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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-

bility, membrane spacing, and temperature difference) are discussed and compared with the results obtained using column transport coefficients empirically determined from steady state flow data (see Part II). Experimental separation factors for the transient operation agree with those predicted using column transport coefficients (*H* and *K*) derived from steady flow studies, providing further support for the use of the Furry, Jones, Onsager approach to the analysis of electrophoresis columns. Failure of quantitative agreement between theory and experiment is attributed to the use of large spacings in obtaining experimental data.

In Part I of this paper, a basic theory of a thermoelectrogravitational electrophoresis column without reservoirs was developed by application of the Furry, Jones, and Onsager procedure of thermal diffusion. In Part II this theory was extended to the continuous-flow operation of the column, and separation factor data as function of flow rate were obtained to compare theory and experiment.

A review of the literature indicates that no theoretical and experimental work has been reported on the transient batch electrophoresis columns without reservoirs. Also, study of the batch transient behavior of the column provides another test of the theoretical development.

The purpose of this part is to apply the transport equation to the theoretical analysis of transient batch operation of the electrophoresis column and compare the theory with experimental data for separation as a function of field strength, temperature difference, membrane spacing and mobility.

THEORY

Experience with thermal diffusion (Jones and Furry, 1946; Powers, 1961; Von Halle, 1959) indicates that the transport equation approach can also be applied to the analysis of transient batch operation of thermoelectrogravitational columns. The transport equation was developed in Part I:

$$\tau = HC - K\frac{dC}{dy} \tag{1}$$

It is possible to incorporate this equation into a material balance to describe the transient operation of the electrophoresis column.

A sketch of the operation of a transient electrophoresis column without reservoirs is given in Figure 1. In this case, the solution is placed in the column at the beginning of the experiment and then subjected to the influence of both an electric field and a temperature difference. During the experiment, no products are removed and no feed is added. However, samples are withdrawn from the column at intervals and the depletion of the solution is made up by entry of feed solution at the feed point in the column. For the purposes of the present analysis, the effect of periodic sample withdrawal and subsequent entry of the feed solution into the column on the transient behavior of the column will be ignored.

The coordinate system to be used for mathematical analysis is slightly different from that in the flow case and is shown in Figure 1, and the column is treated as one section.

Using the transport equation [Equation (1)] developed for batch operation of the column and taking a material balance on a differential element of the column cross section, one obtains in the limit the equation

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

which describes the transient behavior of the column. In the above equation *m* is the mass of solution per unit length of the column and is considered constant. It is also assumed that the system contains either one mobile component or that it can be reduced to a one mobile component (hypothetical) system as explained in Part I. This equation is to be solved with the boundary conditions:

$$\begin{aligned} HC - K\frac{\partial C}{\partial y} &= 0 \qquad \text{at } y = 0,\ L;\ \text{all } t \\ C &= C_0 \qquad \text{at } t = 0,\ \text{all } y \end{aligned} \tag{3}$$

Equations (2) and (3) can be written in more convenient form by substitution of $\Psi = y/L$:

$$\frac{\partial^2 C}{\partial \Psi^2} - 2A\frac{\partial C}{\partial \Psi} - \alpha\frac{\partial C}{\partial t} = 0 \tag{4}$$

$$\frac{\partial C}{\partial \Psi} - 2AC = 0 \quad \text{at} \quad \Psi = 0 \tag{5}$$

