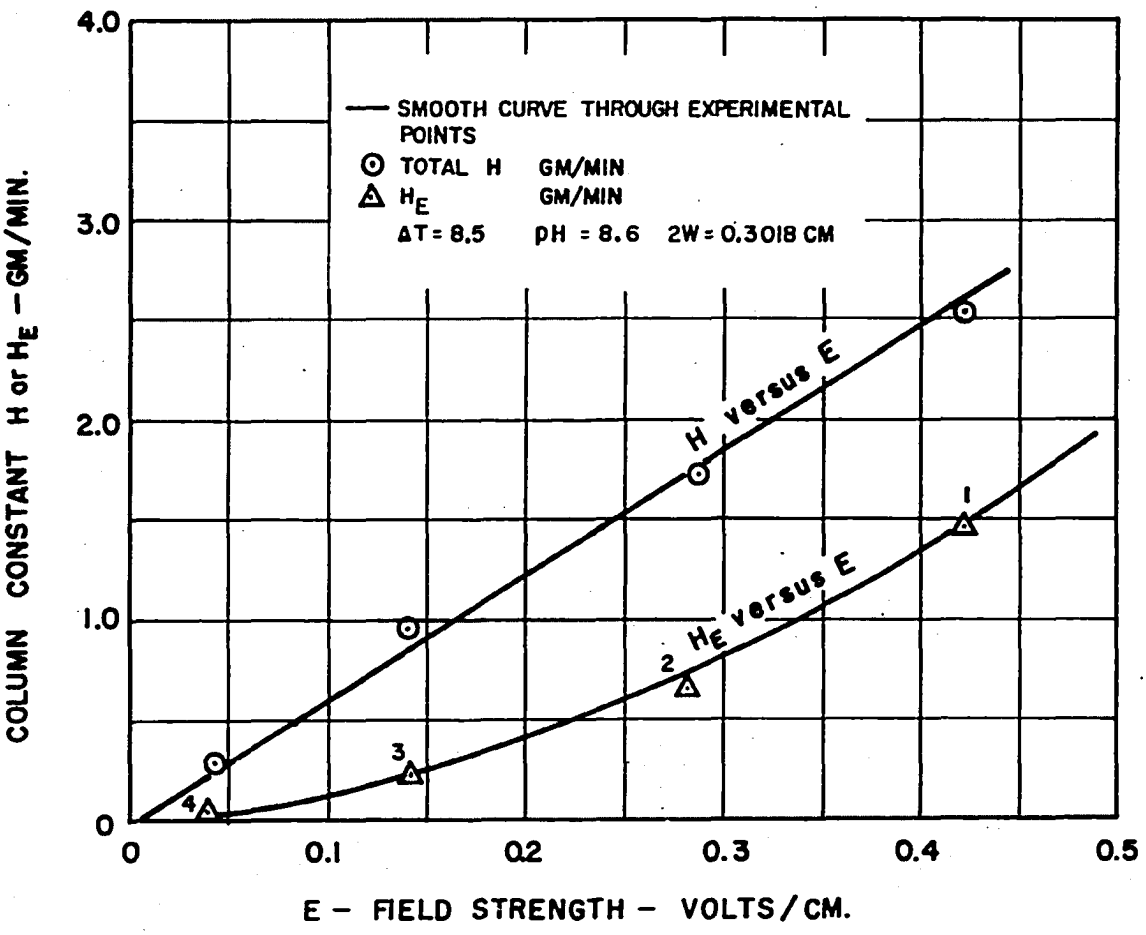
The column constant H (total) consists of two parts: (1) the interaction term involving both ΔT-temperature difference and E-field strength and (2) the electrophoresis term involving only E-field strength. From the equations for each of these it is seen that H_{TE} is proportional to both ΔT and E while the second term H_E is proportional to the square of E. H is given by an equation of the form:

$$H = a \cdot \Delta T \cdot E + bE^2 \qquad 5.5$$

The functional dependence of H_{TE} and H_E is given by equations of the form:

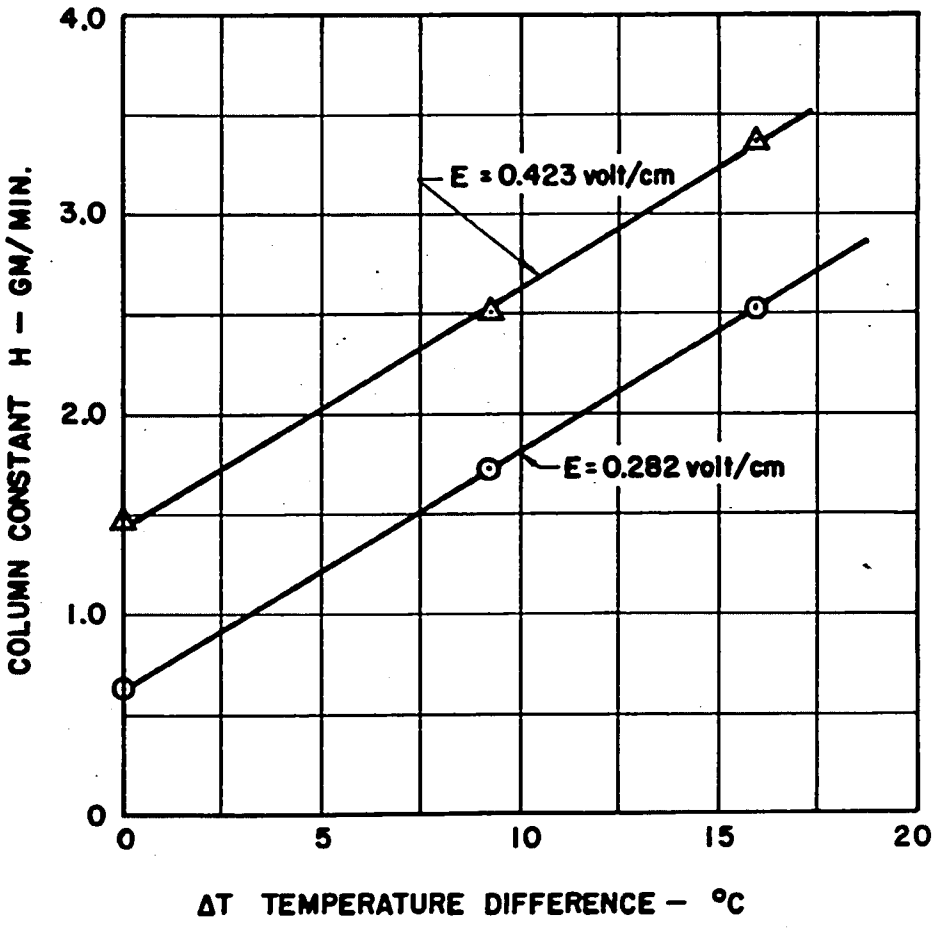
$$H_{TE} = a \cdot \Delta T \cdot E \; ; \quad H_E = bE^2 \qquad 5.6$$

The H value at zero temperature difference consists only of H_E because only the electric force field is operative. The data at zero temperature difference are therefore useful to study the effect of E on $H = H_E$. To test this predicted functional relationship, plots of H, H_{TE} and H_E as functions of E and ΔT are presented in Figures 5-7 and 5-8. In Figure 5-7 curve A represents the dependence of H_{total} on E at $\Delta T = 8.5°C$ and $2\omega = 0.3018$ cm. On curve B, the points 1 and 2 are the experimental points while points 3 and 4 are the extrapolated values from the data at those E values with use of Figure 5-8. In Figure 5-8, total H as a function of ΔT is a straight line plot indicating that functional proportionality expressed by the form of equation 5.6 is satisfied with respect to the effect of ΔT. Since H_E is



H OR H_E AS A FUNCTION OF FIELD STRENGTH

FIGURE 5-7



DEPENDENCE OF TOTAL H ON TEMPERATURE DIFFERENCE

FIGURE 5-8

proportional to E^2, a log-log plot of H_E as a function of E should give a straight line of slope 2. Figure 5-9 shows the dependence of H_E on E at $\Delta T = 0$ and $2w = 0.3018$ cm. The slope of the line is seen to be equal to 2.0 as predicted by the theory. The experimental values of H therefore show that the functional forms of the theoretical equations for the column constants H seem correct.

The functional form of the equation for total K is rather complicated, (equation 3.67 to 3.67e) consisting of six terms. The three terms containing the heat generation term, Q, are according to theory negligibly small in the present work because of the small Q, and, therefore, only the remaining three will be considered here. The contribution to K of parasitic remixing will be ignored (cf. Powers (47)), and the vertical diffusion can be shown to be negligibly small for these experiments. The total K is then given by the equation:

$$K = K_T + K_{TE} + K_E \qquad 5.7$$

where (1) K_T is the contribution to total K due to ΔT alone

(2) K_{TE} is the contribution to total K due to the product of E and ΔT (the interaction term)

(3) K_E is the contribution to total K due to E alone

The above equation 5.7 for K can be written in a functional form of the type:

$$K = a(\Delta T)^2 + b\ \Delta T \cdot E + cE^2 \qquad 5.8$$

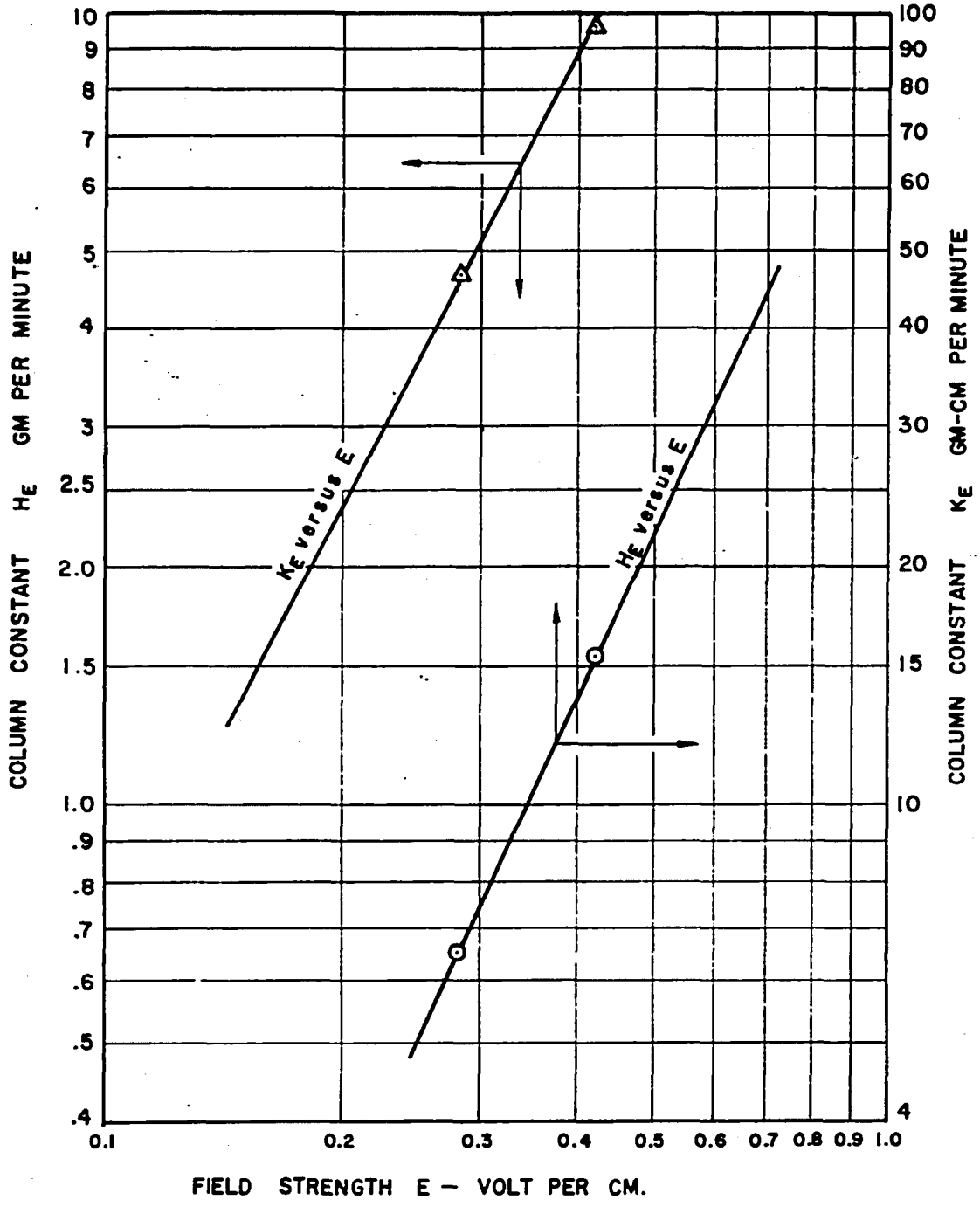
where a, b and c are constants so that the individual contributions to K are represented by the functional forms:

$$K_T = a(\Delta T)^2 \; ; \quad K_{TE} = b\,\Delta T \cdot E \quad \text{and} \quad K_E = cE^2 \qquad 5.9$$

Figure 5-9 shows the functional dependence of K at $\Delta T = 0$ (if $\Delta T = 0$, $K_T = 0$ and $K_{TE} = 0$ and therefore $K = K_E$) on the parameter E on logarithmic co-ordinates. The slope of the line through the two experimental points has a value of 2.0 which indicates that $K = K_E$ satisfies the functional form of its theoretical expression.

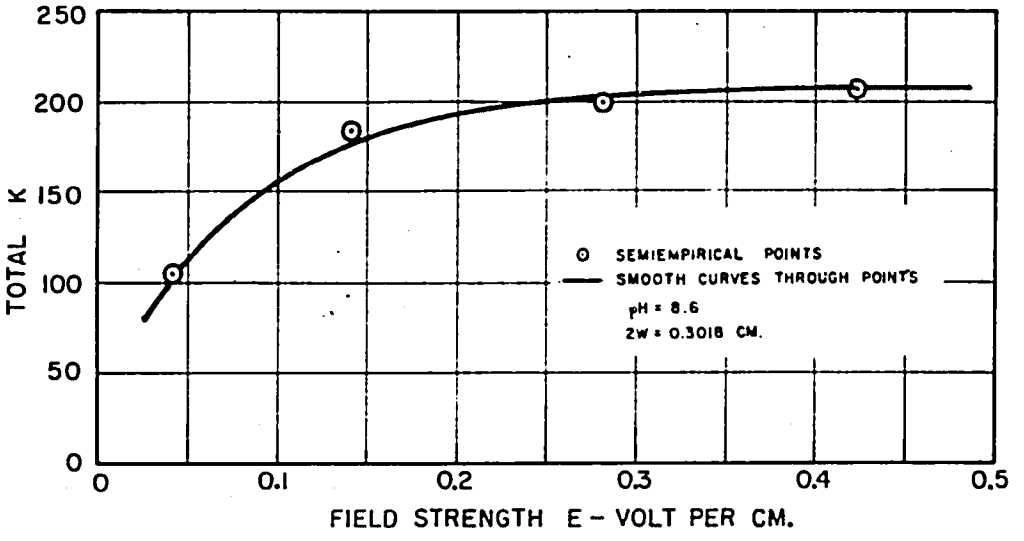
However, the case of total K is different. From Figure 5-10a, (total K as a function of E at $\Delta T = 8.5°C$ and $2\omega = 0.3018$ cm.) it is noticed that the empirical K values differ very little with increase in E. It appears to reach a limiting value which is contrary to theory. This is not, however, surprising since at such large membrane spacing, K values, which are measure of the internal convection flow, are less likely to follow theory. For example, in thermal diffusion a similar situation exists as can be seen from the data of Boyer (7) and others for continuous-flow thermal diffusion columns.

The effect of temperature difference is seen from Figure 5-10b in which are presented the total K values as a function of the temperature difference at two different E values as the constant parameter. In this case also, the value of total K appears to reach a limiting value with increase in ΔT which is contrary to theory. Similar reasoning



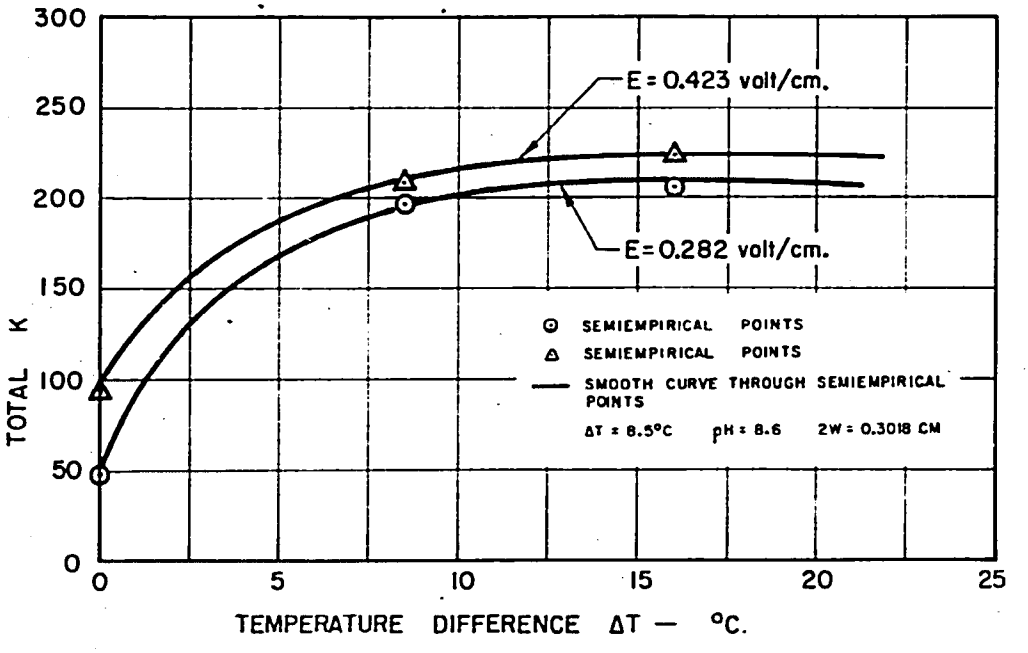
H_E and K_E as FUNCTIONS OF FIELD STRENGTH

