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Part II. Steady State Operation of Continuous Flow Column: Theory and Experiment

In Part I of this paper, a thermoelectrogravitational electrophoresis column without reservoirs was proposed for preparative electrophoretic separations. The Furry, Jones, and Onsager procedure in thermal diffusion was applied to develop a transport equation, and its solution for the steady state batch case was employed to demonstrate some typical effects of temperature difference, electric field strength, and membrane spacing on the predicted steady state batch separation in the column.

In this part, a theory is first developed from mathematical analysis of a continuous-flow thermoelectrogravitational column without reservoirs by modification of the transport equation to take into account the bulk flow through the column.

An experimental center-fed thermoelectrogravitational electrophoresis column and the related equipment used to obtain experimental data to test the theory are described. Further, experimental separation factors as a function of flow rates ranging from 0 to 10 g/min for the bovine albumin system at two *p*H values (8.6 and 6.0) were obtained using two membrane spacings (0.1354 and 0.3018 cm respectively) at four different electric field strengths (0.0423 to 0.423 volt/cm) and for three different temperature differences of 0°C, 8.5°C, and 16°C. Experimental data indicated that meaningful separations could be obtained using thermoelectrogravitational columns but that the temperature difference has an adverse effect on electrophoretic separation.

Analysis of the experimental flow data showed that theory and experiment are not in quantitative agreement. However, there was general, qualitative agreement between theory and experiment for the dependence of separation on field strength, temperature difference, membrane spacing and mobility of the component.

In Part I, a qualitative description of the proposed thermoelectrogravitational electrophoresis column was presented and a mathematical theory was developed for its analysis using the Furry, Jones, and Onsager procedure for thermal diffusion.

The purpose of Part II is to extend the application of the transport equation developed in Part I to the continuous-flow operation of a thermoelectrogravitational column, to describe in detail the construction of such a column to obtain laboratory experimental data, and to present the experimental results and compare them with the theory.

THEORY

Figure 1 illustrates an experimental continuous flow thermoelectrogravitational electrophoresis column. The transport equation derived by consideration of the steady state batch operation was (for the bottom section)

$$\tau_B = H_B C - K_B \frac{dC}{dy} \tag{1}$$

This equation must be modified to account for the flow through the column resulting from continuous withdrawal

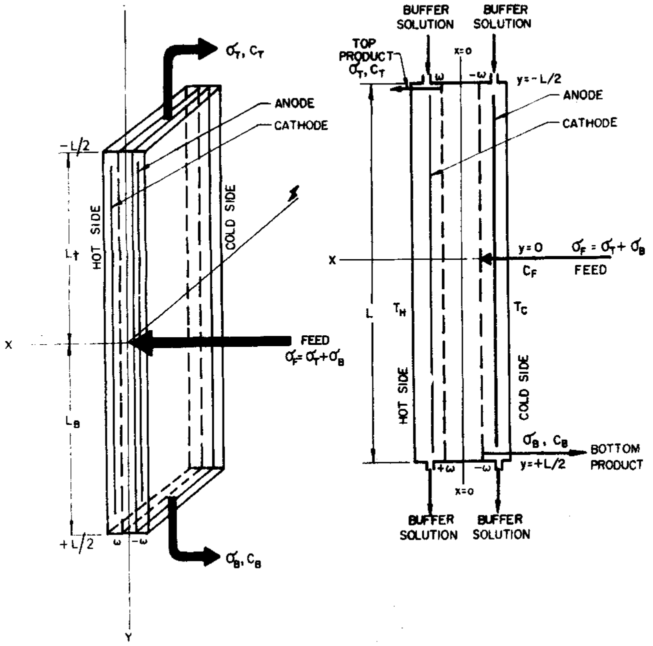

Fig. 1. Coordinate system for continuous-flow electrophoresis column.

of the product. In thermal diffusion, Jones and Furry (1946) have suggested that an additional linear transport may be impressed in the y direction in order to account for this product removal. Because of the assumption that the velocity of the solution is independent of y, the values of transport coefficients H and K in Equation (1) above are applicable to the batch velocity profile only if the net removal of product from the column is small compared to internal convective flow (Jones and Furry, 1946; Furry et al., 1939; Powers, 1954). With this stipulation, when a linear transport τ_Bg/min is impressed in the y direction on the enriching (bottom) section of the electrophoresis column, one obtains the modified transport equation for the continuous-flow case as follows (coordinate system for continuous-flow case is given in Figure 1):

$$\tau_B = H_B C - K_B \frac{dC}{dy} + \sigma_B C \tag{2}$$

Since the total amount removed from the bottom of the column must be the total net transport in the bottom half of the column, Equation (2) can be simplified by putting $\sigma_B C_B$ for τ_B in the case of steady state continuous-flow:

$$\sigma_B (C_B - C) = H_B C - K_B \frac{dC}{dy} \tag{3}$$

where σ_B is the net withdrawal of product from the bottom section per unit time. Similar reasoning applied to the top section of the column yields

$$-\sigma_T (C_T - C) = H_T C - K_T \frac{dC}{dy} \tag{4}$$

where C_T is the concentration of the mobile component in the stream leaving the top section of the column.