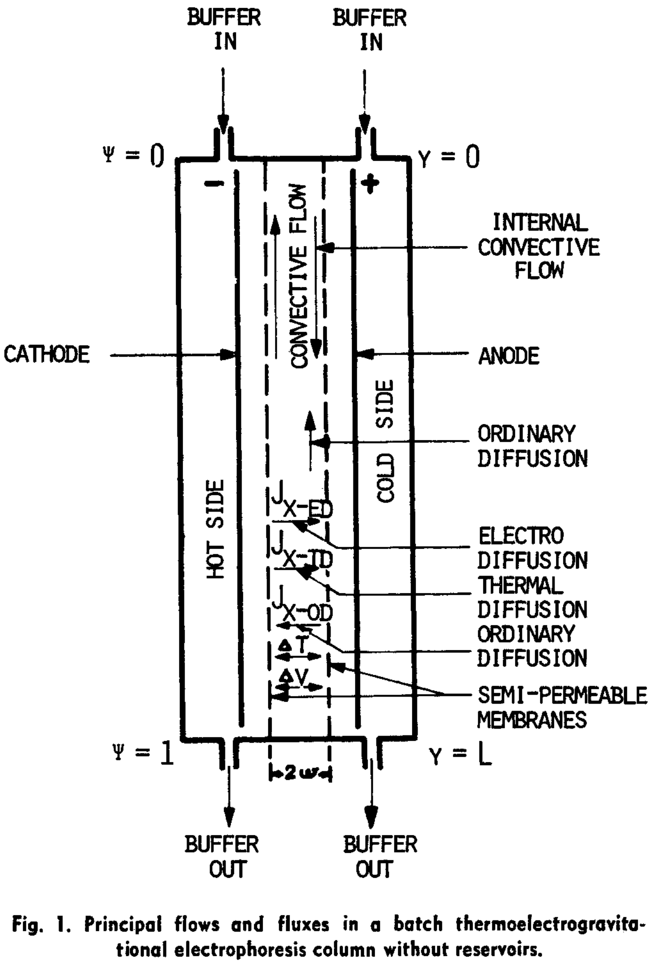

Fig. 1. Principal flows and fluxes in a batch thermoelectrogravitational electrophoresis column without reservoirs.

$$C = C_0 \qquad \text{at} \qquad t = 0, \text{ all } \Psi$$

where

$$\alpha = mL^2/K \tag{6}$$

and

$$A = HL/2K \tag{7}$$

The solution of Equation (4) with the boundary conditions of Equation (5) can be written down from the solution of a similar equation in thermal diffusion first obtained by Bardeen (1940) by use of separation of variables and later with use of Laplace transform by Von Halle (1959). The solution is given by

$$C(\Psi, t) = \frac{2A\, C_0 e^{A\Psi}}{e^{2A} - 1} + 4AC_0 e^{-A\Psi} \sum_{n=1}^{\infty} \left[\frac{n^2\pi^2}{(n^2\pi^2 + A^2)^2} [1 + (-1)^{n+1}\, e^{-A}] \; e^{-\frac{n^2\pi^2 A^2}{\alpha} t} \left(\cos n\pi\Psi + \frac{A}{n\pi} \sin n\pi\Psi \right) \right] \tag{8}$$

From Equation (8), an expression for the separation factor realized at time t can be obtained by dividing the concentration at the bottom ($\Psi = 1$) by concentration at the top ($\Psi = 0$) at time t and is given by

$$q_t = \frac{C(1, t)}{C(0, t)} = (C_B/C_T)_t$$

$$q_t = e^{2A} \left[\frac{1 + S \sum_{n=1}^{\infty} b_n (-1)^n\, e^{-t/t_n}}{1 + S' \sum_{n=1}^{\infty} b_n e^{-t/t_n}} \right] \tag{9}$$

where

$$S = 2e^{-A}\,(e^{2A} - 1) \tag{9a}$$

$$S' = 2\,(e^{2A} - 1) \tag{9b}$$

$$b_n = \sum_{n=1}^{\infty} \frac{n^2\pi^2}{(A^2 + n^2\pi^2)^2} [1 + (-1)^n\, e^{-A}] \tag{9c}$$

and

$$t_n = \frac{\alpha}{(n^2\pi^2 + A^2)} = \frac{mL^2}{K(n^2\pi^2 + A^2)} = \frac{4mK}{H^2} / (1 + n^2\pi^2/A^2) \tag{9d}$$

and the approach to steady state ($t = \infty$) is then given by the equation

$$q_t/q_s = \frac{1 + S \sum_{n=1}^{\infty} b_n (-1)^n\, e^{-t/t_n}}{1 + S' \sum_{n=1}^{\infty} b_n\, e^{-t/t_n}} \tag{10}$$

In Equation (9), the first term represents the steady state or equilibrium term and those remaining are the transient terms, each of them decaying with a characteristic time t_n. If A is not too large, the first transient term will be the most important and then the approach to equilibrium is characterized by a relaxation time t_r given by

$$t_r = t_{(n=1)} = \frac{4mK}{H^2} / (1 + \pi^2/A^2) \tag{11}$$

For large values of A, it is necessary to use more than one term in the series. For smaller values of t/t_n, it can be shown as done by Debye (1939) in thermal diffusion that

$$q_t = 1 + 4H(t/\pi mK)^{1/2} \tag{12}$$

Since both theoretical H and K increase with field strength and are proportional to E^2 (strictly true at $\Delta T = 0$), Equation (12) shows that large separations should result at larger E values and that during the initial period, the incremental separation factor is roughly proportional to E. Equation (12) is independent of L and therefore the initial separation (short time) is independent of length.

In the discussion of the continuous-flow data on the electrophoresis column, it was observed that in order to obtain quantitative correspondence between theory and experimental data, it was necessary to use empirically obtained values of the column transport coefficients H and K to calculate the separation factors from Equation (14) of Powers (1961). A similar procedure must be followed for the transient data in order to obtain agreement between experimental transient separation factors and those calculated by the use of Equation (9).

EQUIPMENT

The thermoelectrogravitational column and the related equipment used to obtain transient batch separation data have been discussed in Part II. Studies of the transient behavior of the column were made possible because the sample tap arrangement permitted direct sampling from the separation space of the column.