FIGURE 5-9



TOTAL K AS A FUNCTION OF FIELD STRENGTH

FIGURE 5-10a



TOTAL K AS A FUNCTION OF TEMPERATURE DIFFERENCE

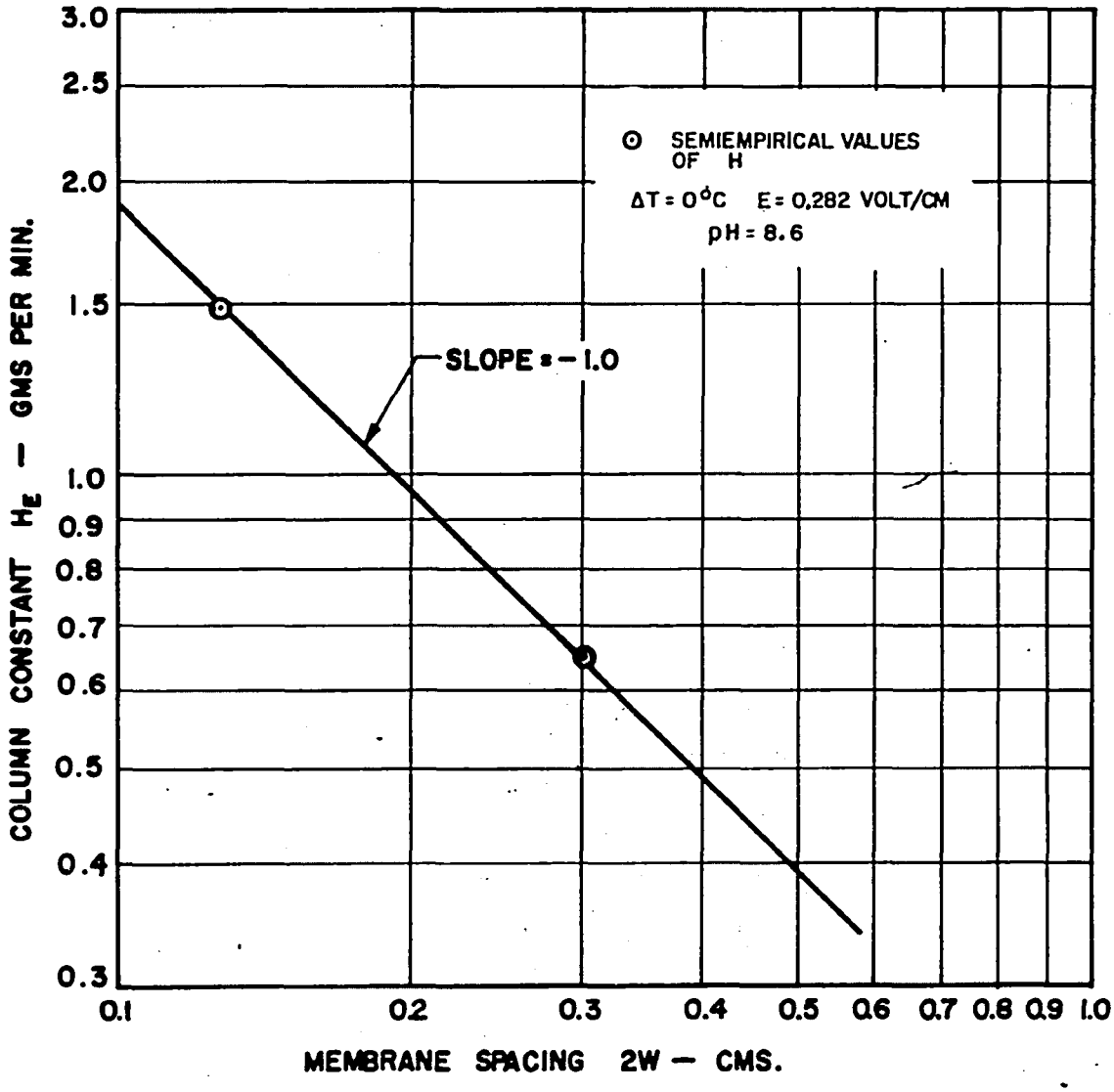
FIGURE 5-10b

as given above in explaining the discrepancy with regard to the effect of E on total K applies here and will therefore not be repeated. The above discrepancies with regard to the dependence of total K on E and ΔT are not however detrimental from the point of the separations desired. An examination of equation 3.88 for separation factors shows that total K appears in the denominator of the exponent and if this remains constant while H increases with increase in either E or ΔT, this results in an increase in value of A ($A = HL/2K$) which means an increase in separation factors.

The various column constants defined have the following functional form when all parameters other than membrane spacing are the same:

$$H_{TE} = a(2\omega)^4 \ , \quad H_E = b(2\omega)^5 \ , \quad K_T = c(2\omega)^7 \ , \quad K_{TE} = d(2\omega)^8 \quad \text{and} \quad K_E = e(2\omega)^9 \qquad 5.10$$

These theoretical functional forms suggest a strong dependence of these constants on the spacing between the membranes. For example, H_E (the value of H at $\Delta T = 0$) contains the term 2ω raised to the fifth power while K_E (the value of K at $\Delta T = 0$) contains the term 2ω raised to the 9th power! An examination of the empirical values of the column constants, however shows that these do not confirm the functionality of their expressions. As an example, the H ($= H_E$) at $\Delta T = 0$ were inversely proportional to the membrane spacing (Figure 5-11). Thus, it is seen that the column constants do not satisfy the theoretical functional relationship with regard to the



COLUMN CONSTANT H AS A FUNCTION
OF
MEMBRANE SPACING

FIGURE 5-11

membrane spacing (2ω). Similar discrepancies between theory and experiment have been indicated by Boyer (7) and others (7,48) in thermal diffusion columns.

To summarize, the following observations about H and K may be made on the basis of the above discussion:

(i) The empirical column constants H and K are practically independent of flow rate σ as expected from theory. Therefore, use of H and K calculated using batch velocity profiles to represent also the flow case seems to be justified.

(ii) The empirical values of column constants, H, H_{TE} and H_E confirm the functional forms of their theoretical equations with regard to their dependence on field strength E and temperature difference ΔT.

(iii) The empirical column constants K at $\Delta T = 0$ ($K = K_E$ in this case) follow the functionality of their theoretical expression, but at other temperature differences and higher field strengths the K values are almost constant and fail to satisfy the theoretical functional relationship.

(iv) For membrane spacing, with all other parameters held constants, it is seen that all empirical column constants H and K do not observe the theoretical functionality.

From what has been said so far about the column constants H and K, it would appear at first that their use for comparison of separation factors might not be too meaningful. However, the failure of empirical column constants to follow their theoretical functional relationships

would not be a serious limitation in determining the separation factors as a function of flow rate (equation 3.88). Further, for a given membrane spacing and ΔT greater than zero, the values of H follow their functional relationship for the parameters E and ΔT while the K remains more or less constant. The values of H will therefore be the more appropriate basis for comparison of the separation factors.

With the above observations about column constants, the separation factors as a function of parameters other than flow rate can be discussed.

Discussion of Separation Factors

Effects of Field Strength

According to the theory higher separation factors should result with an increase in E at constant ΔT other than zero. The separation factors taken at various values of E and temperature difference (8.5°C, 16°C) were presented in Figures 5-3, 5-4, and 5-5 and these seem to satisfy qualitatively their theoretically expected dependence on field strength E.

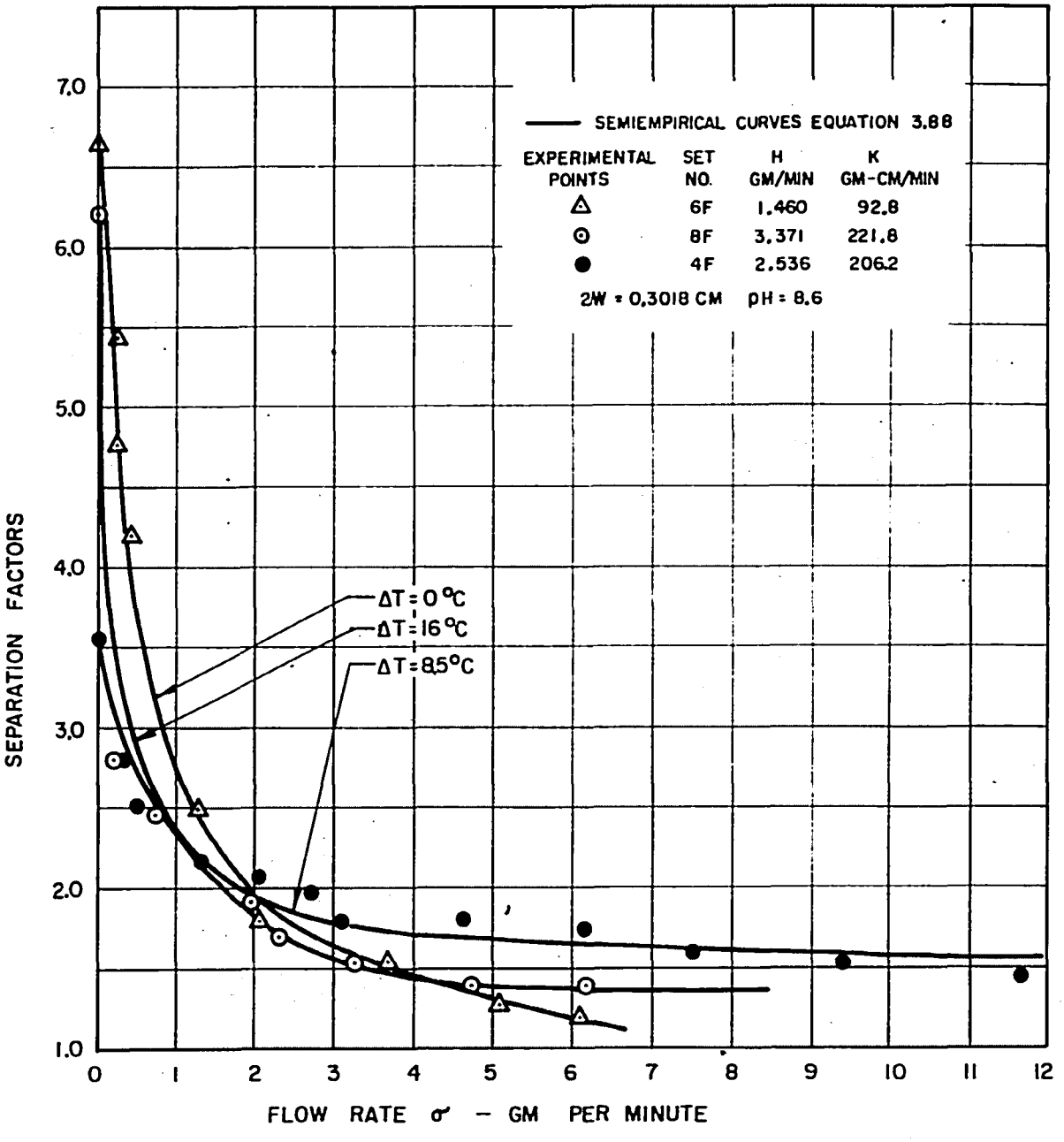
The situation is quite different at $\Delta T = 0$. Both H_E and K_E at this ΔT are functions of E^2. Therefore, from theory it would be expected that for the same values of the parameters other than E, the batch separation factor given by $C_B/C_T = e^{2A} = e^{(2aE^2/bE^2)}$ should be independent of field strength E at $\Delta T = 0$. The theory and experiment do not agree well as can be seen in Figure 5-5 which shows separation

factors obtained as function of two E values at $\Delta T = 0$. The values of A incorporating the ratio H/K for both the E values agree within about 10%; however, the small difference in A is exponentially multiplied in the separation factor, thus resulting in the large difference in the values of the separation factors actually obtained.

Effect of Temperature Difference

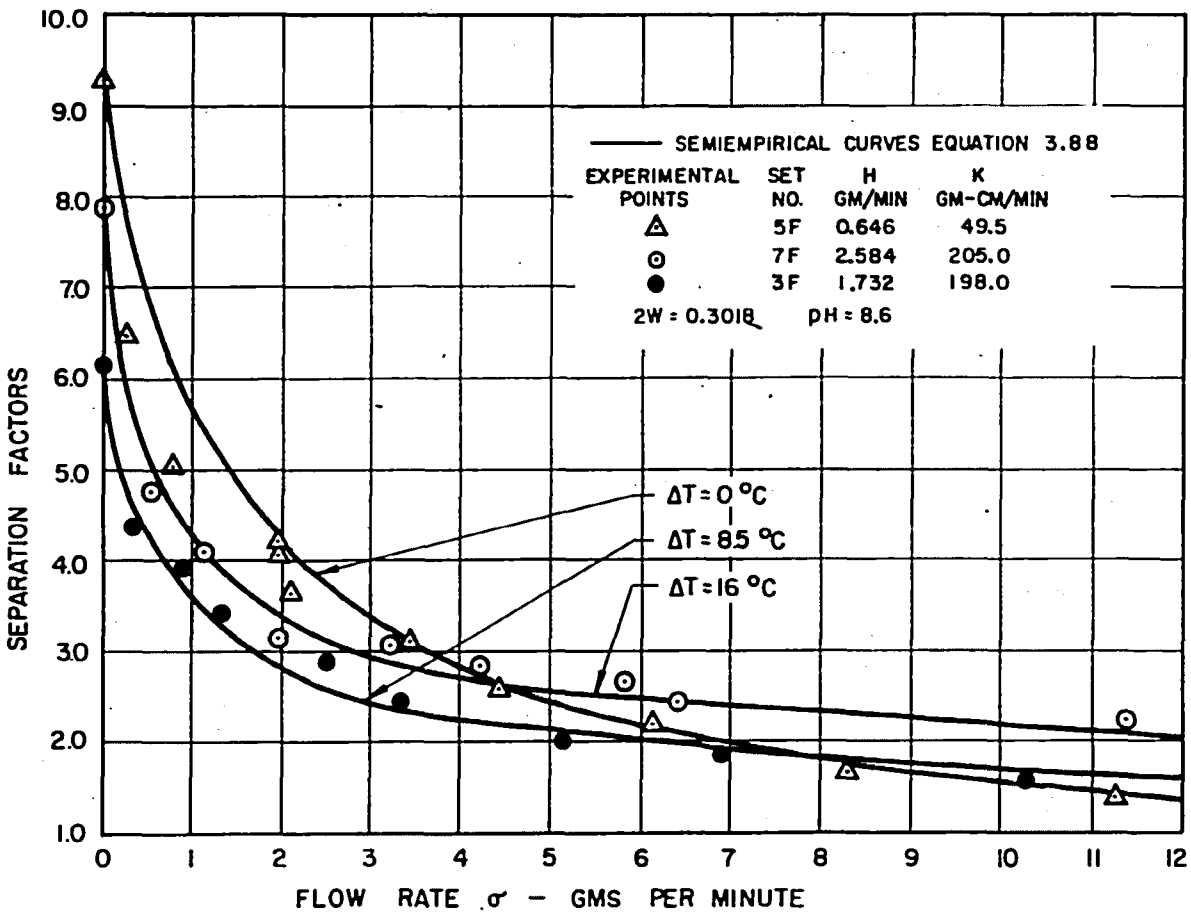
The separation factors as a function of ΔT at two different values of E were presented before in Figures 5-3, 5-4 and 5-5 but for the sake of comparison are again presented in Figures 5-12a and 5-12b. The theory predicts that an increase in ΔT should cause a decrease in the separation factors. Comparing the separation factors obtained at 8.5°C and 16°C respectively at the same E, it is seen that improvement in the separation factors does occur with increase in ΔT, which is contrary to the theory. This occurs for all E values studied.

The above observed discrepancy between theory and experiment as regards the dependence of separation factors on increasing ΔT can be explained on the basis of the behavior of the semiempirical column constants H and K. It has been seen before that semiempirical H is proportional to ΔT while K approaches a limiting constant value as ΔT increases. As a result the value of A given by equation 3.85c also increases resulting in increased separation factors with increase in ΔT in contradiction to theory.



SEPARATION FACTORS AS FUNCTION OF FLOW RATES WITH ΔT AS PARAMETER AT E = 0.282 volt/cm

FIGURE 5—12a



SEPARATION FACTORS AS FUNCTION OF FLOW RATES WITH ΔT AS PARAMETER AT E=0.423 volt per cm.

FIGURE 5-12b

However, it should be remembered here that the temperature differences used in this investigation were small and therefore the contribution to either H or K by the product of ΔT and E was also small. Hence the effect of the thermal field on the separation factors was relatively insignificant.

The influence of ΔT is more significant and more consistent with the theory at low E values and low ΔT values and these results agree with Kirkwood's conclusion that ΔT is not particularly necessary for substantial separation in an electrophoresis column. In contrast, however, it appears that important increases in separation (about 30%) especially at low values, are obtained by the addition of a temperature gradient to increase the convective flow, as the theory predicts.

The experimental separation factors at $\Delta T = 0$ are greater than those obtained at other temperature differences for the same E value, as can be seen by comparing the respective separation factors in Figure 5-12a and 5-12b. This is true for both spacings used in these experiments. This observation is however consistent with the theory which predicts increases in separation factors as ΔT decreases. It is seen from Figure 5-10b, that the value of K drops quickly as the temperature difference decreases to 0 while there is no corresponding quick drop in H values. Higher values of A therefore result in case of small temperature differences in contrast to large temperature differences. As a result higher separation factors result at low

temperature differences. In addition, at $\Delta T = 0$ where both membranes are at 4°C, the viscosity of the medium is higher (15%) and thus a more laminar convection would result reducing parasitic remixing. It seems, therefore, that significantly large temperature differences will be required to yield substantially large contributions to the separation factors by the thermal field since in such cases, K will be small and H values will be large.

On examination of Figures 5-12a and 5-12b, it is also seen that at high flow rates, the behavior of the separation factors as explained above does not hold. There is quite an overlapping of the experimental data for all the temperature differences. It may be observed that the separation factors at $\Delta T = 0$°C become lower than those at 16°C. It appears that the semiempirical theory also does not hold good at high flow rates. A few possible explanations can be given. First, at high flow rates, the use of batch velocity profiles to calculate H and K may not be too accurate. Second, a higher temperature difference would help suitable flow conditions to be established over a wide range of flow rates. Third, anamalous behavior of water at 4°C might be also a factor for the observed discrepancy between the experimentally observed data and the semiempirical theory. Probably a combination of the factors just cited might be the cause of the odd behavior of the separation factors with regard to their dependence on temperature difference at high flow rates.

Effect of Membrane Spacing

Separation factors as a function of membrane spacing at one value of E and two values of temperature difference are presented on Figures 5-13 and 5-14. The curves in these figures represent, as in other cases, the value of the separation factors calculated from the equation using empirical values of H and K.