STEADY STATE SOLUTION FOR THE FLOW CASE

The solution of the transport Equations (4) and (5) will yield the theory of the steady state continuous-flow column. The solution of Equation (5) can be obtained for the top section subject to the boundary condition $C = C_M$ at $y = 0$ (see Figure 1 for coordinates):

$$C = -\frac{\sigma_T C_T}{H_T - \sigma_T} + \left(C_M + \frac{\sigma_T C_T}{H_T - \sigma_T}\right) e^{\frac{H_T - \sigma_T}{K_T} y} \tag{5}$$

The expression relating C_T, the concentration at the top of the column, $y = -L_T$ with C_M, the concentration at $y = 0$, is

$$\frac{C_M}{C_T} = \frac{e^{\frac{H_T L_T}{K_T}\left(1 - \frac{\sigma_T}{H_T}\right)} - \frac{\sigma_T}{H_T}}{1 - \frac{\sigma_T}{H_T}} \tag{6}$$

An analogous treatment for the bottom section yields

$$\frac{C_M}{C_B} = \frac{e^{\frac{-H_B L_B}{K_B}\left(1 + \frac{\sigma_B}{H_B}\right)} + \frac{\sigma_B}{H_B}}{1 + \frac{\sigma_B}{H_B}} \tag{7}$$

Equations (6) and (7) are applicable to a flow column with the feed point located anywhere between the top and bottom ends of the column. However, in the experimental work, a center-fed column was employed to separate the mobile component from a dilute solution.

Flow rate, length, and parameters are the same for both top and bottom sections, hence $\sigma_T = \sigma_B = \sigma$, $H_T = H_B = H$, $K_T = K_B = K$, $L_T = L_B = L/2$. And after defining $A = HL/2K$, Equations (6) and (7) give

$$C_M/C_T = \frac{e^{A(1-\sigma/H)} - \sigma/H}{1 - \sigma/H} \tag{8}$$

$$C_M/C_B = \frac{e^{-A(1+\sigma/H)} + \sigma/H}{1 + \sigma/H} \tag{9}$$

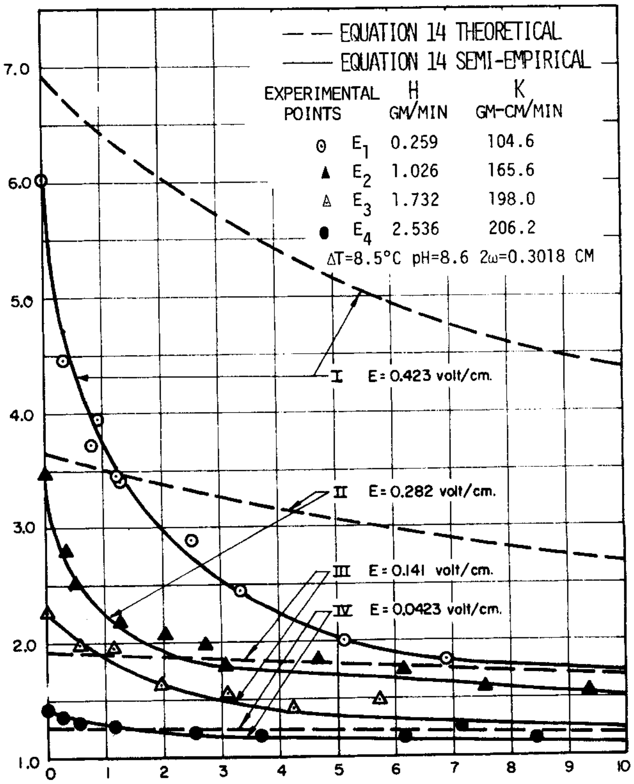

Fig. 2. Theoretical, semiempirical, and experimental separation factors as a function of flow rate σ at four different field strengths.

It is convenient to work with the ratio of the concentrations at the bottom and top and the separation factor q, defined as the ratio of C_B to C_T, which is obtained by dividing Equation (9) into Equation (8):

$$q = C_B/C_T = \frac{(e^{A(1-\sigma/H)} - \sigma/H)(1 + \sigma/H)}{(e^{-A(1+\sigma/H)} + \sigma/H)(1 - \sigma/H)} \quad (10)$$

In the present investigation, Equation (10) will be the most useful form for describing steady state separations obtained in a center-fed continuous-flow thermoelectrogravitational electrophoresis column at low flow rates. The experimental apparatus and procedure have been prepared as a supplement and are on file from the editor.