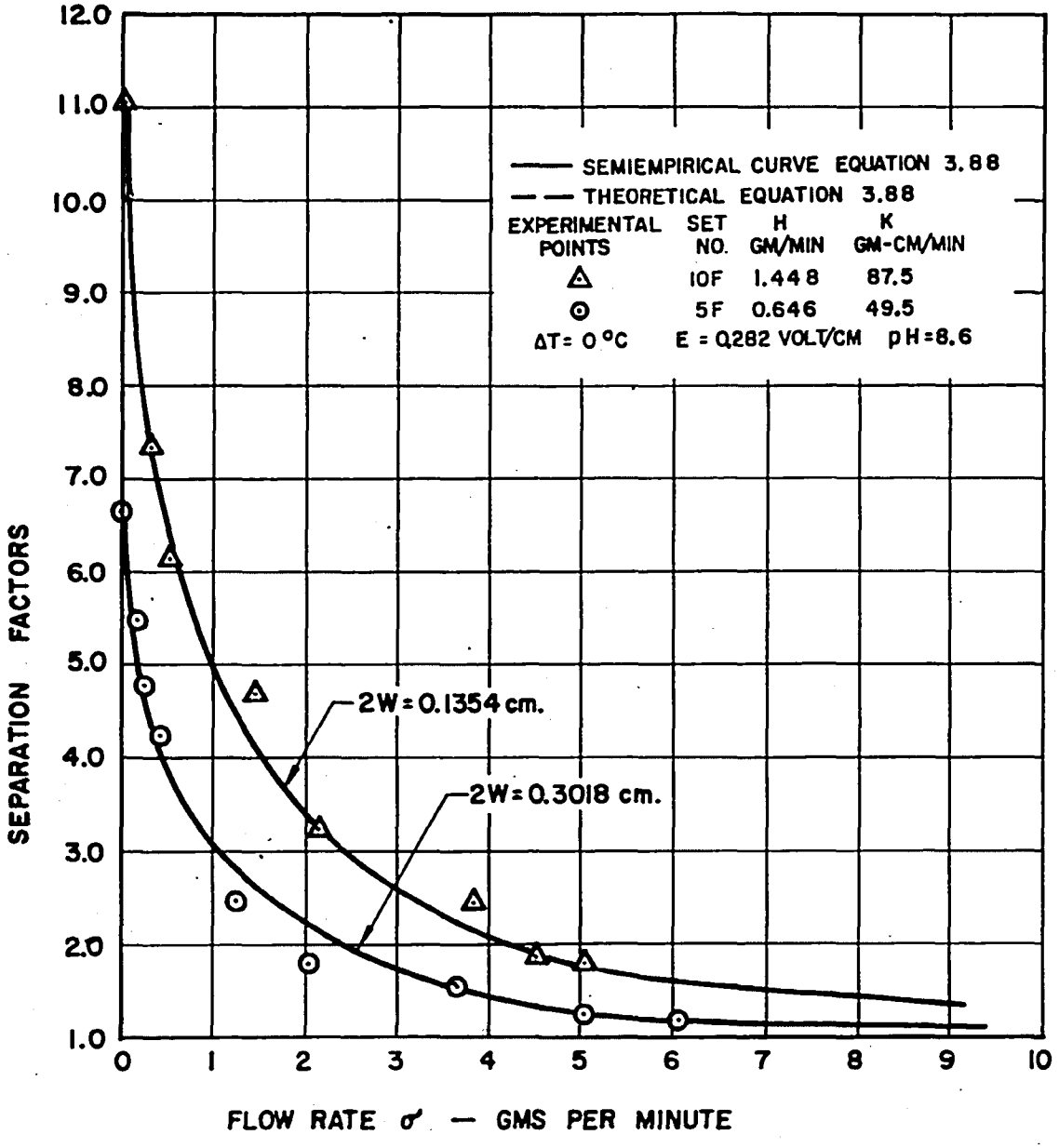
The functionality of column constants shows a strong dependence on membrane spacing and the effect on separation factors should therefore, be strong. Examination of the data and Table 5.1, for empirical column constants, shows increase in separation factors with decrease in membrane spacing. This is in accordance with the theory qualitatively and is clearly indicated in Figures 5-13 and 5-14.

Effect of pH of Solution

The mobility of the protein is a function of the hydrogen ion concentration of the solution. The separation factors therefore should depend upon pH of the solution as they depend upon the mobility. In Figure 5-15 are presented the data at pH 8.6 (boric acid-borax buffer) and at pH (6.0) (phosphate buffer). The mobilities U of bovine albumin are -6.3×10^{-5} and -2.2×10^{-5} cm.^{2}/volt/sec. at pH 8.6 and 6.0 respectively. The theory predicts smaller separation factors at lower mobilities as is shown by Figure 5-15.

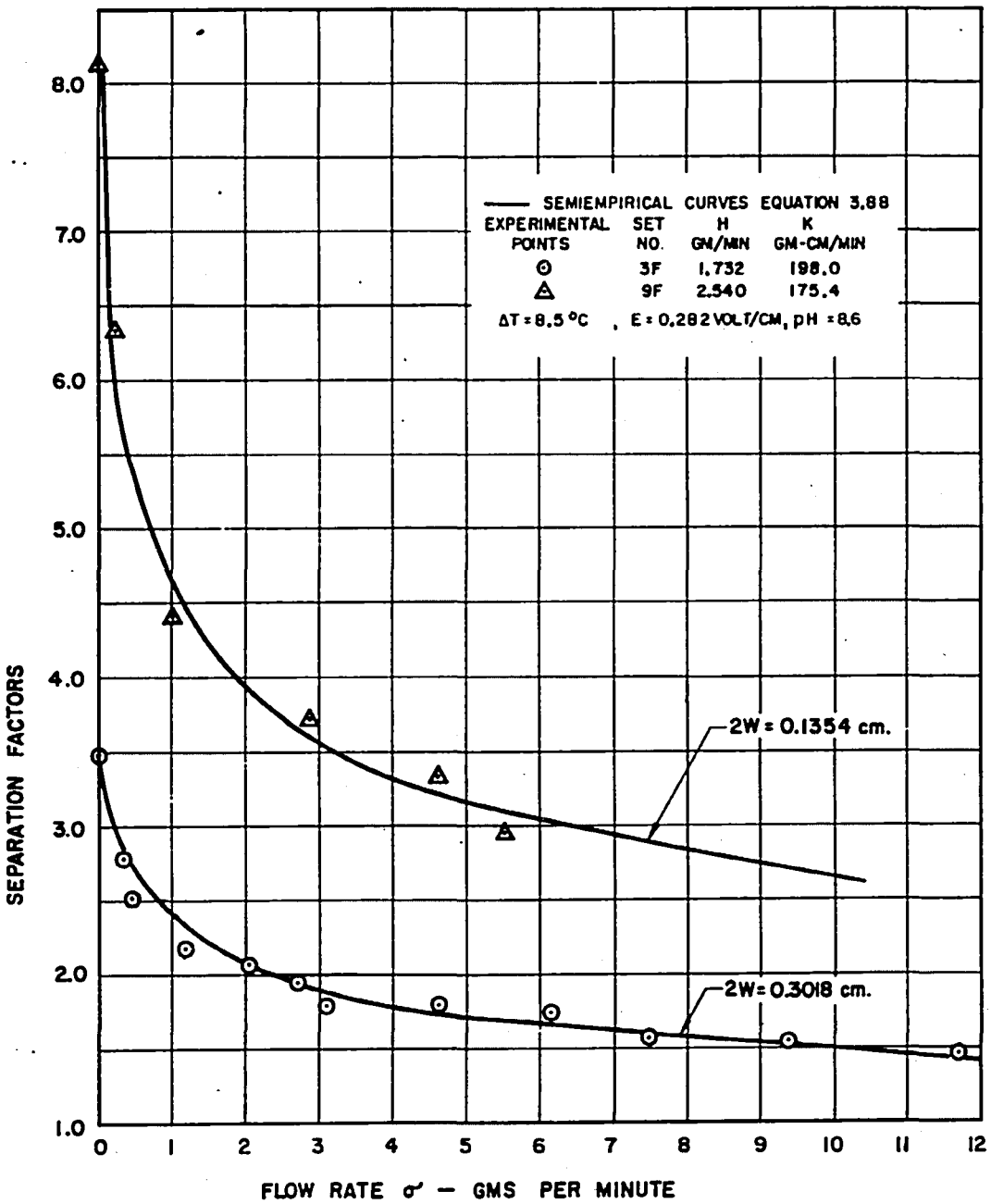
Effect of Column Length

In this investigation column length L was kept



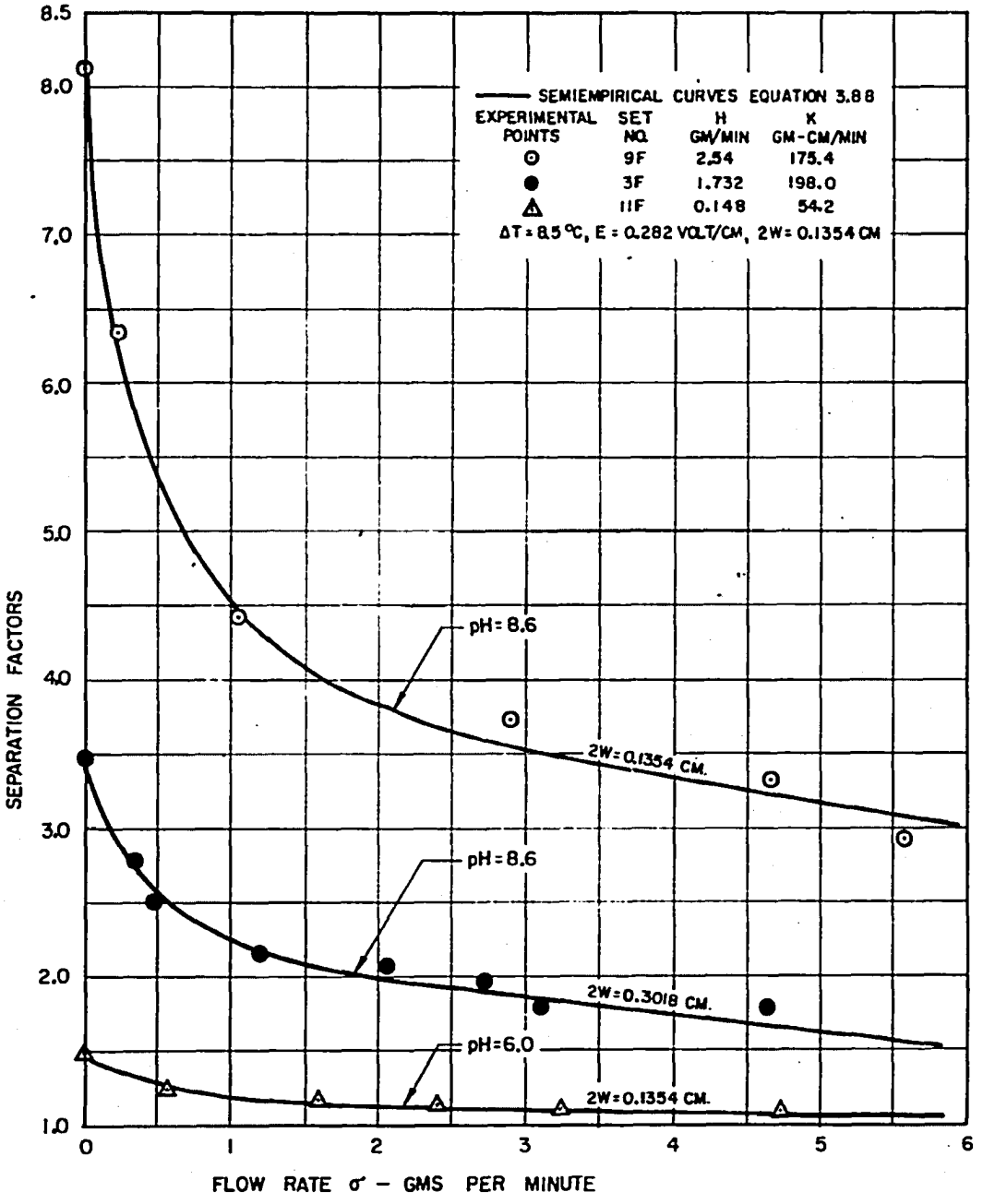
SEPARATION FACTORS AS FUNCTION OF FLOW RATE AT TWO DIFFERENT MEMBRANE SPACINGS

FIGURE 5-13



SEPARATION FACTOR AS FUNCTION OF FLOW RATE FOR TWO DIFFERENT MEMBRANE SPACINGS

FIGURE 5-14



SEPARATION FACTORS AS A FUNCTION OF FLOW RATE AT TWO DIFFERENT pH VALUES

FIGURE 5—15

constant and no experimental data were taken with L as parameter. But its effect is discernible from the equation used to define A because H and K values should be independent of L. The form of the equation suggests that increased separation factors should be obtained with increase in length. (No generalized observations can be made here because no experimental study of this effect was made.)

Discussion of Quantitative Aspect of the Theory

The theoretical development given in detail in Chapter III predicts not only the form of the equation that may be used to represent the experimental data and the functional dependence of the empirically determined terms H and K, but the numerical magnitudes of these terms as well. Comparison of experimental and theoretical values of the various column constants H and K is a convenient method of investigating the quantitative aspects of the theory.

It has already been observed that the experimental separation factors followed the functional form of the equation 3.88 but were in quantitative disagreement. For thermal diffusion columns, many investigators (48,7) have defined correction factors for H and K to bridge the gap between theory and experiment. For example, Powers (48) defined the correction factors as:

$$\varphi_H = \frac{H_{exp}}{H_{theor}} \qquad 5.11$$

$$\varphi_K = \frac{K_{exp}}{K_{theor}} \qquad 5.12$$

where the subscript "exp" and "theor" refer to experimental and theoretical values respectively. He observed that the theory was in quantitative agreement with experiment over certain small values of plate spacings and that it was in good qualitative agreement over a wide range of variables investigated, that the corrections φ_H and φ_K change rapidly with spacing 2ω, that both φ_H and φ_K appear to be independent of L the column length, that the corrections vary little with large changes in the temperature difference and that both φ_H and φ_K are functions of the physical properties of the system.

Since there is considerable similarity between the thermal diffusion column and the electrophoresis column, the same technique of correction factors could be used to interpret quantitative aspects of the electrophoresis column theory. In the present work, the correction factors were calculated for all the sets of data and are listed in Table 5.3.

It has been reported by Powers (48) that the correction factors φ_H and φ_K are empirically related by the equation:

$$\varphi_K = \varphi_H^{1.25} \qquad 5.13$$

For comparison purposes φ_K values were calculated using this relation and these values are also included in Table 5.3 as φ_K calculated. Upon comparison of experimental φ_K values and those calculated by above relation, it is seen that this relationship holds only in case of the lower spacing

TABLE 5.3

Experimental and Calculated Correction Factors φ_H and φ_K

E	2ω	ΔT	H exp.	H theor.	K exp.	K theor.	φ_H	φ_K exp.	φ_K calculated
volt/cm.	cm.	°C	gm./min.	gm./min.	gm.-cm./min.	gm.-cm./min.	gm./min.	gm.-cm./min.	gm.-cm./min.
.0423	.3015	8.5	.259	5.91	104.6	15561	.0438	.0067	.0204
.141	.3018	8.5	1.026	52.59	165.6	110900	.0195	.00149	.00732
.282	.3018	8.5	1.732	199.2	198.0	397591	.0087	.000508	.00268
.423	.3018	8.5	2.536	439.7	206.2	861331	.00567	.00024	.00157
.282	.3018	0	.646	219.7	49.5	420211	.00234	.0001176	.00070
.423	.3018	0	1.460	494.2	92.8	945475	.00295	.000982	.000710
.282	.3018	16	2.584	201.7	205.0	457889	.00128	.000428	.000338
.423	.3018	16	3.371	426.5	221.8	909738	.00792	.000244	.00048
.282	.1354	8.5	2.54	3.87	175.4	334.7	.656	.522	.590
.282	.1354	0	1.448	3.993	87.5	309.4	.363	.283	.281
.282	.1354	8.5	.148	.0997	54.2	16.20	1.485	3.34	1.63

2ω = 0.1354 cm., ΔT = 0°C, ΔT = 8.5°C and E = 0.282 volt/cm. for a solution of pH 8.6. At spacing of 0.3018 cm., the correction factors are numerically extremely small, i.e. the actual values of K as realized in experiment are considerably smaller than their theoretical values.

By comparison of the correction factors given in Table 5.3, the following observations are made:

(1) The relationship between φ_H and φ_K given by Powers (48) in most cases does not hold good for present data.

(2) The φ_K experimental and φ_K calculated from equation 5.13 are in good agreement at the lower spacing of 0.1354 cm. and at temperature differences of 0 and 8.5°C respectively. Although no general conclusion can be drawn from this single agreement with theory, it faintly suggests that Power's relation for φ_H and φ_K might be applicable to electrophoresis columns also at low membrane spacings. In the present work, rather large spacings were used and therefore there is no quantitative agreement between $\varphi_{K_{exp}}$ and φ_K values calculated by using Powers' empirically observed relationship between φ_K and φ_H in thermal diffusion.

(3) The correction factors decrease with increase in field strength and they have very small values at the larger membrane spacing.

The experimental dependence of separation factors upon membrane spacing definitely disagrees with the theory. The membranes may change or bulge while in contact with the solution and the spacing measurement could be in error. The

spacing is measured accurately only at the periphery of the cell. Although this might explain in part the disagreement between theory and experiment, membranes must have been relatively well behaved because of the positive pressure which always forced them against their Flexolith supports, and because of the consistency of the experimental results after two replacements of the membranes which required complete reassembly of the column. Further, the spacing measurement would have to be in error by a factor of 100% to account for the disagreement observed, and the technique of measuring spacing could not possibly be so much in error.

One must, therefore, conclude that at the large membrane spacings used in this investigation, the theory does not correctly predict separation factors as a function of membrane spacing. It may be noted here that similar disagreement with respect to plate spacing also exists between theory and experiment in thermal diffusion.

Transient Analysis of the Electrophoresis Column

It has been shown in Chapter III, how the transport equation obtained by the reduction of the general partial differential equation by assuming steady state batch operation can be used to describe the transient behavior of an electrophoresis column. The transient batch experimental data provide an independent means to check the validity of the theory developed in Chapter III and also to check the applicability of the semiempirical procedure developed in

the earlier part of this chapter.

A transient batch run was made for each set of the separation factors as function of flow rate data to determine the time required to reach the steady state. However, these data are also useful to see how well the semiempirical H and K values obtained from the steady state flow rate data represent the transient batch data.