RESULTS

In experiments with the electrophoresis column, the separations (and separation factors) were determined as a function of flow rate with the membrane spacing 2ω, field strength E, electric mobility U, and temperature difference ΔT as parameters. Changes in E and ΔT could be made very easily but column length was not varied. Mobility of the mobile component was changed by preparing its solution in a boric acid-borax buffer (pH 8.6) used in most of the experiments and a phosphate buffer (pH 6.0).

Flow data were obtained at four different field strengths ranging from 0.0423 to 0.423 volt/cm and for three temperature differences of 0°, 8.5°, and 16°C with two membrane spacings of 0.3018 and 0.1354 cm respectively. Experimental flow rates ranged from 0 to 10 g/min. of 0.956 g/100 ml albumin solution. A complete tabulation of the electrophoresis flow data is given in Prabhudesai (1965).

DISCUSSION OF RESULTS

Figure 2 presents typical steady state separation factors as a function of flow rate, taken at four different values of field strength but constant temperature difference, membrane spacing, and solution pH. The quantitative agreement between separation factors calculated theoretically using Equation (10) (dashed curves) and those determined experimentally (solid curves) is less than satisfactory. However, there appears to be qualitative agreement in the similar shape of the theoretical and experimental curves. Therefore, it seems possible to correlate the experimental data by introducing empirical column transport coefficients H and K following procedures (Powers, 1954; Powers and Wilke, 1957; Boyer, 1961) for thermal diffusion columns.

METHOD OF COMPARISON

Since dependence of separation upon flow rate is important for application of the theory and because there is only qualitative correspondence between theory and experiment, values of column transport coefficients H and K were determined empirically such that separation factors calculated using Equation (10) agreed closely with experimental data over the range of flow rates studied, implying that H and K are independent of flow rate.

A modified form of the procedure used by Powers (1954, 1957) in thermal diffusion was used to obtain empirical values of H and K. Equation (10) was used to obtain H and K for a particular set of separation factors versus flow rate data. The value of $A = HL/2K$ was calculated from the steady state batch separation obtained at zero flow rate. Using A obtained in this manner, H was determined for each run in the set by a trial and error procedure such that the observed separation at a given flow rate agreed closely with that calculated by Equation (10) using the assumed value of H. To obtain the resulting empirical H value for the entire set, an arithmetic average of H values corresponding to all the flow rates in that set was employed. The value of column transport coefficient K was then calculated from the relation:

$$K = HL/2A \quad (11)$$

The experimental separation factor-flow rate data were successfully represented by single curves based on H and K values empirically determined as described above for each set of flow rate data. Figure 2 indicates the closeness of fit obtained. The theory predicts the effect of the operating parameters on column operation and these empirical column transport coefficients can be used to check these predictions.

The shape of the semiempirical curves obtained and the excellent fit 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 12 g/min. A comparison of H and K values obtained at different flow rates with other parameters kept constant showed that H and K were independent of flow rate within experimental error. This is in agreement with the theory which predicts no dependence of H and K on flow rate, and this justifies the use of H and K calculated on the basis of batch velocity profiles. This supports the use of the transport equation approach for mathematical analysis of electrophoresis columns.

FUNCTIONAL DEPENDENCE OF EMPIRICAL TRANSPORT COEFFICIENTS *H* AND *K*

Effect of ΔT and E on H and K

Examination of the empirical H and K values for their functional dependence on the parameters, E ΔT, 2ω and U provides further checks on the applicability of the theory which also predicts the functional dependence of H and K on various operating parameters.

The column transport coefficient H is a sum of two terms H_{TE} and H_E whose functional dependence on E and

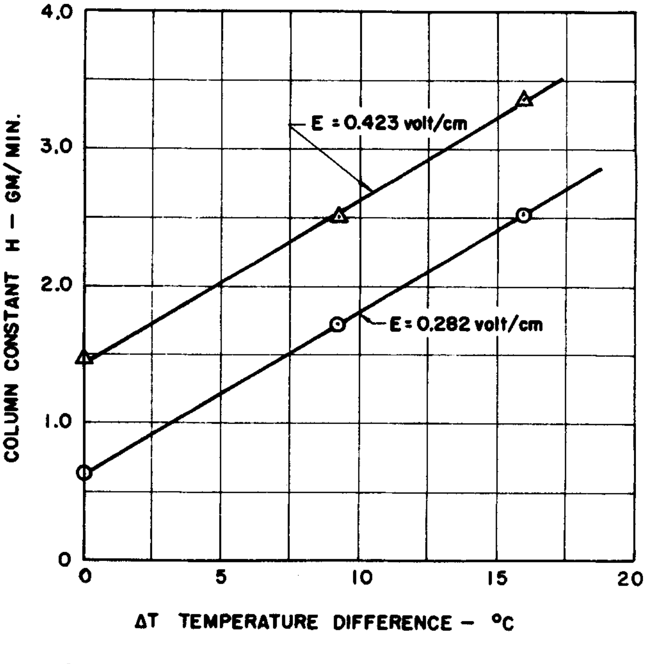

Fig. 3. Dependence of total H on temperature difference.

ΔT with other parameters constant is given by

$$H_{TE} = a\Delta TE, \quad H_E = bE^2 \qquad (12)$$

and H_E is the value of H at zero ΔT.

Figure 3 shows H to be a linear function of ΔT for two constant field strengths and constant 2ω and U as predicted by Equation (12). Figure 5 shows that H is proportional to E at constant ΔT, and Figures 4 and 5 show that H_E, (the value of H at $\Delta T = 0$), is proportional to E^2 as suggested by Equation (16). Hence, the empirical H and H_E values seem to satisfy the functional dependence predicted by the theory.

The functional form of the equation for K is rather complicated, consisting of six terms as shown in Part I. By neglecting the terms containing heat generation, parasitic remixing, and the vertical diffusion term K_d, the total K is given by the equation:

$$K = K_T + K_{TE} + K_E \qquad (13)$$

The functional dependence of K_T, K_{TE} and K_E on ΔT and E, with other parameters constant can be expressed as

$$K_T = a\Delta T^2; \quad K_{TE} = b\ TE \quad \text{and} \quad K_E = cE^2 \qquad (14)$$

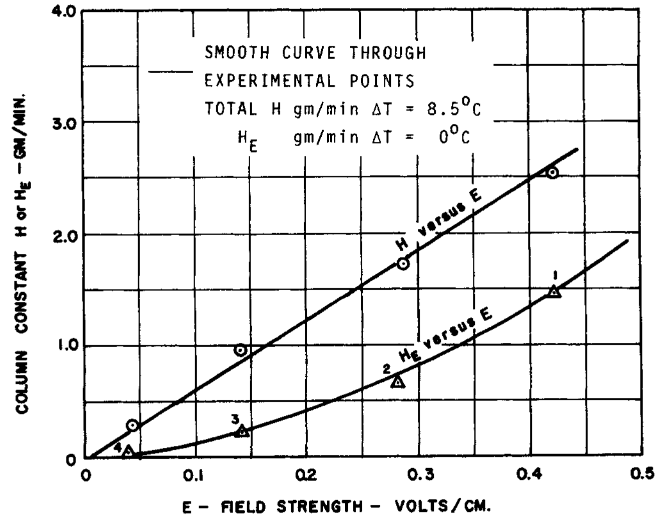

Fig. 4. H or H_E as a function of field strength E.

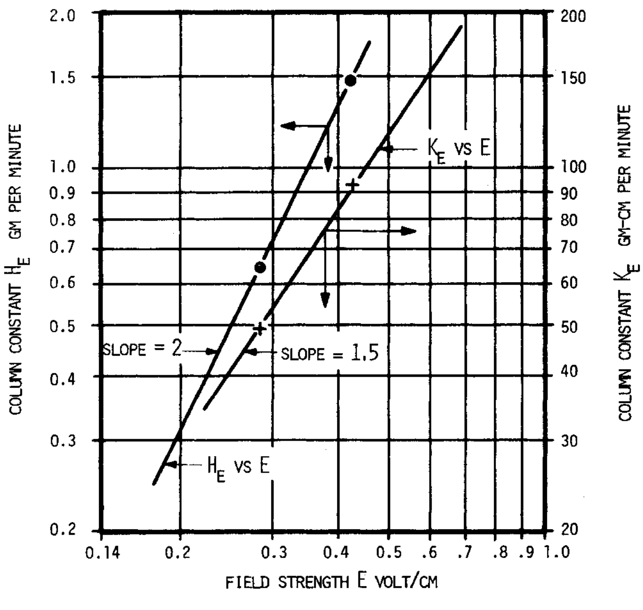

Fig. 5. Column constants H_E and K_E as a function of field strength E.

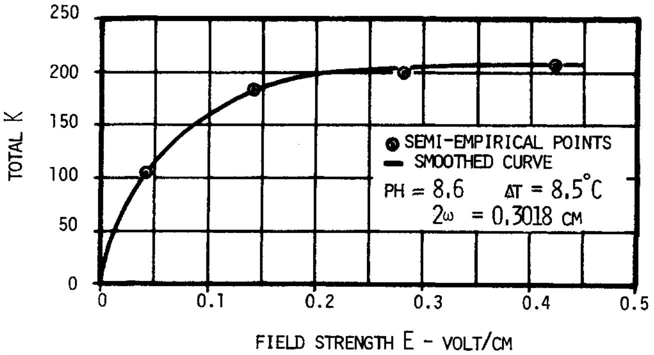

Fig. 6a. Total K as a function of field strength E.