The transient separation factor as a function of column parameters and time has been shown to be given by equation 3.114 as:

$$f = \frac{C_B}{C_T} =$$

$$\frac{\frac{2AC_o e^{2A}}{e^{2A}-1} + 4AC_o e^{A} \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2+n^2\pi^2)^2}\left[1+(-1)^{n+1}e^{-A}\right](-1)^n e^{-\frac{(A^2+n^2\pi^2)K}{mL^2}t}}{\frac{2AC_o}{e^{2A}-1} + 4AC_o \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2+n^2\pi^2)^2}\left[1+(-1)^{n+1}e^{-A}\right]e^{-\frac{(A^2+n^2\pi^2)K}{mL^2}t}} \qquad 3.114$$

where C_B is the concentration of the component in question at the bottom of the column and C_T is that at the top and α as defined before:

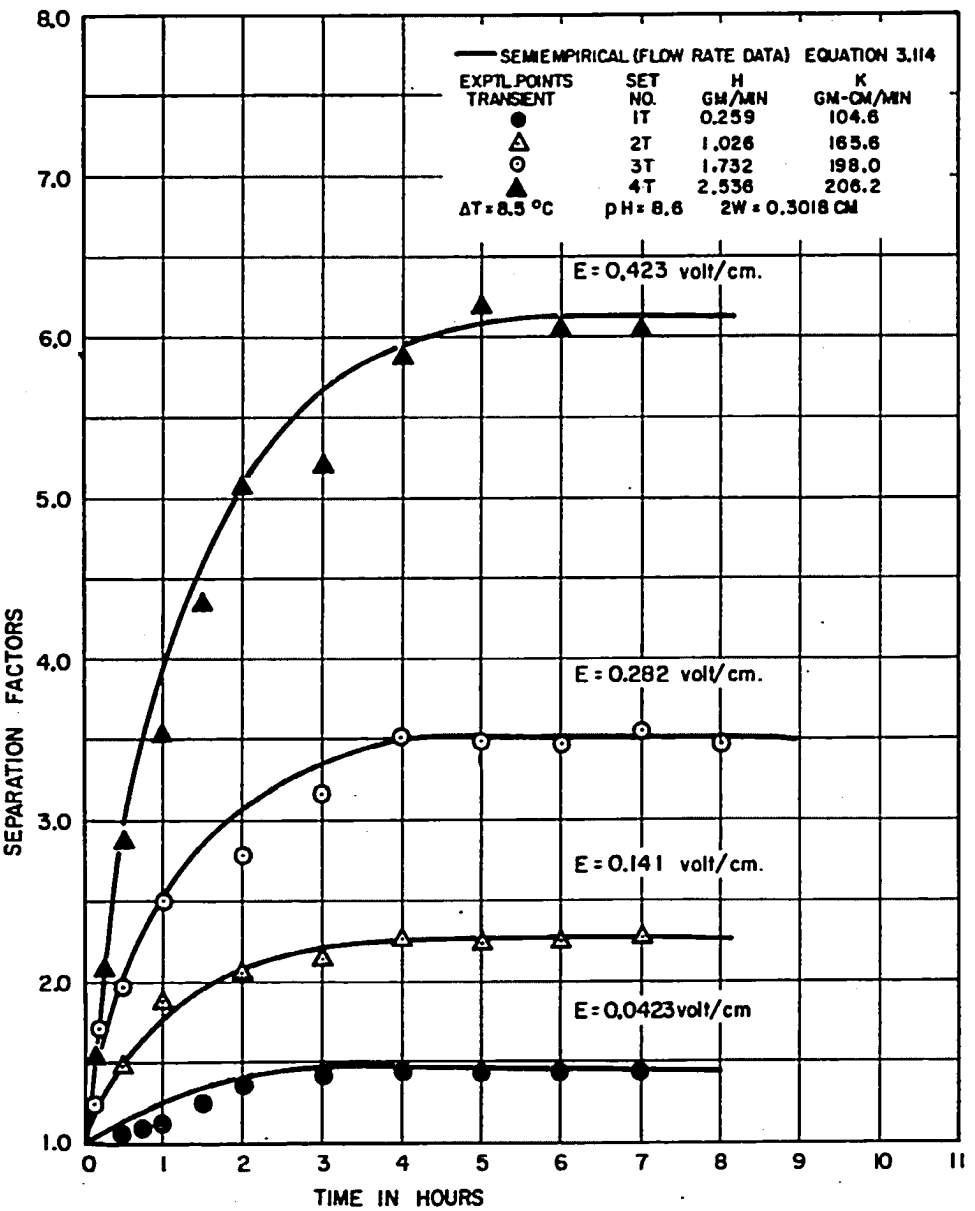
$$\alpha = \frac{mL^2}{K} \qquad 3.98$$

For a column of a given length, an examination of the transient part of equation 3.114 shows that the relaxation time (time to reach the steady state) depends upon the values

of A and K. For the cases studied here, however, A is small, and we need discuss only K. The theoretical values of K for the conditions used in the present experiments were quite large, indicating that very short times would be required to reach the steady state. However, the times observed were considerably longer than these and it is therefore evident that the transient theory and transient experiment do not quantitatively agree. Because of the disagreement between theory and experiment in the case of transient experimental data also, it is considered interesting to compare these independently obtained transient experimental data with the results calculated by using the semiempirical H and K values which were derived from the flow rate data. Such a comparison will now be made first with regard to the effect of the various parameters on the relaxation time for the column.

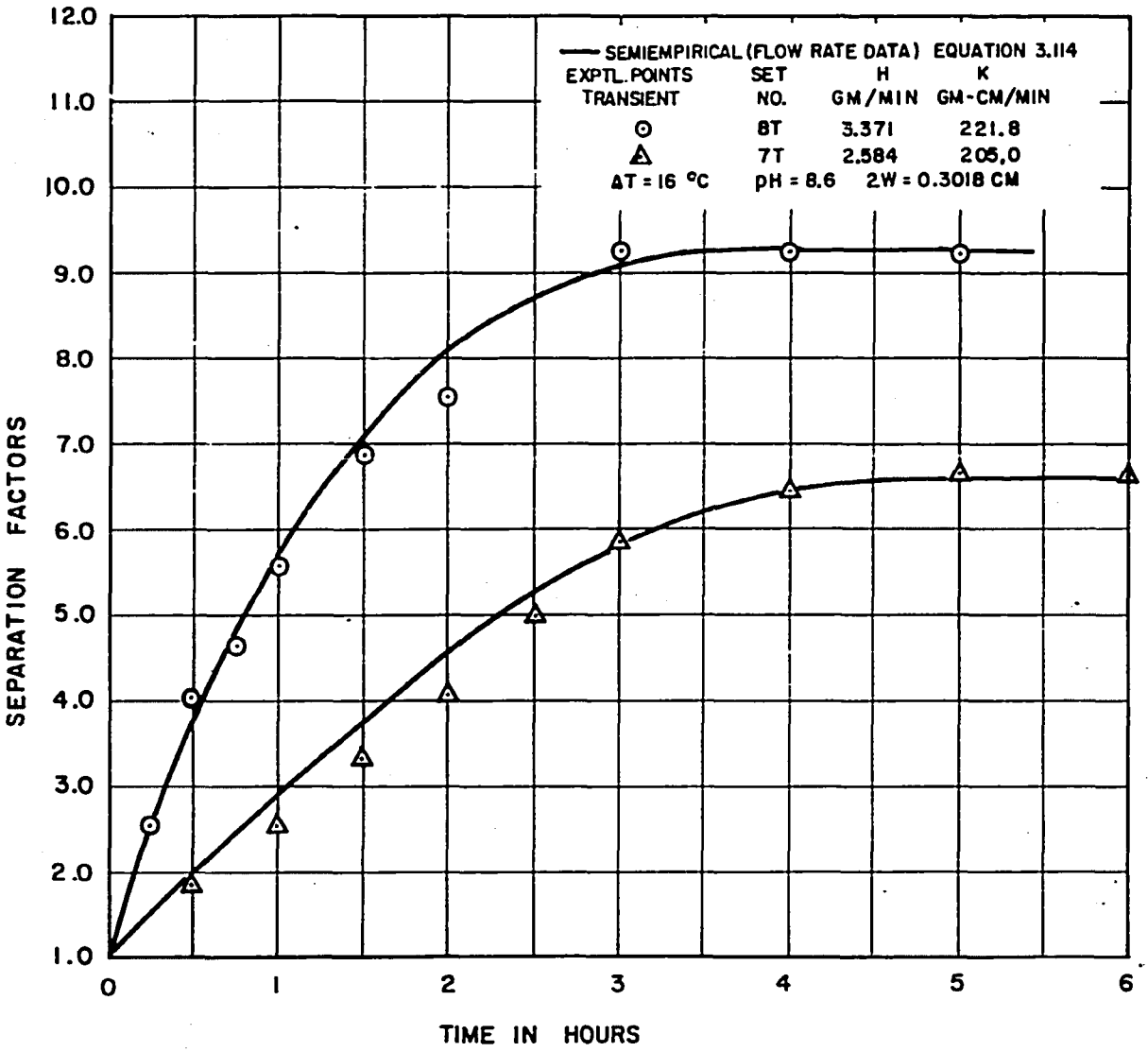
Effect of Field Strength

In Figures 5-16, 5-17 and 5-18 are presented the experimental transient data obtained at four different field strengths and temperature differences with all other parameters held constant. The solid curves in these figures represent the separation factors as a function of time obtained using the semiempirical H and K values from the flow rate data. Since semiempirical values of K increase with increase in E and approach a limiting value at fairly high values of E, the relaxation time should be practically independent of E. At low E values however, the relaxation



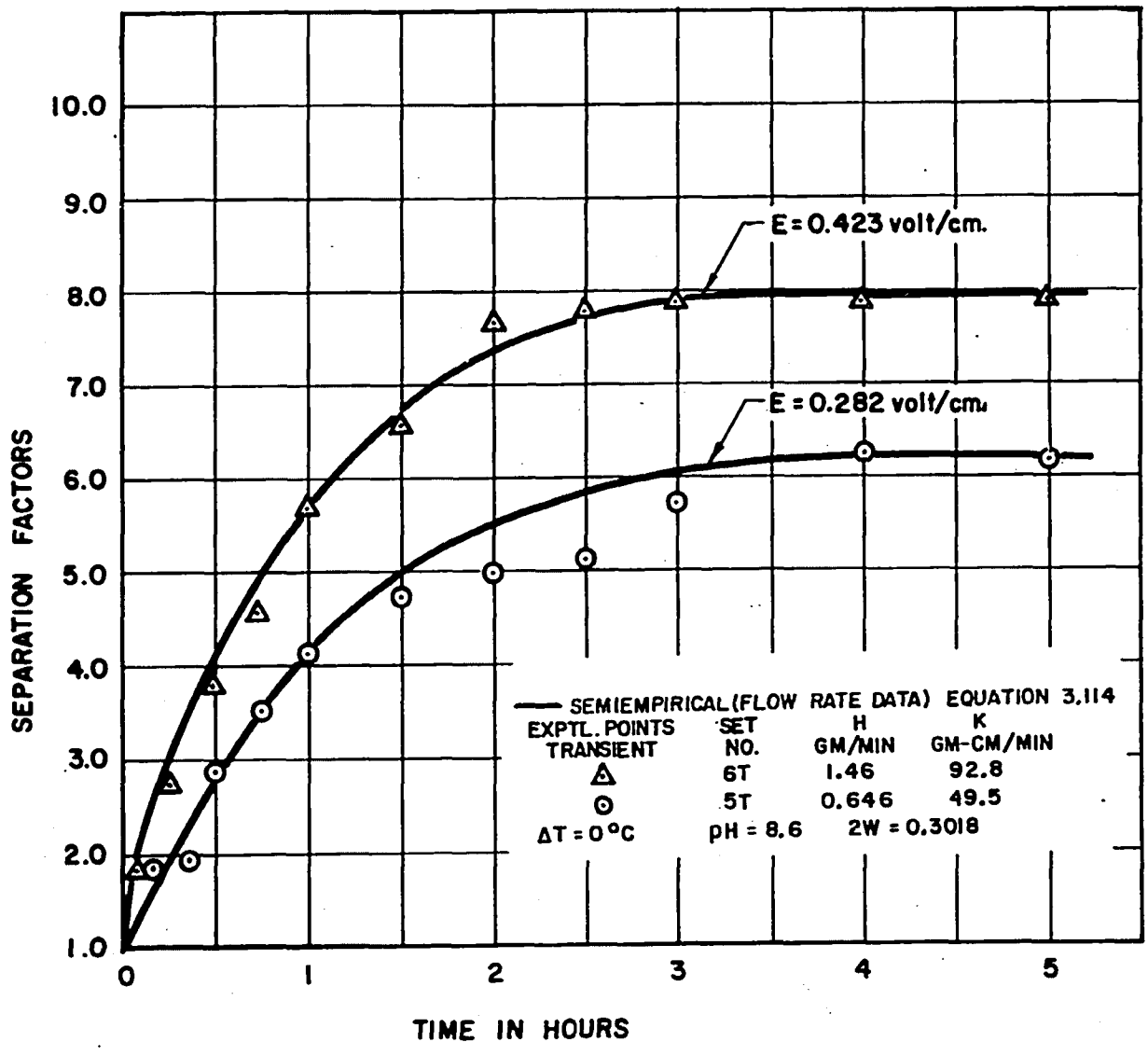
TRANSIENT SEPARATION FACTORS AT VARIOUS FIELD STRENGTHS

FIGURE 5–16



TRANSIENT SEPARATION FACTORS AT ΔT = 0 °C

FIGURE 5—17



TRANSIENT SEPARATION FACTORS AT $\Delta T = 16°C$

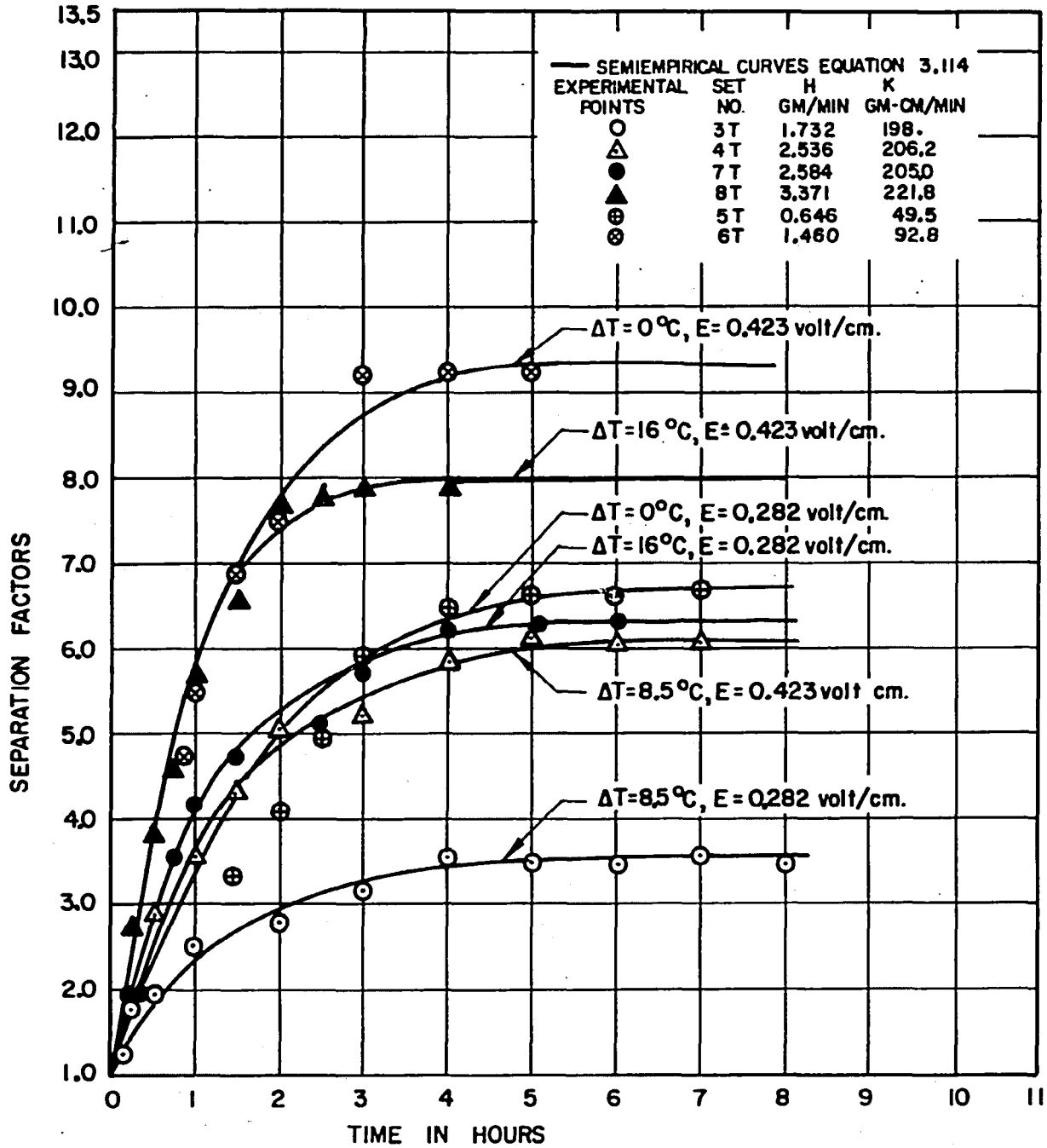
FIGURE 5-18

times should increase with decrease in E. The data presented in Figure 5-16 nearly confirm the above observations. In Figure 5-16, it is rather difficult to see the difference in the times required for reaching steady state but semiempirical calculations showed that the relaxation time was 7.5 hours at E = 0.0423 volt/cm., 6.5 hours at E = 0.141 volt/cm., 6 hours at E = 0.282 volt/cm. and about 6 hours at E = 0.423 volt/cm. These semiempirical values of the relaxation times agree closely with the experimental relaxation times as will be seen from Figure 5-16. The data presented in Figures 5-17 and 5-18 also substantiate the agreement between the semiempirical theory and the data.

Effect of Temperature Difference

Theory predicts an increase in K with increase in ΔT. The relaxation times should therefore decrease with increase in ΔT. However, it may be recalled here that the semiempirical K values increase with increase in ΔT but tend to approach a constant value at sufficiently large temperature differences. As a result, except at very small temperature differences, according to the semiempirical theory, the relaxation times should be about independent of the temperature difference.

Figure 5-19 presents the separation factors as a function of time at temperature differences of 8.5°C, 16°C and 0°C respectively and two different values of the field strength (0.282 volt/cm. and 0.423 volt/cm.) with other parameters held constant. It is seen from this that the



TRANSIENT SEPARATION FACTORS AT THREE DIFFERENT TEMPERATURE DIFFERENCES

FIGURE 5—19

relaxation times at ΔT of 8.5°C and ΔT of 16°C are not very different. However at ΔT of 0°C, the relaxation time is larger than that at either ΔT of 0°C or ΔT of 16°C. This is in accordance with the semiempirical theory derived from the flow rate data which predicts a similar behavior. Here again there is quite good agreement between transient experimental data and the results calculated using semiempirical H and K values from the separation factors as a function of flow rate data.