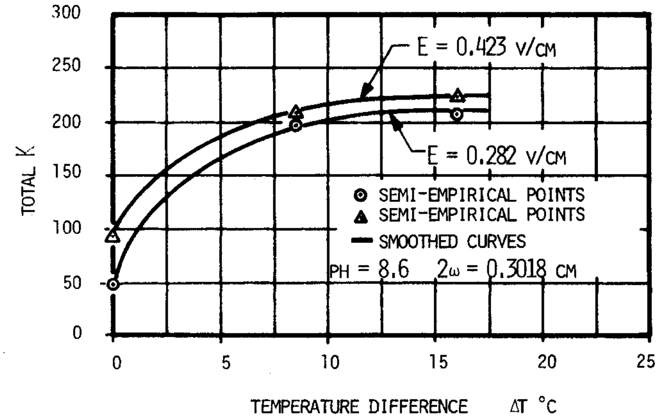

Fig. 6b. Total K as a function of temperature difference ΔT.

and K_E is the value of K at zero ΔT.

The logarithmic plot of K_E versus E (Figure 5) does not yield a straight line of slope 2 indicating that functional form of K_E as given by Equation (18) is not satisfied. In Figures 6a and 6b empirical K values are plotted as a function of E at constant ΔT, and as a function of ΔT at constant E. It is seen that K appears to reach a limiting value, which is contrary to theory. A similar discrepancy for separations in thermal diffusion columns has been reported (Powers, 1954; Powers and Wilke, 1957; Boyer, 1961).

EFFECT OF MEMBRANE SPACING ON EMPIRICAL COLUMN CONSTANTS *H* AND *K*

The column constants have the following functional forms when all parameters other than membrane spacing are the same.

$$H_{TE} = a(2\omega)^4; \quad H_E = b(2\omega)^5$$

$$K_T = c(2\omega)^7 \ K_{TE} = d(2\omega)^8 \text{ and } K_E = e(2\omega)^9 \qquad (15)$$

These suggest a strong dependence of these parameters on the membrane spacing. H_E consists of the term 2ω raised to fifth power while K_E contains the 2ω term raised to the ninth power. An examination of the empirical values of the column constants, however, shows that these do not conform to the functionality of their expressions. As an example, values of H_E (H at $\Delta T = 0$) are inversely proportional to 2ω and K_E (K at $\Delta T = 0$) are proportional to $2\omega^{-0.75}$ (Figure 7). Similar discrepancies between theory and experiment at large spacings have been observed in the case of thermal diffusion columns with large plate spacings (Furry et al., 1939; Powers, 1954).

DISCUSSION OF EXPERIMENTAL SEPARATION FACTORS

Effect of Field Strength

Separation factors as a function of flow rate at various field strengths and at a temperature difference of 8.5°C have been presented in Figure 8. Theory predicts higher separation factors with an increase in E at constant ΔT. The separation factors presented in Figure 2 satisfy qualitatively this expected dependence on field strength E.

The theory is at variance with the experiments for steady state batch separations at $\Delta T = 0$. Both H_E and K_E are functions of E^2. Therefore, for constant parameters other than E the steady state 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 in Figure 7 which shows the separation factors at $\sigma = 0$ are not the same. This may be explained by the fact that the empirical H values (that is, H_E when $\Delta T = 0$) reaches a limiting value. Thus, an increase in H accompanied by no increase in K would give higher batch separation factors with an increase in E.

EFFECT OF TEMPERATURE DIFFERENCE

The separation factors as a function of flow rate with ΔT as a parameter at constant E, 2ω, and pH are presented in Figure 9. The formal theory predicts that an increase in ΔT should cause a decrease in the separation factors. The data show that except at $\Delta T = 0$ the separation factors at low flow rates seem to increase with increase in ΔT. *This* discrepancy for the dependence of separation factors on ΔT at high ΔT levels can be explained on the basis of the behavior of semiempirical column constants H and K. Since H is proportional to ΔT while K approaches a constant value as ΔT increases, the value of $A = HL/2K$ increases, resulting in increased separation factors. The experimental separation factors at $\Delta T = 0$ and at low flow rates are greater than those obtained at other temperature differences for the same E value as can be seen by comparing the separation factors presented in Figure 9. This observation is in accordance with the formal theory which predicts an increase in separation factors at ΔT decreases.

At high flow rates, this behavior of separation factors does not hold. There is an overlapping for all the temperature differences and the separation factors at $\Delta T = 0$°C become lower than those at 16°C.

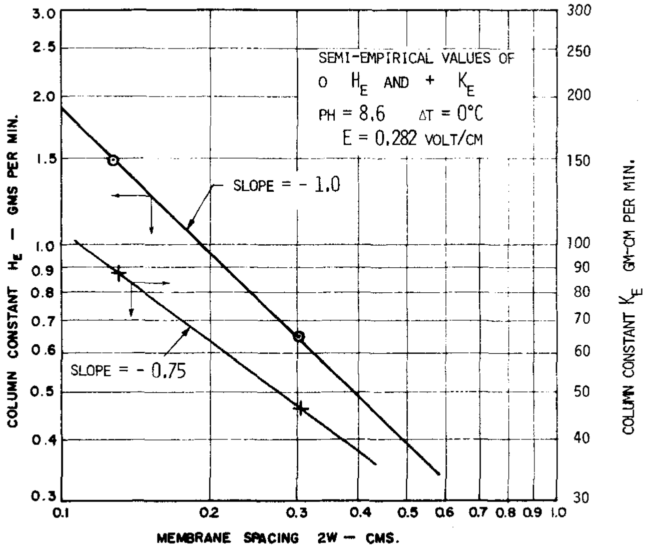

Fig. 7. Column constants H_E and K_E as a function of membrane spacing.

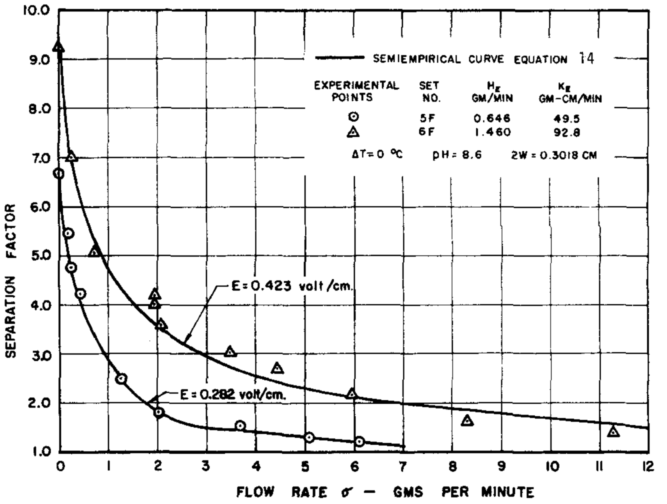

Fig. 8. Comparison of empirical and experimental separation factors at $\Delta T = 0$ for two values of field strength.

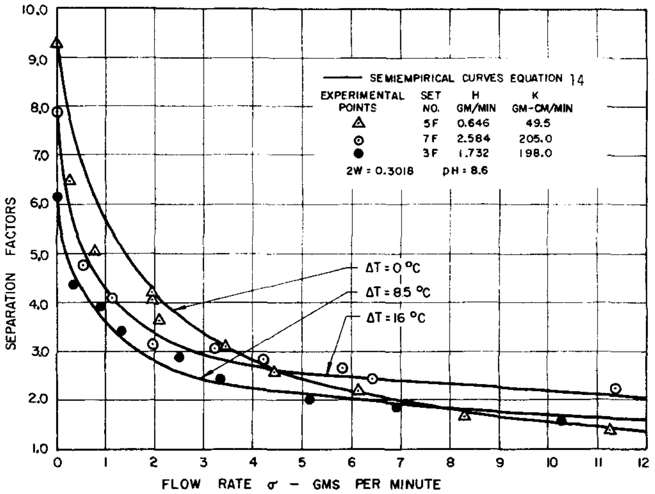

Fig. 9. Separation factors as a function of flow rates with ΔT as parameter at E — 0.423 volt/cm.

In the present work, only small temperature differences could be used and the contribution to either H or K due to ΔT was small. Hence, the effect of ΔT on the separations was relatively small when compared to that of E.

EFFECT OF MEMBRANE SPACING

Separation factors as a function of membrane spacing at constant E, (0.282 volt/cm), ΔT (0°C) and pH = 8.6 are presented in Figure 9. Formal theory indicates a tremendous influence of membrane spacing (2ω) on separations in the electrophoresis column. However, the empirical column constants were found not to be so strong a function of spacing. The separation factors decrease with increase in membrane spacing in accordance with both formal and empirical theories as shown in Figure 10.

The disagreement between theory and experiment for the dependence of separation factors on membrane spacing is unlikely to be caused by an error in the spacing measurement. The membranes cannot bulge because of the positive pressure which forces them against their Flexolith supports, and the experimental results were consistent after two replacements of the membranes which required complete reassembly of the column. The spacing measurement would have to be in error by a factor of 100% to account for the disagreement observed, and it is believed that the technique of measuring spacing is much more accurate than that.

It is concluded that at large membrane spacings, the theory does not correctly predict separation factors as a function of membrane spacing. A similar disagreement with respect to plate spacing also exists between theory and experiment in the case of thermal diffusion (Powers, 1954; Powers and Wilke, 1957; Boyer, 1961).

EFFECT OF SOLUTION *p*H

The electric mobility is a function of solution *p*H and Figure 10 presents the separation factor data at a *p*H of 8.6 and a *p*H of 6.0. The electric mobilities of bovine albumin are -6.3×10^{-5} and -2.2×10^{-5} cm/volt/s respectively. The theory predicts smaller separation factors at lower mobilities as shown in Figure 11.