Effect of Membrane Spacing

Theory predicts considerable influence of membrane spacing on the values of K and the relaxation times should be considerably affected by a change in membrane spacing. Theoretically the relaxation times should increase with a decrease in membrane spacing. Semiempirical values of K at the two membrane spacings used in the present work, which are given in Table 5.1 are repeated below for immediate reference:

Values of Total K at Two Membrane Spacings

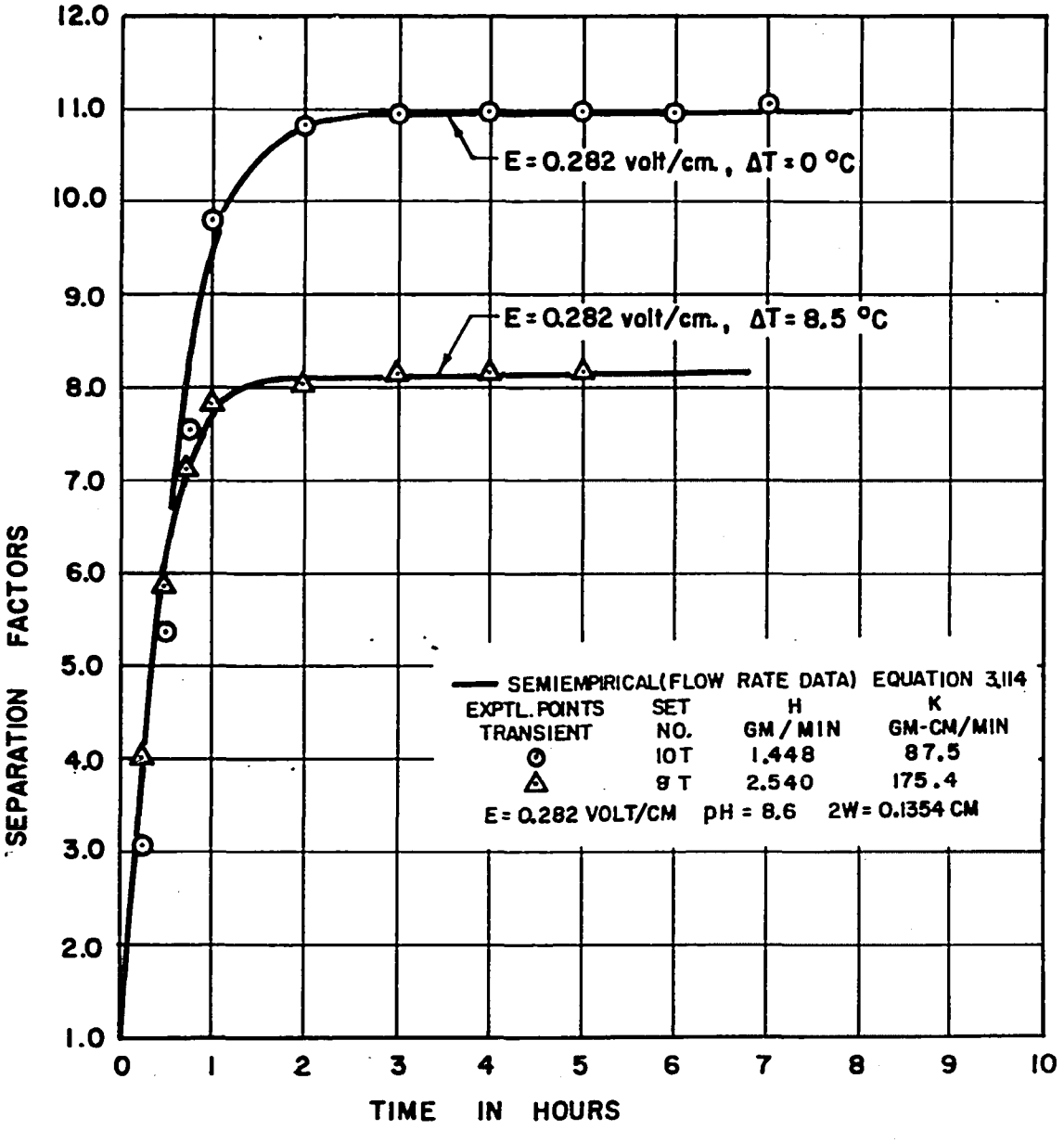
Spacing cm.	K at ΔT = 8.5°C gm.-cm./min.	K at ΔT = 0°C gm.-cm./min.
0.3018	198.0	49.5
0.1354	175.0	87.5

For a temperature difference of 0°C, the semiempirical value (87.5 gm.-cm./min.) of K at the spacing of 0.1354 cm. is greater than that (49.5 gm.-cm./min.) at the spacing of

0.3018 cm. The relaxation time should therefore be less for the smaller spacing as is seen from Figure 5-16 discussed before and from Figure 5.20 by a comparison of the corresponding curves ($\Delta T = 0°C$ and $\Delta T = 8.5°C$). The K values at the other temperature difference are about the same and therefore the relaxation times should be about the same. However, comparison of the curves of separation factors as function of time for ΔT of $8.5°C$ given in Figure 5-16 and a similar curve given in Figure 5-20 shows that for both the membrane spacings the relaxation times appear to be about the same. These observations are in complete agreement with the semiempirical theory derived from flow data but are in disagreement with the results calculated from the theory developed in Chapter III. This is not however surprising since, as has been already shown, the experimental data do not follow the theoretical dependence of membrane spacing.

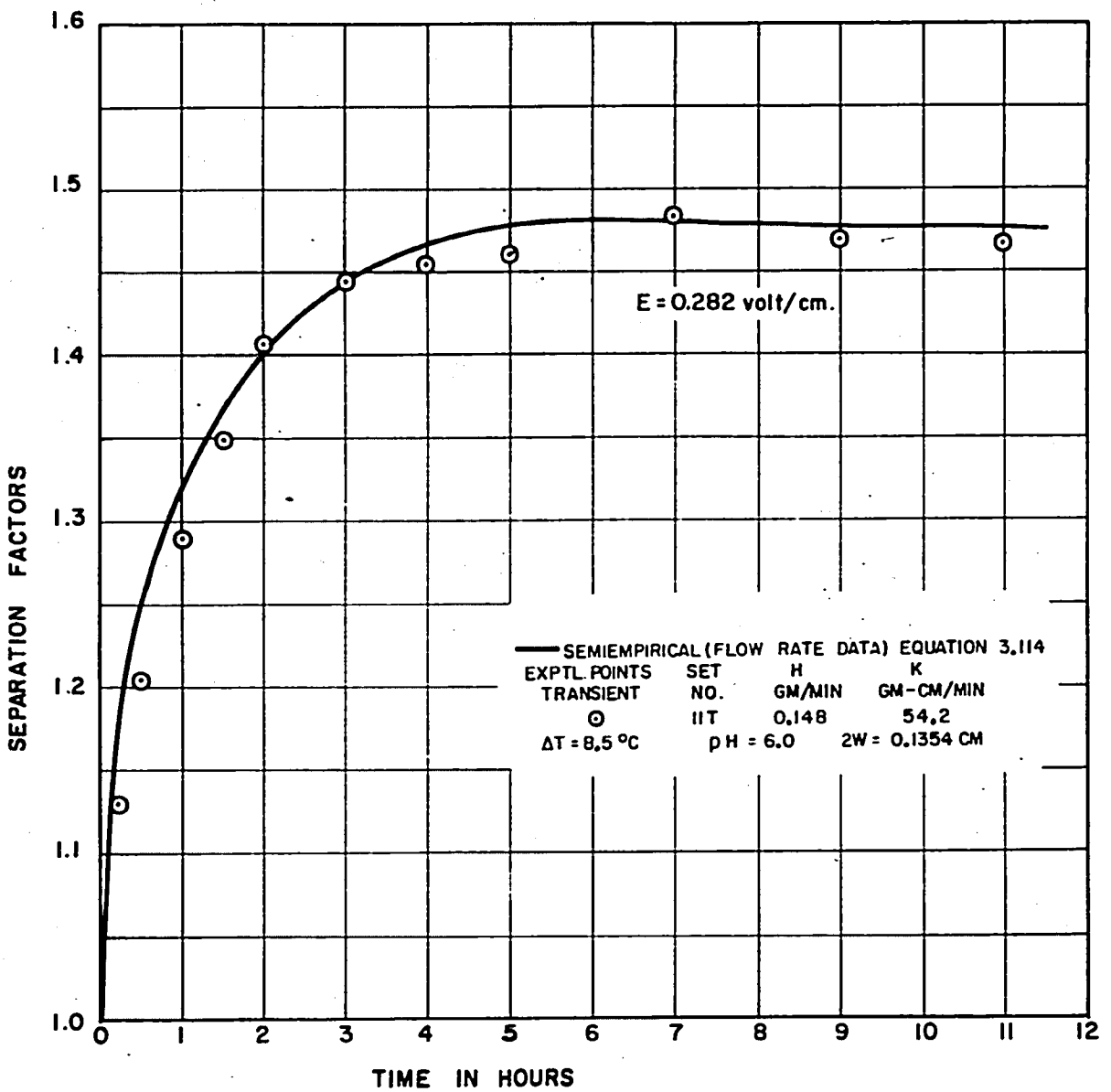
Effect of pH of the Solution

The effect of pH is reflected in the value of mobility and since K increases with increase in mobility when other parameters are held constant, the relaxation time should decrease with increase in mobility. In Figure 5-21 the experimental data for a mobility of -2.22×10^{-5} cm.^{2}/sec.-volt (pH of 6.0) are presented for a temperature difference of $8.5°C$, E of 0.282 volt/cm. and membrane spacing of 0.1354 cm. A similar curve for a mobility of -6.3×10^{-5} cm.^{2}/sec.-volt (pH of 8.6) at the same experimental conditions has been



TRANSIENT SEPARATION FACTORS
AT SPACING OF 0.1354 CM.

FIGURE 5 – 20



TRANSIENT SEPARATION FACTORS AT pH 6.0

FIGURE 5-21

presented in Figure 5-20. The relaxation time is about five hours for the lower mobility and about two hours for the higher mobility in agreement with the theory.

Transient Behavior of Column

It is seen from Figures 5-16 to 5-21 that the solid curves prepared by using semiempirical H and K values derived from flow data represent the transient separation factors very well. Except in few cases, the agreement between semiempirical theory and experiment is excellent.

The few discrepancies observed (cf. Figures 5-18, 5-20, 5-21) may be either due to experimental disturbances or they may be the result of effects caused by frequent sampling which rendered the run not truly batch. Vichare and Powers (65) have given a theoretical treatment to estimate the amounts of the samples to be withdrawn and the frequency of withdrawal from the thermal diffusion column in a transient batch experiment. This analysis could not be profitably used in the present investigation because of two reasons: (1) Vichare's analysis pertains to a column where the assumption of the constancy of the product C_1C_s could be made and where a symmetry about the midpoint of the column could be assumed. (2) In actual experimentation, it was necessary to withdraw comparatively larger amounts of the samples (from 0.75 ml. to 1 ml.) for analytical purposes since 0.5 ml. or more of the protein solution was required to develop a measurable color intensity.

In spite of the difficulties with regard to the sampling procedure and barring the few discrepancies mentioned above, the semiempirical H and K values obtained from flow rate data were capable of representing the transient batch data with excellent agreement. The agreement of the column data for the steady state and the excellent prediction of the transient results from the steady state column constants is very significant. It definitely supports the conclusion that the FJO procedure may be applied to describe the transient and steady state batch as well as steady state flow operations of a thermoelectrogravitational electrophoresis column.

Conclusions

(i) The experimental separation factor data on the performance of the thermoelectrogravitational electrophoresis column used in this investigation show that it is possible to obtain reasonable separation under a variety of conditions of electrical field strengths, temperature difference and membrane spacing.

(ii) Equation 3.80 developed in Chapter III for the separation factor fits the experimental data very well qualitatively, but there is no quantitative agreement between original theory and experiment.

(iii) Semiempirically calculated average values of H and K fit the experimental separation factor data very well. The empirical H and K are also reasonably independent of flow rates in agreement with the theory. Therefore H and K

calculated using batch velocity profiles are applicable to the flow case.

(iv) The functional forms of the empirically determined column constants H, H_{TE}, H_E and K were qualitatively in agreement with their theoretical expressions as functions of the parameters field strength (E) and temperature difference, ΔT.

The column constants K, however, appear to reach a limiting value with increase in either E or ΔT. This discrepancy is attributed to large membrane spacing for which the theory does not seem to be either qualitatively or quantitatively correct.

The values of the empirical column constants H and K do not follow their theoretical functionality with regard to the membrane spacing even qualitatively.

(v) As predicted by the theory, the separation factors increase with:

(a) increase in field strength,

(b) decrease in temperature difference,

(c) increase in electric mobility of the component,

and (d) decrease in membrane spacing.

(vi) At large temperature differences, the separation factors increase with increase in temperature difference, which contradicts theory. This is because K does not increase proportionately but tends to reach a constant value as ΔT increases.

The separation factors at $\Delta T = 0$ were higher than

those at the other temperature differences. This is attributed to the more orderly laminar convection that would result because of increase in viscosity and thus reduce parasitic remixing. Significantly large temperature differences will be required to give a substantial contribution to the separation process. However, at low values of E and flow rate, the contribution of ΔT is significant portion of the total effect. At large temperature differences, K remains constant and H increases with ΔT, therefore increase in separation factors are obtained.

(vii) The rate of approach to steady state in an electrophoresis column could be represented by equation 3.114, given in Chapter III. The empirical H and K values derived from steady state flow rate data represented the experimental transient batch data very well, and the application of the FJO procedure is justified.

CHAPTER VI

SUMMARY

Electrophoresis, an elegant method of separation of charged species from their mixtures by application of an electric field, has been extensively used in biochemical analysis since the introduction of Tiselius apparatus. Only recently this has begun to receive attention for preparatory purposes. A review of the preparative electrophoresis work indicated that most of the workers have developed convection free, low flow batch apparatus and have not taken the advantage of gravitational reflux action due to convection. Using the principle of Clusius-Dickel column, Kirkwood et al. developed a batch thermogravitational electro-convection apparatus with reservoirs for the separation of proteins and derived a mathematical theory to describe the behavior of their column. No theoretical or experimental work has been so far reported on the possible development of continuous-flow electrophoresis columns similar to Clusius-Dickel continuous-flow thermal diffusion columns.

In the present work, in order to develop new continuous-flow electrophoresis apparatus for preparative purposes, a center-fed thermoelectrogravitational

continuous-flow electrophoresis column using the principle of the Clusius-Dickel column was proposed. To explain the separation obtained in the column upon application of electric and thermal fields, a theory was developed using the Furry, Jones and Onsager procedure to yield a transport equation, which can be used to describe both the continuous-flow and transient operation of the column. Only systems containing one mobile component have been considered in the development of the theory.

An electrophoresis column containing Visking cellulose semipermeable membranes was constructed and utilized to obtain experimental transport data for bovine albumin. The experiments determined the effect on column operation of flow rate, field strength, temperature difference, membrane spacing and the pH of the solution. The experimental data were then compared to the theory developed.

Qualitative experimental confirmation of the theory proposed was found for both steady state continuous-flow and transient batch electrophoresis column operation. All the experimental data confirmed the functional relationships of separation factors developed in the theory for all the parameters, membrane spacing, flow rate, field strength, temperature difference and pH of the solution. Few discrepancies with regard to the dependence of separation factors on ΔT were observed. It was found that quantitative agreement between theory and experiment was not satisfactory. This might be due to the fact that the basic

thermogravitational theory of Furry, Jones and Onsager as applied to electrophoresis is not itself quantitative because of assumed approximations in its development, and because of the fact that in the present investigation comparatively large plate spacings were used, at which FJO theory has been shown to be not quantitative in thermal diffusion.

DEFINITION OF SYMBOLS

DEFINITION OF SYMBOLS

A = $HL/2K$ defined by equation 3.85e. $A_T = A$ for top; $A_B = A$ for bottom.

a = general constant

b = general constant

B = column width

B = subscript to identify streams leaving the bottom of the column

B' = general constant

c = general constant

C = concentration

C_o = concentration (original) in feed

$\bar{C}$ = Laplace transform of C

C_1 = concentration of component 1

C_2 = concentration of component 2

C_M = concentration of component at feed point of the column

C_B = concentration of component 1 leaving bottom section

C_T = concentration of component 1 leaving top section

C_F = concentration of component 1 in feed

$\overline{C}$ = average concentration

C' = general constant

d = general constant

D = diffusion constant without subscript

D_{1s} = diffusion constant with subscript, also D_{12}, D_{13}, etc.

D' = general constant

e = general constant

e' = general constant

F' = general constant

f = separation factor

g = local acceleration due to gravity

g_c = dimensional constant

G' = general constant

H = column constant defined by equation 3.70

H_{Th} = column parameter in thermal diffusion

H_E = column constant given by equation 3.73b

H_{TE} = column constant given by equation 3.73a

H_T = column constant representing thermal diffusion term in H

j = subscript to indicate solution component

k = thermal conductivity of solution

K = constant defined by equation 3.74

K_c = constant defined by equation 3.23

K_d = constant defined by equation 3.75d

K_T = constant defined by equation 3.75a

K_E = constant defined by equation 3.75c

K_{TE} = constant defined by equation 3.75b

K_{Th} = column parameter in thermal diffusion

L = total column length

L_T = length of top section

L_B = length of bottom section

$L/2$ = half the column length

m = amount of solution per unit length of column

P = pressure in the column

P_1 = protein, also P_2 . . . P_n

Q = heat generated in the column

s = transform of t

s = membrane spacing measurement

t = time

T = temperature

T = subscript indicating top section of electrophoresis column

T_1 = temperature of hot membrane

T_2 = temperature of cold membrane

$\overline{T}$ = integral average temperature

ΔT = mean temperature difference between hot and cold membrane

U = electrical mobility, also U_{1s}, U_{12}, U_{13}, etc.

$v(x)$ general velocity distribution function

V = voltage

x = axis normal to membranes

y = axis parallel to membranes

z = axis perpendicular to x,y plane

α = a constant defined by the relation $\alpha = mL^2/K$ of equation 3.98

α_{1s} = thermal diffusion coefficient, also α_{12}, α_{13}, etc

β_T = change in density with temperature

β_C = change in density with concentration

η = coefficient of viscosity

ρ = density

σ = average mass flow rate

σ_B = mass flow rate from the bottom of the column

σ_T = mass flow rate from the top of the column

σ_F = mass flow rate of feed

τ = amount of component one passing through a cross-section of the electrophoresis column normal to the membranes

τ = amount of component one passing through a cross-section of a thermogravitational column normal to plates

φ = dimensionless length given by equation 3.93

φ_H = correction factor defined by equation 5-10

φ_K = correction factor defined by equation 5-11

2ω = spacing between hot and cold semipermeable membranes

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APPENDIX A

COLUMN AND SYSTEM PARAMETERS FOR SETS OF EXPERIMENTAL DATA

TABLE A.1

Column and System Parameters for Sets of Experimental Data

Exp. Set.	$2w$ Mean Membrane Spacing	ΔT Mean Temp. Difference	B Column Width	C Feed Concentration	pH of Solution	E Electrical Field Strength
No.	cm.	°C	cm.	$\frac{\text{gm.}}{100\ \text{ml.}}$		$\frac{\text{volts}}{\text{cm.}}$
1	0.3018	8.5	10.2	0.956	8.6	0.0423
2	0.3018	8.5	10.2	0.956	8.6	0.1407
3	0.3018	8.5	10.2	0.956	8.6	0.280
4	0.3018	8.5	10.2	0.956	8.6	0.421
5	0.3018	0	10.2	0.956	8.6	0.282
6	0.3018	0	10.2	0.956	8.6	0.423
7	0.3018	16.0	10.2	0.956	8.6	0.282
8	0.3018	16.0	10.2	0.956	8.6	0.423
9	0.1354	8.5	10.2	0.956	8.6	0.282
10	0.1354	0	10.2	0.956	8.6	0.282
11	0.1354	8.5	10.2	0.956	6.0	0.098

Note: Column length L was held constant at 145 cm. for all experimental runs.

APPENDIX B

TABLES OF EXPERIMENTAL DATA

TABLE D.1

EXPERIMENTAL DATA SET - 1F

Conditions of Experiment

Field Strength ---- 0.0423 volt/cm.
Temp. Difference -- 8.5°C.
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.295	0.283	0.289	1.102	0.811	1.360
2	0.541	0.501	0.521	1.087	0.826	1.316
3	1.125	1.249	1.187	1.072	0.841	1.275
4	2.765	2.306	2.536	1.051	0.862	1.218
5	3.686	3.721	3.704	1.031	0.881	1.170
6	6.025	6.349	6.187	1.024	0.889	1.152
7	8.801	8.034	8.418	1.020	0.893	1.142
8	8.801	8.034	8.418	0.984	0.929	1.060

TABLE D.2

EXPERIMENTAL DATA SET - 2F

Conditions of Experiment

Field Strength ---- 0.141 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.584	0.572	0.578	1.269	0.644	1.97
2	1.177	1.083	1.130	1.260	0.653	1.93
3	1.833	2.095	1.964	1.182	0.731	1.618
4	3.035	3.312	3.124	1.160	0.753	1.540
5	4.357	4.116	4.237	1.119	0.794	1.410
6	5.562	6.010	5.781	1.135	0.778	1.460
7	6.897	7.443	7.17	1.059	0.854	1.240
8	10.783	12.019	11.401	1.0425	0.870	1.198

TABLE D.3

EXPERIMENTAL DATA SET - 3F

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.371	0.324	0.348	1.408	0.505	2.79
2	0.487	0.494	0.491	1.368	0.545	2.51
3	1.376	1.217	1.297	1.307	0.605	2.16
4	1.819	2.297	2.058	1.290	0.623	2.07
5	2.723	2.726	2.730	1.267	0.646	1.96
6	3.177	3.009	3.093	1.227	0.686	1.79
7	4.645	4.650	4.648	1.230	0.683	1.80
8	6.438	5.926	6.182	1.215	0.698	1.74
9	7.329	7.681	7.505	1.169	0.744	1.57
10	8.265	10.504	9.385	1.157	0.756	1.53
11	11.931	11.051	11.691	1.132	0.781	1.45

TABLE D.4

EXPERIMENTAL DATA SET - 4F

Conditions of Experiment

Field Strength ---- 0.423 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.371	0.302	0.337	1.562	0.350	4.46
2	1.078	0.717	0.898	1.526	0.387	3.94
3	1.288	1.313	1.301	1.479	0.434	3.41
4	1.163	1.398	1.281	1.481	0.434	3.43
5	2.535	2.475	2.505	1.420	0.432	2.88
6	3.749	2.924	3.337	1.357	0.493	2.44
7	4.541	5.680	5.110	1.268	0.556	1.966
8	6.802	6.991	6.897	1.240	0.645	1.843
9	9.331	11.185	10.258	1.199	0.673	1.680

TABLE D.5

EXPERIMENTAL DATA SET - 5F

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 0°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.178	0.162	0.170	1.616	0.297	5.44
2	1.280	1.260	1.270	1.363	0.549	2.48
3	2.253	1.855	2.054	1.225	0.688	1.78
4	3.964	3.397	3.681	1.154	0.759	1.521
5	5.057	5.069	5.063	1.066	0.846	1.260
6	6.395	5.780	6.088	1.035	0.877	1.180
7	0.411	0.43	0.421	1.546	0.367	4.21
8	0.231	0.214	0.223	1.581	0.332	4.76

TABLE D.6

EXPERIMENTAL DATA SET - 6F

Conditions of Experiment

Field Strength ---- 0.423 volt/cm.
Temp. Difference -- 0°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.288	0.225	0.257	1.673	0.240	6.47
2	0.681	0.800	0.741	1.597	0.316	5.05
3	1.473	1.489	1.981	1.544	0.369	4.18
4	1.473	1.489	1.981	1.534	0.379	4.05
5	1.998	2.196	2.097	1.447	0.416	3.60
6	3.373	3.516	3.445	1.437	0.476	3.020
7	4.288	4.628	4.458	1.372	0.540	2.540
8	5.815	6.121	5.968	1.304	0.609	2.140
9	8.293	8.351	8.322	1.194	0.719	1.660
10	11.188	11.372	11.28	1.092	0.821	1.330

TABLE D.7

EXPERIMENTAL DATA SET - 7F

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 16°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	$\frac{gm.}{min.}$	$\frac{gm.}{min.}$	$\frac{gm.}{min.}$	$\frac{gm.}{100\ ml.}$	$\frac{gm.}{100\ ml.}$	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.223	0.196	0.210	1.407	0.506	2.78
2	0.717	0.727	0.722	1.362	0.551	2.47
3	1.670	2.207	1.939	1.253	0.659	1.90
4	2.151	2.294	2.228	1.204	0.708	1.70
5	3.078	3.484	3.281	1.148	0.765	1.51
6	4.746	4.670	4.708	1.112	0.800	1.39
7	6.211	6.151	6.181	1.109	0.804	1.38

TABLE D.8

EXPERIMENTAL DATA SET - 8F

Conditions of Experiment

Field Strength ---- 0.423 volt/cm.
Temp. Difference -- 16°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.584	0.572	0.578	1.581	0.333	4.74
2	1.173	1.082	1.128	1.536	0.377	4.08
3	1.833	2.098	1.966	1.451	0.462	3.14
4	3.312	3.173	3.243	1.444	0.469	3.08
5	4.537	4.166	4.237	1.413	0.499	2.83
6	5.562	6.010	5.786	1.389	0.524	2.65
7	6.069	6.623	6.346	1.354	0.559	2.42
8	10.783	12.019	11.401	1.319	0.594	2.22

TABLE D.9

EXPERIMENTAL DATA SET - 9F

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 0°C
Membrane Spacing -- 0.1354 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.333	0.283	0.308	1.683	0.229	7.34
2	0.619	0.490	0.555	1.665	0.268	6.13
3	1.347	1.441	1.394	1.575	0.337	4.67
4	2.176	1.859	2.018	1.460	0.453	3.22
5	3.959	3.809	3.884	1.355	0.558	2.43
6	4.643	4.415	4.529	1.246	0.666	1.87
7	5.135	5.033	5.084	1.227	0.686	1.79

TABLE D.10

EXPERIMENTAL DATA SET - 11F

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.1354 cm.
pH of Solution ---- 8.6

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.223	0.214	0.219	1.652	0.261	6.34
2	1.117	0.981	1.049	1.559	0.354	4.41
3	3.025	2.751	2.889	1.509	0.404	3.74
4	4.827	4.482	4.655	1.470	0.443	3.32
5	5.467	5.651	5.559	1.427	0.685	2.94

TABLE D.11

EXPERIMENTAL DATA SET - 11F

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.1354 cm.
pH of Solution ---- 6.0

STEADY STATE FLOW DATA

Run	Bottom Flow Rate	Top Flow Rate	Average Flow Rate	Bottom Concentration	Top Concentration	Separation Factor
	gm./min.	gm./min.	gm./min.	gm./100 ml.	gm./100 ml.	f
No.	σ_B	σ_T	σ	C_B	C_T	C_B/C_T
1	0.576	0.558	0.567	1.051	0.862	1.220
2	1.62	1.572	1.596	1.029	0.886	1.165
3	2.424	2.413	2.419	1.016	0.896	1.134
4	3.254	3.239	3.247	1.001	0.913	1.096
5	4.642	4.840	4.741	0.985	0.928	1.062

TABLE D-1A

EXPERIMENTAL DATA SET - 1T

Conditions of Experiment

Field Strength ---- 0.0423 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100\ \text{ml.}}$	$\frac{\text{gm.}}{100\ \text{ml.}}$	f
		C_B	C_B	C_B/C_T
1	30	0.985	0.928	1.062
2	45	0.995	0.918	1.083
3	60	1.015	0.898	1.130
4	90	1.063	0.850	1.250
5	120	1.102	0.811	1.360
6	180	1.117	0.795	1.405
7	240	1.126	0.787	1.430
8	300	1.124	0.788	1.426
9	360	1.125	0.787	1.428
10	420	1.126	0.787	1.430

TABLE D.2A

EXPERIMENTAL DATA SET - 2T

Conditions of Experiment

Field Strength ---- 0.141 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100 \text{ ml.}}$	$\frac{\text{gm.}}{100 \text{ ml.}}$	f
		C_B	C_T	C_B/C_T
1	30	1.135	0.7744	1.47
2	60	1.244	0.669	1.86
3	120	1.286	0.627	2.05
4	180	1.302	0.611	2.13
5	240	1.324	0.589	2.25
6	300	1.321	0.592	2.23
7	360	1.321	0.590	2.234
8	420	1.321	0.584	2.262

TABLE D.3A

EXPERIMENTAL DATA SET - 3T

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100\ \text{ml.}}$	$\frac{\text{gm.}}{100\ \text{ml.}}$	f
		C_B	C_T	C_B/C_T
1	5	1.063	0.850	1.250
2	10	1.205	0.707	1.704
3	30	1.264	0.669	1.946
4	60	1.366	0.547	2.500
5	120	1.407	0.506	2.780
6	180	1.453	0.461	3.160
7	240	1.440	0.423	3.520
8	300	1.486	0.427	3.480
9	360	1.485	0.427	3.476
10	420	1.492	0.421	3.544
11	480	1.485	0.428	3.472

TABLE D.4A

EXPERIMENTAL DATA SET - 4T

Conditions of Experiment

Field Strength ---- 0.423 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100\ \text{ml.}}$	$\frac{\text{gm.}}{100\ \text{ml.}}$	f
		C_B	C_T	C_B/C_T
1	5	1.154	0.759	1.52
2	15	1.288	0.625	2.06
3	30	1.414	0.498	2.86
4	60	1.490	0.423	3.52
5	90	1.554	0.359	4.33
6	120	1.598	0.315	5.07
7	180	1.604	0.309	5.19
8	240	1.635	0.278	5.89
9	300	1.646	0.266	6.18
10	360	1.641	0.272	6.04
11	420	1.640	0.272	6.03

TABLE D.5A

EXPERIMENTAL DATA SET - 5T

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 0°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100\ \text{ml.}}$	$\frac{\text{gm.}}{100\ \text{ml.}}$	f
		C_B	C_T	C_B/C_T
1	30	1.239	0.674	1.84
2	60	1.369	0.543	2.52
3	90	1.469	0.444	3.31
4	120	1.534	0.379	4.05
5	150	1.593	0.319	4.99
6	180	1.632	0.280	5.82
7	240	1.656	0.257	6.44
8	300	1.662	0.251	6.62
9	360	1.661	0.252	6.60
10	420	1.662	0.250	6.64

TABLE D.6A

EXPERIMENTAL DATA SET - 6T

Conditions of Experiment

Field Strength ---- 0.423 volt/cm.
Temp. Difference -- 0°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100 \text{ ml.}}$	$\frac{\text{gm.}}{100 \text{ ml.}}$	f
		C_B	C_T	C_B/C_T
1	15	1.371	0.542	2.53
2	30	1.533	0.380	4.03
3	45	1.573	0.340	4.63
4	60	1.620	0.292	5.56
5	90	1.669	0.243	6.86
6	120	1.688	0.224	7.52
7	180	1.726	0.186	9.26
8	240	1.726	0.187	9.24
9	300	1.725	0.187	9.21

TABLE D.7A

EXPERIMENTAL DATA SET - 7T

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 16°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{gm.}{100\ ml.}$	$\frac{gm.}{100\ ml.}$	f
		C_B	C_T	C_B/C_T
1	10	1.231	0.682	1.805
2	20	1.252	0.660	1.896
3	30	1.420	0.493	2.880
4	45	1.491	0.421	3.540
5	60	1.541	0.372	4.140
6	90	1.581	0.333	4.740
7	120	1.592	0.321	4.960
8	150	1.604	0.313	5.120
9	180	1.621	0.286	5.710
10	240	1.668	0.264	6.230
11	300	1.646	0.266	6.180

TABLE D.8A

EXPERIMENTAL DATA SET - 8T

Conditions of Experiment

Field Strength ---- 0.423 volt/cm.
Temp. Difference -- 16°C
Membrane Spacing -- 0.3018 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{gm.}{100\ ml.}$	$\frac{gm.}{100\ ml.}$	f
		C_B	C_T	C_B/C_T
1	5	1.233	0.679	1.830
2	15	1.399	0.514	2.720
3	30	1.514	0.399	3.800
4	45	1.569	0.344	4.560
5	60	1.627	0.286	5.690
6	90	1.659	0.254	6.540
7	120	1.691	0.221	7.640
8	150	1.700	0.218	7.790
9	300	1.698	0.215	7.894
10	360	1.696	0.216	7.840

TABLE D.9A

EXPERIMENTAL DATA SET - 9T

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 0°C
Membrane Spacing -- 0.1354 cm.
pH of Solution

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100\ \text{ml.}}$	$\frac{\text{gm.}}{100\ \text{ml.}}$	f
		C_B	C_T	C_B/C_T
1	15	1.441	0.472	3.05
2	30	1.626	0.269	5.67
3	45	1.689	0.224	7.54
4	60	1.736	0.177	9.80
5	120	1.751	0.162	10.81
6	180	1.753	0.160	10.94
7	240	1.758	0.174	10.96
8	300	1.756	0.174	10.95
9	360	1.751	0.162	10.96
10	420	1.756	0.173	11.02

TABLE D.10A

EXPERIMENTAL DATA SET - 10T

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.1354 cm.
pH of Solution ---- 8.6

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100\ \text{ml.}}$	$\frac{\text{gm.}}{100\ \text{ml.}}$	f
		C_B	C_T	C_B/C_T
1	15	1.532	0.381	4.02
2	30	1.636	0.277	5.90
3	45	1.678	0.235	7.13
4	60	1.696	0.216	7.84
5	120	1.701	0.212	8.04
6	180	1.703	0.210	8.11
7	240	1.703	0.209	8.13
8	300	1.703	0.210	8.11

TABLE D.11A

EXPERIMENTAL DATA SET - 11T

Conditions of Experiment

Field Strength ---- 0.282 volt/cm.
Temp. Difference -- 8.5°C
Membrane Spacing -- 0.1354 cm.
pH of Solution ---- 6.0

TRANSIENT BATCH DATA

Sample No.	Time	Bottom Concentration	Top Concentration	Separation Factor
	Minutes	$\frac{\text{gm.}}{100\ \text{ml.}}$	$\frac{\text{gm.}}{100\ \text{ml.}}$	f
		C_B	C_T	C_B/C_T
1	15	1.015	0.898	1.130
2	30	1.045	0.868	1.204
3	60	1.078	0.835	1.290
4	90	1.099	0.814	1.350
5	180	1.120	0.793	1.412
6	300	1.135	0.778	1.460
7	420	1.143	0.770	1.484
8	540	1.138	0.775	1.468
9	660	1.137	0.776	1.465

APPENDIX C

PHYSICAL PROPERTIES OF BOVINE ALBUMIN SYSTEM

TABLE A.2

Physical Properties of Bovine Albumin Solution

Property or Other Item	Value
Concentration of bovine albumin in solution	0.956 gm./100 ml.
Density of solution at pH 8.6 (Boric acid-Borax Buffer)	1.024 gm./cm.^{3}
Density of solution at pH 6.0 (Phosphate Buffer)	1.044 gm./cm.^{3}
Viscosity of solution at pH 8.6 and at a temperature of 281.25°K	1.4348 C.P.
Viscosity of solution at pH 8.6 and at a temperature of 285°K	1.295 C.P.
Viscosity of solution at pH 8.6 and at a temperature of 277°K	1.626 C.P.
Viscosity of solution at pH 6.0 and at a temperature of 285.25°K	1.445 C.P.
Diffusion Coefficient of Bovine Albumin at 20°C	5.9×10^{-5} cm.^{2}/sec.
Coefficient of density increment (for plasma proteins)	0.25 gm./cm.^{3}-gm.
Electrophoretic mobility of bovine albumin at pH 8.6	-6.3×10^{-5} cm.^{2}/volt-sec.
Electrophoretic mobility of bovine albumin at pH 6.0	-2.2×10^{-5} cm.^{2}/volt-sec.

Note:

Values for the density ρ and the viscosity η of Bovine Albumin Solution were determined in laboratory. The values of the temperature coefficient of expansion $\beta = -\left(\frac{d\rho}{dT}\right)$ were obtained by determining the slopes of the temperature-density data for water given in Perry's Handbook for Chemical Engineers (1). Data presented by Greenberg (2) were the sources of diffusion coefficient values. Mobilities were obtained from the book "Electrophoresis of Proteins" (3).

References:

(1) Chemical Engineer's Handbook, McGraw-Hill Book Co., New York, 1963.

(2) Greenberg, D. M., Amino Acids and Proteins, Thomas Publisher, New York.

(3) Electrophoresis of Proteins, H. A. Abramson, Reinhold Publications Corporation, 1942.

APPENDIX D

SOME SAMPLE CALCULATIONS

Sample Calculations

Estimation of Average H for a Set of Data

Set 9 is chosen to illustrate this calculation. A value of A = HL/2K was calculated first using the relation:

$$\left(\frac{C_B}{C_T}\right)_{\sigma=0} = e^{2A} \qquad 5.1$$

when $\sigma = 0$ and when steady state is achieved.

$A = -1.15 \times \log C_B/C_T$

$= 1.21$

Then for each steady state flow run of the set, the procedure of calculation explained in Chapter V was followed. A value of H was assumed and for the above value of A and for the assumed value of H, the separation factor C_B/C_T was calculated. If this value did not agree closely with the experimental value the calculation was repeated for a new assumed value of H. When agreement occurred, that H value was taken as appropriate value for that run. Similar calculations were done for the other observations. Table below gives the calculations for set 9.

TABLE A.3

Flow Rate σ	Assumed Value of H for final gm./min.	Computed $\frac{\left(\frac{C_B}{C_T}\right)_\sigma}{\left(\frac{C_B}{C_T}\right)_{\sigma=0}}$	$\frac{\left(\frac{C_B}{C_T}\right)_{exp}}{\left(\frac{C_B}{C_T}\right)_{\sigma=0}}$	Expt $\frac{C_B}{C_T}$	Computed $\frac{C_B}{C_T}$
0.308	1.024	0.809	0.811	7.34	7.326
0.555	1.180	0.676	0.678	6.130	6.127
1.394	1.782	0.515	0.516	4.670	4.658
2.018	1.412	0.360	0.356	3.220	3.260
3.884	1.827	0.264	0.257	2.430	2.396
4.529	1.412	0.208	0.206	1.870	1.889
5.084	1.482	0.201	0.198	1.790	1.820

Average value of H is 1.448 gm./min.