SUMMARY

In Part II, the transport equation developed in Part I is extended to the continuous-flow case of the thermoelectrogravitational column without reservoirs. A laboratory electrophoresis column was used to obtain experimental data on separation factors for the bovine albumin system, as a function of field strength, electric mobility, temperature difference, and membrane spacing.

From the analysis of the experimental results the following conclusions were drawn:

1. The theory developed using the transport equation approach is not quantitative but predicts the trend for the effect of the parameters E, ΔT, and 2ω and U.
2. The empirical column constants H and K are practically independent of flow rate σ as expected from theory. Therefore, the use of H and K calculated using batch velocity profiles to represent the flow case is justified.
3. The empirical column constants H, H_{TE}, and H_E conform to their theoretical functional dependence on field strength E and temperature difference ΔT.

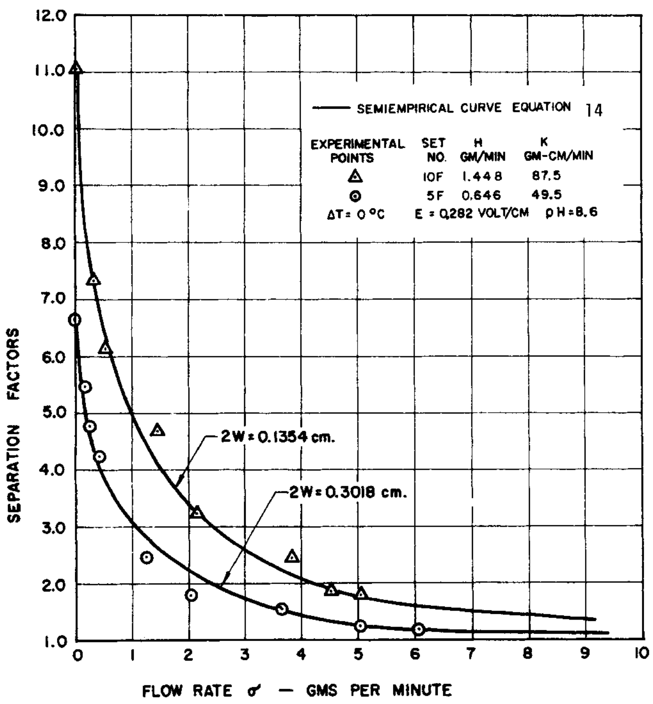

Fig. 10. Separation factors as a function of flow rate at two different membrane spacings.

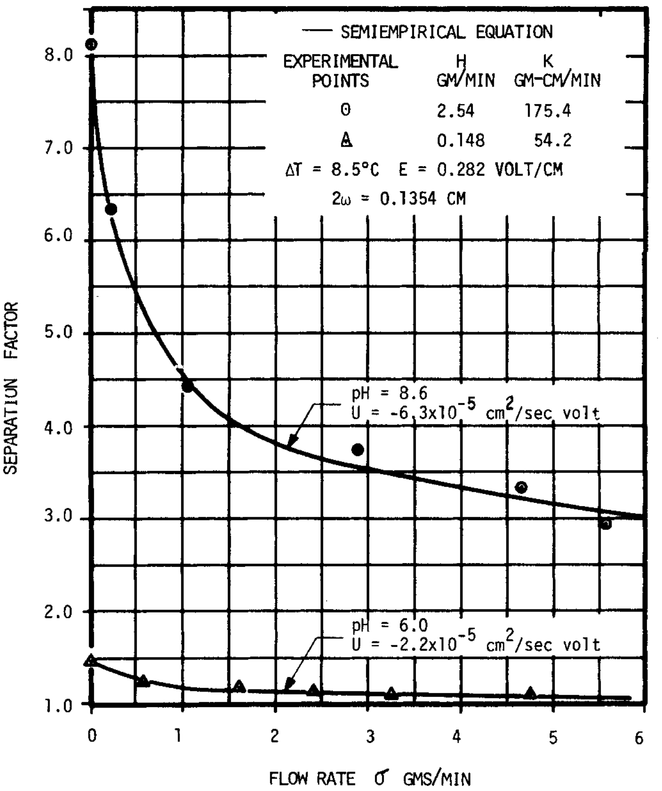

Fig. 11. Separation factors as a function of flow rate σ at two *p*H values.

4. The empirical column constants K_E follow their theoretical expression, but the total K values approach a constant value with increase in ΔT or E.
5. None of the column constants conform to their theoretical dependence on membrane spacing.
6. As predicted by the theory, the separation factors increase with increase in field strength, decrease in temperature difference, increase in electric mobility of the component and decrease in membrane spacing.
7. At large temperature differences, the separation factors increase with ΔT contrary to the theory. This follows because K reaches a constant value as ΔT increases.
8. At moderately low temperature differences that are possible in a low temperature electrophoretic separation of albumin, ΔT has an adverse effect on the separation.