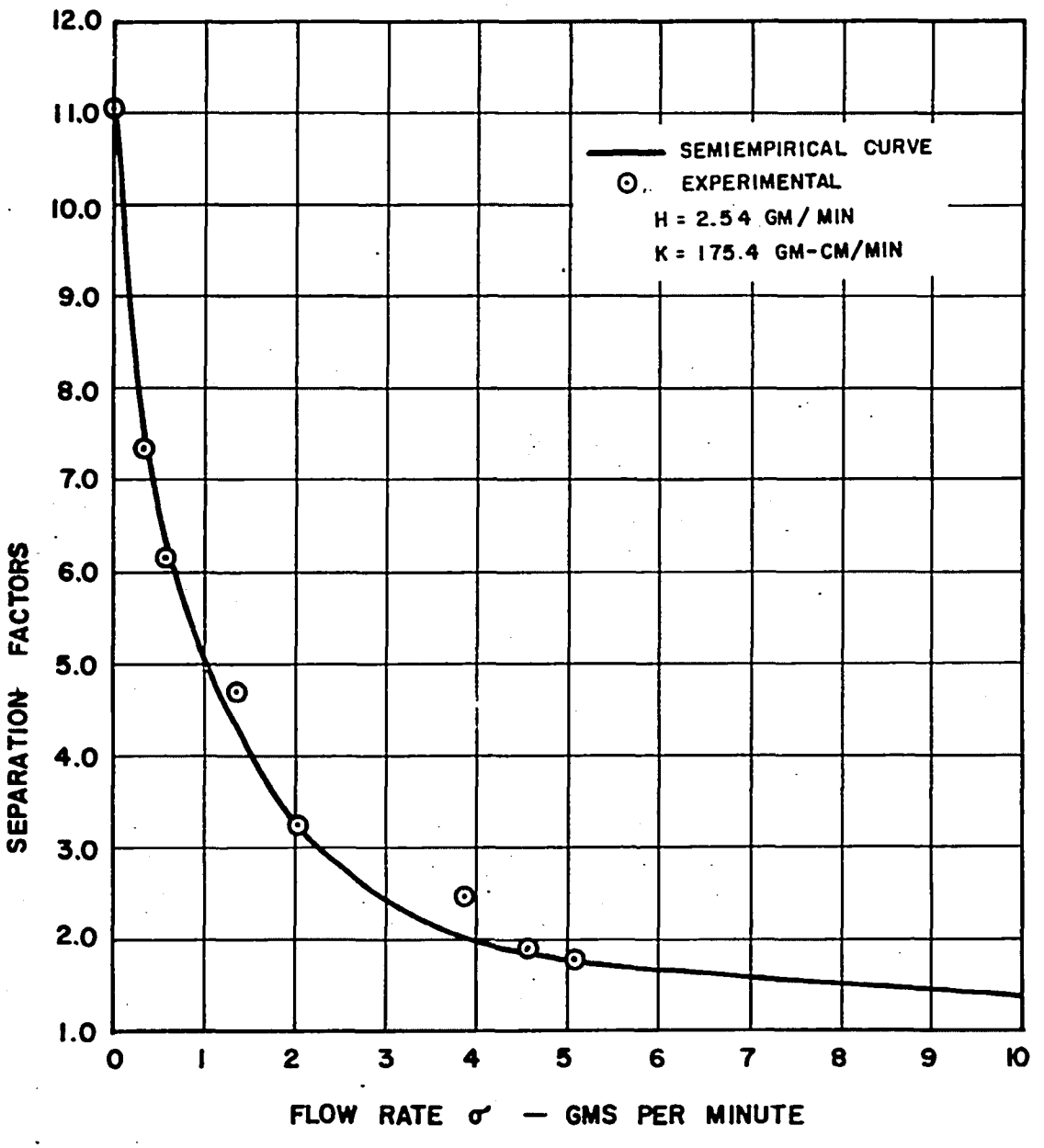
Using the value of A and average H obtained in the above manner, the separation factors were calculated as a function of flow rate and are shown on Figure A-1 along with the experimental separation factors. The fit obtained is excellent and therefore assumed values of A and H are satisfactory. Knowing A and H, K_{exp} can be calculated as:

$$K_{exp} = \frac{H_{exp} \times L_T}{2A}$$

$$= \frac{1.448 \times 145.0}{2 \times 1.2}$$

$$= 87.5 \text{ gm.-cm./min.}$$

where $H_{exp} = 1.448$ gm./min.



SAMPLE FIT USING EXPERIMENTAL H AND A OF DATA SET 9

FIGURE A-1

Calculation 2

Sample Calculation of K_E and H_E

A: K_E theoretical $= \frac{B\rho}{D}\left(\frac{g}{\eta}\cdot\frac{0.3UE\beta_C C_o}{D}\right)^2 \frac{(2\omega)^9}{9!}$

$E = 0.282$ volt/cm.

$\beta_C = 0.25$ gm./cm.^{3}-gm.

$\rho = 1.024$ gm./cm.^{3}

$g = 980$ cm./sec.^{2}

$B = 10.2$ cm.

$U = -6.3 \times 10^{-5}$ cm.^{2}/sec./volt

$C_o = 0.009382$ mass fraction

$2\omega = 0.1354$ cm.

$D = 4.18 \times 10^{-5}$ cm.^{2}/sec.

$\eta = 1.4348 \times 10^{-2}$ gm./cm.-sec.

When these values are substituted in the above equation along with a factor of 60 sec./min., the result is

K theoretical = 309.43 gm.-cm./min.

B: H_E theoretical $= B\rho\left(\frac{UE}{D}\right)\left(\frac{g}{\eta}\cdot\frac{0.3UE\beta_C C_o}{D}\right)\frac{(2\omega)^5}{6!}$

When appropriate values of the variables are substituted along with a factor of 60 sec./min., the result is:

H = 3.9926 gm./min.

From previous sample calculation

$$(H_E)_{exp} = 1.448 \text{ gm./min.}$$

$$\text{Therefore } \varphi_H = \frac{1.448}{3.992} = 0.363$$

Calculation of φ_K:

$$\varphi_K = \varphi_H^{1.25}$$

$$\varphi_K = (0.363)^{1.25} = 0.281$$

Calculations of other constants could be done in the same manner.

APPENDIX E

LIST OF ASSUMPTIONS

APPENDIX E

List of Assumptions

The assumptions that were made in the development of equation are given below. They were used as and when required in the development of the theory. The equations used to compare theory and experiment are summarized following this list.

Assumptions Used in Derivation of Transport Equation

1. Field strength E is constant.
2. $T = T(x)$ only and $\frac{dT}{dx}$ can be replaced by $\overline{\frac{dT}{dx}}$.
3. Any variance in the direction normal to x-y plane may be neglected.
4. Temperature and concentration dependence of D, U, α_l, β_T and β_C can be ignored.
5. The temperature level T can be replaced by its integrated average value $\overline{T}$.
6. $v = v(x)$ only.
7. $\frac{\partial C}{\partial t} = 0$ (steady state).
8. The column width B is constant in any one section.
9. The vertical diffusion term $D \frac{\partial^2 C}{\partial y^2}$ is small compared to the convective velocity term, $v(x) \frac{\partial C}{\partial y}$
10. Laminar flow conditions exist between the plates.
11. $\frac{\partial C}{\partial y}$ is independent of x (basis of development by FJO).
12. Concentration of mobile component is small (dilute solution).

13. A mean density ρ can be used to convert the volumetric flow rates to mass flow rates.

14. The product $\overline{C_l C_s}$ is not a function of x.

15. $\overline{C}$ - mean concentration at x = 0 is satisfactory in defining y dependence of composition.

16. The term $\frac{\alpha_{ls}\Delta T}{T \cdot 2\omega} << \frac{UE}{D}$ and therefore can be neglected.

Assumptions for the Flow Case

1. $C_s \cong 1$

2. $\sigma_B = \sigma_T = \sigma$

3. $L_{Top} = L_{Bot} = L/2$

4. $B_{Top} = B_{Bottom}$

Equations Used to Compare Theory and Experiment

All equations are based on the assumption that $C_s \cong 1$

1. Steady state separation factor:

$$\frac{C_B}{C_T} = \frac{\left(e^{A\left(1 - \frac{\sigma}{H}\right)} - \frac{\sigma}{H} \right)\left(1 + \frac{\sigma}{H}\right)}{\left(e^{-A\left(1 + \frac{\sigma}{H}\right)} + \frac{\sigma}{H} \right)\left(1 - \frac{\sigma}{H}\right)}$$

2. Batch steady state:

$$\left(\frac{C_B}{C_T} \right)_{\sigma=0} = e^{2A}$$

3. Transient batch separation factor:

$$C_B/C_T =$$

$$\frac{e^{2A} + 2e^{A}(e^{2A} - 1) \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(n^2\pi^2 + A^2)^2} (-1)^n \left[1 + (-1)^{n+1} e^{-A}\right] e^{-\frac{n^2\pi^2 + A^2}{\alpha} t}}{1 + 2(e^{2A} - 1) \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(n^2\pi^2 + A^2)^2} \left[1 + (-1)^{n+1} e^{-A}\right] e^{-\frac{n^2\pi^2 + A^2}{\alpha} t}}$$

APPENDIX F

DISCUSSION OF KIRKWOOD'S THEORY AND SOME REMARKS ON ITS APPLICATION TO COLUMNS WITHOUT RESERVOIRS

APPENDIX F

Discussion of Kirkwood's Theory and Some Remarks on Its Application to Columns Without Reservoirs

Kirkwood and his associates developed a mathematical theory of transport in an electrophoresis column with reservoirs which was valid under certain limiting and ideal conditions. Since the assumed conditions were only approximately realized in his experiment, exact quantitative agreement with the theory was not expected. However, since it yields times of transport and separation factors of correct magnitude, the theory was useful as guide in designing equipment and planning fractionations. Temperature was not taken into account because it was found to be unnecessary.

In developing the theory they made the following assumptions:

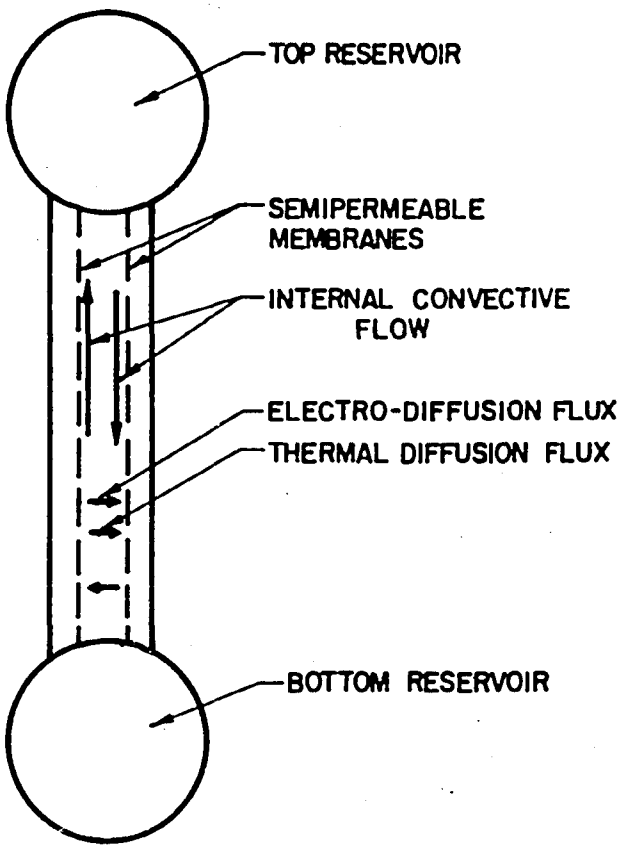
(i) There is no thermodynamic interaction between the mobile components, and they obey ideal diffusion laws.

(ii) The flow in the vertical channel is laminar and the following relations are satisfied:

$$\frac{U_{max}Ea}{D_{min}} << 1$$

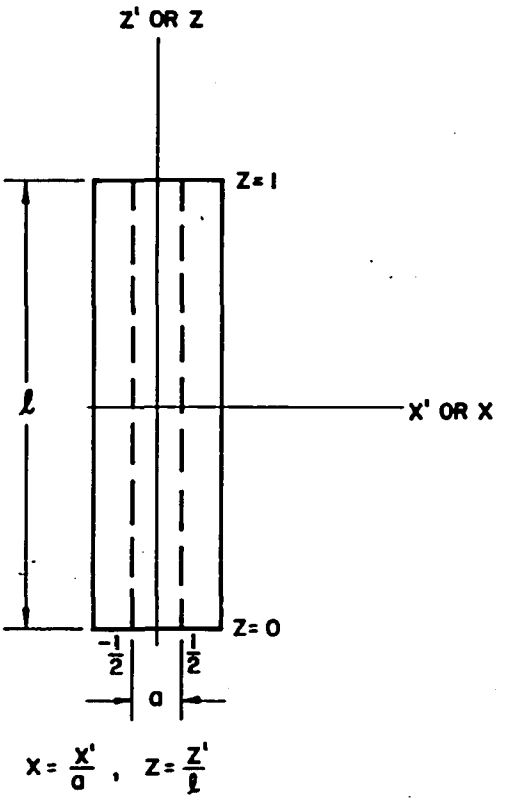
$$\frac{h}{a} << 1$$

$$h = \left(\frac{4\eta \ell D_{max}}{\alpha_{min}\rho_o g C_o} \right)^{\frac{1}{4}}$$



a.

Principle of electrophoresis column with reservoirs



b.

Coordinate system used by Kirkwood

PRINCIPLE OF KIRKWOOD'S APPARATUS AND COORDINATE SYSTEM USED BY HIM FOR MATHEMATICAL ANALYSIS

FIGURE A-2

where a is the channel width, ℓ its length, E the electric field strength, C_o the total protein concentration, η and ρ viscosity and density of solvent, and g the acceleration of gravity. U_{max} is the absolute magnitude of the greatest of the mobilities of the components and D_{min} and D_{max} are the smallest and largest values of the diffusion constants of the components. α_{min} is the least value of $\left(\frac{\partial \rho}{\partial C_K}\right)_{T,P}$ of the components.

Investigation of Transport in the Channel

The system of coordinates and the principles of batch apparatus used by Kirkwood and his associates are shown in Figure A-2. The problem was to investigate the transport of a system of ions at concentrations C_1, C_2, . . . C_K in a conducting solvent by concentration, diffusion and electrical migration in a vertical rectangular channel of width a, length ℓ, connecting two reservoirs of volume V. An electric field E volts/cm. is applied across the membranes.

The general equations of transport as written down by Kirkwood are:

$$\rho \frac{d\vec{U}}{dt} = -\rho g \vec{I}_z - \nabla P + \eta \nabla^2 \vec{v} + \frac{\eta}{3} \nabla \nabla \vec{U} \qquad \text{la}$$

$$\frac{d\rho}{dt} = -\rho \nabla \vec{v} \qquad \text{lb}$$

$$\frac{\partial C_K}{\partial t} = \nabla \cdot \left\{ D_K \nabla C_K - (\vec{U_K} E + \vec{v}) C_K \right\} \quad ; K = 1 \ldots n \qquad \text{lc}$$

where the different symbols mean the following:

v - convective velocity of the solution

ρ - density of solution

η - coefficient of viscosity

D - the diffusion constant

U_K - electrophoretic mobility of the ion constituent K

g - acceleration due tc gravity

I_z - unit vector in the z direction

Equations 1a, 1b and 1c are to be solved subject to the following boundary conditions:

(i) $v = 0$ at the channel walls

(ii) $C_K(U_K\vec{E} + \vec{v}) - D\nabla C_K = 0$ at the channel walls

The edge and end effects will be ignored. The reduced variables are now introduced as follows:

$$x = x/a$$

$$-\tfrac{1}{2} \leq x \leq +\tfrac{1}{2}$$

$$z = z'/\ell \qquad 2$$

$$0 \leq z \leq 1$$

After suitable mathematical manipulation by introducing equations 2 into 1 and assuming that terms involving a^2/ℓ^2 are negligible, the following equations are obtained for the quasi-stationary case $\frac{dU}{dt} = 0$, $\frac{\partial C_K}{\partial t} = 0$:

$$\frac{\partial^3 v}{\partial x^3} - \sum_{K=1}^{n} \frac{\alpha_K \rho_o g a^3}{\eta} \cdot \frac{\partial C_K}{\partial x} = 0 \qquad 3a$$

$$\alpha_K = \frac{1}{\rho}\left(\frac{\partial \rho}{\partial C_K}\right)_{T,P,C_j} ; \quad v = (\pm\tfrac{1}{2}, z) = 0 \qquad 3b$$

$$\frac{\partial}{\partial x}\left(\frac{\partial C_K}{\partial x} - \frac{aU_K E}{D_K} C_K\right) - \frac{a^2 v}{\ell D_K}\frac{\partial C_K}{\partial z} = 0 \qquad K = 1 \ldots U \qquad 3c$$

$$\frac{\partial C_K}{\partial x} - \frac{aU_K E C_K}{D_K} = 0 \qquad x = \pm\tfrac{1}{2} \qquad 3d$$

The horizontal component of convective velocity as well as terms involving vertical diffusion are neglected since they are of the order of a^2/ℓ^2, relative to the terms retained. The solutions are sufficiently dilute, therefore α_K can be considered constant.

Equations 3 are nonlinear but become linear in the first order when v and C_K are expanded in powers of field strength E, in the form:

$$v = \frac{\rho_o g a^3}{\eta} U^{(1)} \lambda(z) \left(\sum_K \frac{\alpha_K C_K^o}{D_K}\right) F(x)\, E + O(E^2) \qquad 5a$$

$$C_K = C_K^o \lambda_K(z) + \frac{U_K a E C_K^o}{D_K} \varphi_K(x,z) + (O(E^2)) \qquad 5b$$

$$U^{(1)} = \sum_{K=1}^{n} g_K U_K \qquad 5c$$

$$g_K(z) = \frac{\left[\dfrac{\alpha_K C_K^o \lambda_K(z)}{D_K}\right]}{\displaystyle\sum_\ell \frac{\alpha_\ell C_\ell^o \lambda_\ell}{D_\ell}} \qquad 5d$$

$$\lambda(z) = \frac{\displaystyle\sum_K \frac{\alpha_K C_K^o \lambda_K}{D_K}}{\displaystyle\sum_K \frac{\alpha_K C_K^o}{D_K}} \qquad 5e$$

where $O(E^2)$ means terms depending upon second and higher powers of field strength E and C_K^o is the initial uniform concentration.

$U^{(1)}$ - mean value of the mobility with respect to distribution function g_K

Differentiating equation 3 with respect to x and then substituting the power series, given by equation 5, into the resulting equation yields:

$$\frac{d^4F}{dx^4} + 4\beta_o^4 F = 0 \qquad 6a$$

$$F(\pm\tfrac{1}{2}) = 0 \left(\frac{d^3F}{dx^3}\right)_{n=\pm\frac{1}{2}} = 1 \qquad 6b$$

$$F(x) = \frac{1}{\beta^3} \cdot \frac{\sin\frac{\beta}{2}\sinh\frac{\beta}{2} - \sin\frac{\beta}{2}\sin\beta x}{\sin\frac{\beta}{2}\cosh\frac{\beta}{2} + \sin\frac{\beta}{2}\cos\frac{\beta}{2}} \qquad 6c$$

$$\beta = (1 + i)\beta_o \qquad 6d$$

$$\beta_o^4 = -\frac{a^4}{h^4} \cdot \frac{d\lambda}{dz} \qquad 6e$$

$$h = \left[\frac{4\eta\ell}{\rho_o g \sum_K \alpha_K C_K^o / D_K}\right]^{\frac{1}{4}} \qquad 6f$$

Use of the power series E, of equation 5 in remaining equation 3, yields after integration of the equation for each C_K from $-\frac{1}{2}$ to x

$$\frac{d\varphi_K}{dx} = \lambda_K(z) - \frac{U^{(1)}}{U_K} \lambda \frac{d\lambda_K}{d\lambda} G(x) \qquad 7a$$

$$G(x) = 4\beta_o^4 \int_{-\frac{1}{2}}^{x} F(x)\, dx \qquad 7b$$

$$G(x) = 1 - \frac{\sin\frac{\beta}{2}\cosh\beta x + \sinh\frac{\beta}{2}\cos\beta x}{\sin\frac{\beta}{2}\cosh\frac{\beta}{2} + \sinh\frac{\beta}{2}\cos\frac{\beta}{2}} \qquad 7c$$

The total current of any component K following in the negative z direction through section of the channel of breadth b is given by:

$$J_K = -ab \int_{-\frac{1}{2}}^{+\frac{1}{2}} C_K v\, dx \qquad 8a$$

and also

$$\int_{-\frac{1}{2}}^{+\frac{1}{2}} v\, dx = 0 \qquad 8b$$

Equation 8b denotes that for batch operation, the net flow through the column is zero.

By partially integrating equation 8a and using relations of equation 5 to 7, one obtains:

$$J_K = -\frac{ab\ell U_K U^{(1)} C_K^o \lambda E^2}{D\left(\frac{d\lambda}{dz}\right)} \int_{-\frac{1}{2}}^{+\frac{1}{2}} G(x)\frac{d\varphi_K}{dx}\, dx + O(E)^2 \qquad 9$$

Evaluation of integral in equation 3 yields the result:

$$J_K = -\frac{ab\ell U^{(1)} C_K^o U_K \lambda E^2}{D_K\left(\frac{d\lambda}{dz}\right)} \left\{\lambda_K(z) - \frac{U^{(1)}}{U_K}\lambda\frac{d\lambda_K}{d\lambda} - \frac{2h}{a}\frac{1}{\left(-\frac{d\lambda}{dz}\right)^{\frac{1}{4}}}\left[K_o\lambda_K - \frac{5}{4}\frac{U^{(1)}}{U_K}K_1\lambda\frac{d\lambda_K}{d\lambda}\right]\right\} \qquad 10a$$

where $$K_o = \frac{\sinh^2 \frac{\beta_o}{2} \cos^2 \frac{\beta_o}{2} + \cosh^2 \frac{\beta_o}{2} \sin^2 \frac{\beta_o}{2}}{\sinh \frac{\beta_o}{2} \cosh \frac{\beta_o}{2} + \sin \frac{\beta_o}{2} \cos \frac{\beta_o}{2}} \qquad 10b$$

$$K_1 = K_o - \frac{2\beta_o}{5} \frac{\sinh \beta_o \sin \beta_o}{[\sinh \beta_o + \sin \beta_o]^2} \qquad 10c$$

Equation 10a is the equation obtained by Kirkwood and associates for the transport of a component in a vertical parallel membrane column in a multicomponent system.

The above can further be simplified by the assumption that functions K_1 and K_o can be approximated by unity if

$$-\frac{d\lambda}{dz} > \left(\frac{h}{a}\right)^4 \qquad 11$$

and therefore:

$$J_K = -\frac{ab\ell U^{(1)} U_K C_K^o E^2}{D_K \left(\frac{d\lambda}{dz}\right)} \left\{\lambda_K(z) - \frac{U^{(1)}}{U_K} \lambda \frac{d\lambda_K}{d\lambda} - \frac{2h}{a} \frac{1}{\left(-\frac{d\lambda}{dz}\right)^{\frac{1}{4}}} \left[\lambda_K - \frac{5}{4} \frac{U^{(1)}}{U_K} \lambda \frac{d\lambda_K}{d\lambda}\right]\right\} \qquad 12$$

After this, Kirkwood and associates proceed with application of the above equation to the case of electrophoresis column with reservoirs. The original paper may be consulted for further details of this development.

At present the application of the above equation to the case of columns without reservoirs is of special interest and will therefore now be considered.

When both sides of equation 12 are summed over all ion constituents, the initial term independent of h/a vanish and we have:

$$\dot{J} = \frac{2hb\ell U^{(1)2}\lambda^2 E^2}{\left(-\frac{d\lambda}{dz}\right)^{5/4}} \sum_{K=1}^{m} \frac{\alpha_K C_K^o}{D_K} \qquad 13a$$

$$\frac{d\lambda}{dz} = -\left(\frac{U^{(1)}}{U_o^{(1)}}\right)^{8/5} \frac{\lambda^{8/5}}{L} \qquad 13b$$

where $$L = \left[\frac{hb\ell U_o^{(1)2} E^2}{2J} \sum_{K=1}^{m} \frac{\alpha_K C_K^o}{D_K}\right]^{4/5} \qquad 13c$$

The above equation was derived with the assumption of quasistationary state in which the velocity and concentrations were assumed to be functions only of vertical and horizontal position and did not change with time in the vertical channel connecting the two reservoirs. This transport was then subjected to a boundary condition allowing a slow accumulation or depletion of material in the reservoirs. The solution of the above equation with this boundary condition (which change slowly with time) described the transport of a mobile component from the top to the bottom reservoir. The assumption of quasistationary state therefore implies that at the ends of the channel, the concentrations must be variant and the net flux down the column must be of finite magnitude. The assumption of stationary state results in an invariant concentration at the ends and requires a constant flux down

the column. However, the net flux (total current $\dot{J}$) at the ends of the column must be zero at steady state for columns without reservoirs, and since in this condition J_K are independent of z, the net flux everywhere becomes zero. Kirkwood's above results cannot accommodate such a boundary condition since if $J = 0$, then $\lambda = 0$, which means no separation would result. Since this is meaningless, it is concluded that for the batch steady state as well as transient case Kirkwood's theory is inapplicable for the case of continuous flow operation of the column.

In case of flow case, the currents J_K are of finite values of say σC_K. In this case, the equation appears to be valid for $\sigma > 0$. However, it would not be general description of the electrophoresis process since the flow case should yield the expression for batch steady state as the external flow approaches zero and it is established above that at this condition, Kirkwood's equations cannot be solved.

It is therefore concluded that the Kirkwood's procedure of linearization of the equations, based on the assumption of quasistationary state cannot be used to describe a batch or continuous-flow electrophoresis column without reservoirs since in both cases it is impossible to satisfy the condition that $J = 0$ at both ends of the column and still give a valid solution of the equation.

Therefore, only remaining way is to solve the nonlinearized original partial differential equations numerically. This procedure, however, is tedious and must

therefore yield to some other simpler procedure. Such a procedure is that of Furry, Jones and Onsager, which was utilized to describe the electrophoresis column after simplifying the system under study by making certain assumptions. This development has been discussed in detail in Chapter III.

APPENDIX G

SUPPLEMENTARY REVIEW ON ELECTROPHORESIS

APPENDIX G

General Review of Electrophoresis

In Chapter II of this thesis a review of the literature pertaining to "preparative electrophoresis" as applied to large scale preparation has been given. However, electrophoresis has been mostly used extensively for small scale preparation, for analytical and characterization purposes in many different ways. The literature in this field is enormous and its total review as a part of this thesis is not considered appropriate. However, a short summary is given below as a handy reference with this thesis to give a general idea of the work which is done or is being done in this vast field of electrophoresis. Reviews and testbooks on some or all aspects of electrophoresis are readily available for detailed information (1,2,3,4,5).

Processes going on between the electrodes due to electrical force fields are termed either Electrophoresis or Ionophoresis. Ever since their discovery by Reuss (5) in 1808, they have been used in several fields of study. Ionophoresis relates to Crystalloids while in Electrophoresis one has either colloids or microscopic particles under observation. The principle of both of these is, however, the same. Electrophoresis involves the migration of charged (positively or negatively) particles or molecules in solution towards electrodes bearing opposite charges when direct current potential field is applied across the solution. The

particles move not only in different direction but at different velocities depending upon the values of their electrical mobilities in the given medium. The usual objectives of Electrophoresis experiments are: (i) to obtain information on the electrical double layers surrounding the charged particles, (ii) the analysis and characterization of mixtures or their separation of constituents from their solution.

The electrical mobility of an ion or charged particle is a function of the viscosity of the medium, and size, shape and concentration of the particles or ions in solution, which play the usual hydrodynamic role, the pH and concentration of the electrolyte. Therefore, in essence electrophoretic separation depends upon the difference in rate and direction of migration of different components. The electrophoretic method is potentially very elegant and efficient method of separation since it can separate substances which are either chemically similar or too mobile to be separated by any other method. Widespread application of Electrophoresis as an analytical as well as separational tool covers such diverse fields as Biology, Biochemistry, Pathology and Physical Chemistry. Outstanding contributions have been made in the analysis and characterization of fluids as blood sera, plasma, hemoglobin, egg and milk components and several types of tissue components. Experimental observations are very often reported.

The electrophoretic methods described in literature

are generally classified into the following general groups:

(a) Moving Boundary Electrophoresis.

(b) Paper electrophoresis.

(c) Zone electrophoresis with or without supporting media.

(d) Preparative Electrophoresis.

Moving Boundary Electrophoresis

It consists of forming a boundary between the macromolecular solution to be studied and a suitable buffer solution. Once the sharp boundary has been formed, an electric field is applied and if the solution contains a number of different molecular species, the originally sharp boundary will spread and finally split into a number of distinct boundaries moving with velocities characteristic of the different types of boundaries. The movement of boundaries is followed by a suitable optional technique (8,13,7). This method allows determination of mobilities, the identification of the constituents and under certain conditions separation. This method suffers from the fact that complete separation is never achieved.

Microscopic Method

An excellent review of the method and its application are given by Brinton and Lauffer (11). The method consists in direct observation of microscopically visible particles as they migrate under the influence of an electric field. The particles are suspended in a suitable solution and placed

in a flat or cylindrical transparent cell through which direct current is passed. This enables study and characterization of the particles of microscopic size like bacteria, erythrocytes, viruses and colloids. This method could not of course be employed for preparative purposes. Numerous papers have been published on the use of this method and the reader is again referred to the above mentioned references for details.

Analytical Method

After Hittorf (9,12) first observed concentration changes on passage of a current in a solution, this method has been subsequently used to advantage in many problems. This method although useful in some special applications (9) is not useful for preparative purposes.

Even before adaption of the electrophoresis method for analysis by Tiselius (6) there were numerous attempts to utilize it for small scale preparative purposes. Concurrent attempts at preparative electrophoresis by compartment type apparatus from 1912 to 1940s have been reviewed by Swensson (10). The period which followed has witnessed a collosal development of different types of electrophoretic apparatus. The existing apparatus can be classified as follows:
(1) Free Electrophoresis (2) Moving Boundary.

The Moving Boundary Method

If concentration gradients are present initially in the liquid portion of the circuit, these are generally

affected by the passage of the direct current. This is the moving boundary method for the study of electrophoretic behavior of the proteins. Separations are carried out in a U-shaped channel and because of migration of proteins density gradients are formed in the solution medium. Different proteins concentrate in different regions because of which the density and refractive index of the regions of the solution change. These are observed by optical methods such as Achlieren. Convective circulation is avoided by creating density gradients in such a manner that lighter solution is on the top of the denser portion of the solution at each boundary. This stabilization of the boundary system with respect to gravity by density increments is characteristic of moving boundary method. It is also sometimes referred to as free electrophoresis or Tiselius method. Complete separations are not generally possible. Slowest and faster moving proteins separate from each other but others form overlapping boundaries.

The technique of moving boundary method has been fully described by Alberty (15). This method has been identified with Tiselius (16,17) but many other workers have contributed to its development and improvement (18). Few attempts were made to employ this method for preparative purposes. Its use for analytical work is however standardized one and is invariably made use of in determining concentrations of amino acids, protein constituents and their electric mobilities.

Zone Electrophoresis

The technique of causing accumulation of various components in different regions of solution space is called zone electrophoresis. A variety of method has been developed to complete separation of proteins into sharp zones. This is achieved by preconditioning the supporting medium in various ways. Object is to obtain gravitational stability of the zones. The first method involves creation of a gravitationally stable density gradient in a vertical direction by appropriate admixture of the buffer solution and the same liquid admixed with a non-electrolyte added to increase the density. Convection is thus avoided by having a negative gradient (22-24). The density increments of the solutions to be separated are now undesirable. As the separation proceeds, no positive density gradients should develop. The method requires small amounts of samples for analysis. Another method is to superimpose a pH gradient on the density gradient prior to electrophoresis as suggested by Kolin (25). One then obtains what are termed isoelectric spectra. A protein concentrates on migration at the level in solution at which it is isoelectric. Thus proteins of different isoelectric points concentrate at different levels and concentration accompanies separation.

Yet another method of zone electrophoresis is to superimpose a conductivity gradient on the density gradient and to obtain mobility spectra of Kolin.

In another type of zone electrophoresis the medium

is stabilized against convective mixing by using solid packing instead of gravity. Several materials such as glass spheres, foam rubber, paper, starch, agar, gelatin, etc. have been used as packing materials. This method also serves as an analytical tool.

Free electrophoresis has also been adapted for recovery of pure proteins in small amounts. The amount of pure material that can be recovered is limited, however. An improvement is obtained by using movement of the boundaries against the flow of solution in the opposite direction. The boundary is given an apparent velocity by injection or by withdrawal. This is termed as counter-current electrophoresis. It has been used in the resolution of proteins. Tiselius' apparatus has been modified for continuous removal of a fraction as it is separated electrophoretically. The method is not, however, useful for large scale operation.

Paper Electrophoresis

Filter paper electrophoresis is described in detail in a number of books and manuals (26). The first indication of using "paper" as a supporting medium was given by Konig in 1939 (24). The paper electrophoresis has been used in very many forms of techniques. Noteworthy contributions have come from symporium Ulrecht (28). Antonini and Piva (29), Wunderley (30), Fischer (31), Kirk and Duggan (32), Durram (27,33,34). The various techniques, however, differ only in minor details.

Paper electrophoresis has also been adopted for preparative separations. A number of continuous flow apparatus have been developed and differ only in minor details (30,38). In essence, the protein mixture is made to flow within a narrow streak down a vertical or inclined sheet of filter paper with a buffer solution of appropriate pH as background electrolyte. Under the action of transverse electric field different protein fractions spread out into individual strips. The separated components drip from the edge of the paper which is serrated specially for the purpose. The capacity of paper electrophoresis is very low and therefore it is unsuitable for large scale preparation. The importance of paper electrophoresis can be easily judged from the enormous amount of work in that field. C. H. Wunderley has listed 239 references (39). Paper electrophoresis carried out carefully and with automatic technique is capable of giving reliable reproduction of the results.

Electrophoresis with Supporting Media

Investigations concerned with the development of new supporting media for electrophoresis separation continues unabated. This development has been stimulated by an effort to avoid limitations of filter paper electrophoresis and to obtain large scale separation. Several methods are used to suppress convection. These include tubes or columns packed with asbestos fiber, glass powder, grannular starch, filter

paper, cellulose and polyvinyl particles. Methods for the use of supporting media are as follows:

(1) Open block or trough system

(2) Horizontal half cylinder method

(3) Vertical column electrophoresis

(4) Segmented sponge electrophoresis

(5) Gel electrophoresis

Open Block or Trough Method

This is the commonest method and has been used mainly for rapid analysis of small samples. The method consists simply of packed material on a glass or plexiglass plate across which D.C. electric field can be applied. Various ways of preparation have been suggested (40-42). The limitations of this method are numerous. Separation depends upon the experience and technique of the investigator. Simplicity however has resulted in its wide application.

Horizontal Half Cylinder Method

This is very simple and requires little special equipment (43-44). Two curved cylindrical troughs are used. It can be adapted to preparative scale but is useful only for small scale analysis. This method has been utilized for the determination of isoelectric points conveniently.

Vertical Column Electrophoresis

Several researchers have described electrophoresis methods using vertical columns (45) and treated this subject

theoretically. The most widely used procedure is that suggested by Porath (46,48) and Flodin and Kupke (47). The usual columns are 105 cm. in length and 3.4 cm. wide. But considerably large types have been used. Use of such columns indicates probability of large scale preparative electrophoresis but heating effects producing asymetrical effects represent a serious limitation. Porath has most recently studied temperature distribution within cylindrical electrophoresis column of large capacities to explore the possibility of large scale operation (48). The greatest advantage of column procedure is the ready recovery of material after electrophoretic separation. In this separation it resembles column chromatography. Despite the limitations and relative complexity of the apparatus required, this column electrophoresis promises a great many applications.

Continuous Flow Method

The application of this principle to continuous electrophoresis was done by Durrum et al. (49) and received widest application. A number of modifications are used (50-51). Other supporting media have been applied by other workers such as Brattsten (53,54) whose apparatus is complex but he has been able to remove most of the pitfalls of continuous flow in electrophoresis. A simplified apparatus has been described by Bradish and Smart (55) which eliminates multiple inflow tubes. Brattsten's apparatus has the combined advantage of capacity and separating power.

Segmented Sponge Method

Application of this method which uses sheets of foam rubber (56) as the supporting medium is very difficult from point of operation. Main advantage is the case of obtaining samples by simply squeezing out the sponge segments following electrophoretic separation. Convection is a problem because of large pore size.

Gel Electrophoresis

Many methods of electrophoretic separation in gels have been reported (57,58). They differ essentially in the type of gel used, concentration of the gel and the presence or absence of filler substances. Gels used by different workers are of four types.

Simple Agar

This method is primarily analytical but isolation of separated fractions is possible. Thus Faure and his associates (59) were able to recover various types of hemaglutinins. A procedure for preparative electrophoresis has been described by Kawarau (60) who employed a semi-continuous system. Fluid Agar has been used by Ressler and co-workers (61) while Bernfeld and Nisselbaum (62) used starch gels in low concentration for isolation of relatively large amounts of serum proteins. Smithies and Poulik (63) have used concentrated starch gels for separation of serum proteins but much more work needs to be done to see its applicability to large scale isolation work.

Most of the equipment described above is a batch type or low flow apparatus. The main objective is the preparation of small amount of pure sample for analysis and characterization. The main feature of all this apparatus is that an attempt is done to eliminate convection.

APPENDIX H

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