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APPENDIX: EXPERIMENTAL APPARATUS

The electrophoresis column is shown diagrammatically in Figure 1. The working space between the membranes was about 10.2 cm wide and 145.7 cm long. Because the product and sample ports were not quite at the ends of the working space of the column, 145 cm was the effective length for the separation zone between the top and bottom sample ports. For constructional details of the various column components, Prabhudesai (1965) may be consulted. The membranes were prepared from Visking Cellulose Dialysis (Viscose Corporation, Chicago, Illinois) tubing by spreading it in a rectangle 20.5 cm by 160 cm. These membranes contained the protein components and prevented flow between the separation space and the buffer compartments. Their pore size allowed only buffer salt ions to pass through so that the *p*H in the separation space would be maintained constant by flow of ions through the membranes and the action of the buffering compound.

Since the membranes were not mechanically strong and large flow rates of cooling buffer might be required, these membranes were supported by Flexolith filter plates. The membrane spacing was varied by using plexiglass spacers of different thicknesses as suggested by Fleming (1961) and the spacing was measured using liquid displacement as described by Boyer (1961).

The electrodes were platinized titanium sheets each of 0.040 cm thickness and were situated in the buffer jackets coextensive with the membranes. Two thermocouples and two electrical probes were located on each Flexolith filter.

The solution separation space was defined by the membranes and the spacer used, while the buffer compartments were formed by a plexiglass buffer jacket covered with a plexiglass coverplate. The entire assembly was held together by stainless steel bolts spaced every 3 inches around the periphery of the column. Each cover plate was supported by a ⅛ in. thick stainless steel frame to give sufficient rigidity to the cell assembly. The cover plate was sealed to the buffer compartment along the periphery by a nylon filled neoprene gasket, 0.015 in. thick.

All material other than the gasket in contact with the working fluid (protein in buffer solution) or the buffer solution was either cellulose, stainless steel insulated with acrilon spray, impolene, Teflon, Plexiglass, Polyethylene, Penton (Polypropylene) or glass. The feed entered the column through holes drilled in the front face of the feed header and products were withdrawn from ports drilled in the product headers.

Temperature controlled, cooled, buffer solutions were circulated through the buffer compartments past the Flexolith filters to maintain the two membranes at desired temperatures and to maintain the *p*H of the solution in the separation zone. Temperatures were obtained by measuring thermocouple voltages using a Leeds and Northrup Portable potentiometer (No. 8662). Temperature measurements were accurate within ± 0.2°C.

The direct current field was applied across the membranes at the two platinized titanium electrodes by an electronic, constant voltage, D.C. power supply rated as 36 volts at 5 amp. The current through the circuit was measured by a calibrated ammeter while the voltage across the membranes was indicated by calibrated voltmeters and was measured by the Leeds Northrup precision potentiometer.

Materials and Analysis

Purified bovine albumin was used as a single mobile component (0.956 gm/100 ml in concentration) in two different suitable buffer solutions of *p*H 8.6 (borax-boric acid) and 6.0 (phosphate buffer) to obtain experimental transport data on the separation behavior of the electrophoresis column without reservoirs. Electric mobilities and diffusivity of bovine albumin were obtained from the literature (Greenberg, 1900) while the densities and viscosities of the solutions were determined experimentally. The physical property data used in the calculations is listed in Prabhudesai (1965).

Sample Analysis

Concentrations of bovine albumin in samples of feed solution and in product samples from the column were estimated with an accuracy of ± 1.5% colorimetrically using a modified biuret method of Robinson and Hogden (1940).

Experimental Procedure

After the membrane-spacing determination had been made, a series of tests were made to determine the steady state separation as a function of flow rate. Before the flow runs for a particular set of parameters such as field strength, temperature difference, *p*H, and membrane spacing were made, a transient batch run with zero flow rate was carried out to determine the time to reach steady state. This time period was used to determine the time period after which the first sample for the flow run should be removed from the column. The column and all lines were purged with the solution to be investigated. For flow runs, the column was center-fed and two samples were taken after steady state was reached, at an interval of one hour or more between them. Sufficient time was allowed to purge the headers and eliminate any disturbances introduced by the sampling procedure. Before collecting samples, the sample needles were purged. 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. During the time interval between samples taken under steady state conditions, rotameter readings were made and the actual product-flow rates were measured by collecting and weighing samples. Other readings taken at this time included the temperatures of the buffer solutions entering and leaving the column, the controller readings and the voltages of the four thermocouples located at the surface of the Flexolith filters and the voltage drop across the membranes.

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Part III. Transient Behavior of a Batch Column

A theory has been developed for the mathematical analysis of the transient behavior of a thermoelectrogravitational electrophoresis column without reservoirs using the transport equation approach. Experimental data were obtained with a parallel membrane batch electrophoresis column to test the theory.

The transient batch separation factor data obtained for a bovine albumin system under different values of the parameters (electric field strength, mo-