REAGENTS AND ANALYSIS

Materials used for the transient batch studies, the various physical property data and the method of analysis of samples withdrawn from the column were the same as described in Part II.

EXPERIMENTAL PROCEDURE

A transient batch run was made for each set of the parameters E, ΔT, 2ω, and U selected for a series of steady state flows to study the transient behavior of the column and to determine the time required to reach the steady state concentration at the top and at the bottom of the column. For a particular run, the column and product lines were first purged by starting the flow of solution through the column. The circulation of the background buffer solutions was then started and about one hour was allowed for the temperature gradient to establish. The flow of solution through the column was then stopped by closing valves on product lines and about two more hours were allowed before any potential was applied to stabilize any disturbances in the temperature field. Samples were then taken from both top and bottom of the column. These samples collected at the beginning of each transient experiment after stabilization of the temperature field never showed any separation. In a few experiments, the electric field was not applied for a period of 10 to 12 hours. The samples taken under these conditions also showed no separation, indicating negligible thermal diffusion effect for albumin. After taking the first samples, an additional half hour was allowed and then the electric field was applied across the membranes by turning on the power supply at the desired voltage. Samples were collected after each hour until a particular transient run was stopped after the time required to reach steady state in a transient run carried out previously at a lower field strength.

EXPERIMENTAL RESULTS

The transient batch separation data were obtained at four different field strengths ranging from 0.0423 to 0.423 volt/cm and for the temperature differences of 0°, 8.5° and 16°C respectively with the cooler wall always at 0°C.

Membrane spacings (2ω) were 0.1354 cm and 0.3018 cm and the electric mobilities were ($U = -6.3 \times 10^{-5}$ cm²/s volt and -2.2×10^{-5} cm²/s volt). All the transient batch separation data have been tabulated in Prabhudesai (1965).

DISCUSSION OF RESULTS

As in the case of continuous-flow electrophoresis data, a comparison of experimental batch separation data with the theoretically calculated results for corresponding conditions of the parameters did not show quantitative agreement with the theory. Since the transient data were obtained under the same conditions as the continuous flow data, the experimental separations were compared with the results calculated using values of the empirical column transport coefficients H and K derived from corresponding continuous-flow data.

The experimental transient separation data are compared in Figure 2a with the theoretical results obtained by using the empirical H and K at four different field strengths. In all cases it is found that the experimental data fit remarkably well with the curves. Similar correspondence was obtained for all other transient data. The effect of the parameters on the experimental transient separation will, therefore, be considered in connection with Equations (9), (10), and (11) using the empirical H and K values.

DISCUSSION OF TRANSIENT SEPARATION FACTORS

Effect of Field Strength

The transient separation factors at four different field strengths (with other parameters constant) is presented in Figure 2a, and the corresponding fractional approach to steady state presented in Figure 2b show that the times to reach equilibrium strongly depend upon field strength E. According to the semiempirical theory, empirical values of H increase with E, and K approaches a limiting constant value. Thus, A increases with E. Equation (11) predicts that higher separations should occur at larger E values, and Figure 2a substantiates this. However, Equation (11) also predicts that the steady state should be reached sooner for higher E values and Figure 2b contradicts this. This disagreement may occur because the theory assumes that the velocity profile is already developed when the electric field is applied at time zero, as was done in the theory for thermal diffusion. This is reasonable for thermal diffusion, but in electrophoresis the major force generating the velocity profile is the density difference resulting from the changes in concentration as the separation develops, and one could assume that the velocity profile was developing as long as the concentration profile was developing. Since the larger separations would have the more profound velocity profile, they would take longer to develop at higher E, while at lower E and smaller separations convection would be stabilized sooner and steady state conditions attained sooner.

Effect of Temperature Difference

According to the theory, A decreases with increase in ΔT and higher separations are expected at lower values of ΔT. Data presented in Figure 3a indicate that at constant E, separations at $\Delta T = 0$ are higher which is in accordance with the theory. However, the separation factors at $\Delta T = 8.5$°C are lower than those at $\Delta T = 16$°C. This is consistent with the empirical H and K derived from flow data because K remains substantially constant with increasing ΔT while H increases with ΔT. This causes an increase in A resulting in increased separations at higher ΔT values.

Because of the complicated way in which ΔT appears in Equation (10), it is rather difficult to draw general conclusions for the effect of ΔT on the approach to steady state. The data at three different ΔT values presented in Figure 3b indicate that the time required to reach steady state is shortest at $\Delta T = 0$, while at $\Delta T = 16$°C it is shorter than the time at $\Delta T = 8.5$°C. The experimental data indicate that the time required to reach steady state depends upon the degree of separation obtained, larger times being required if the separation is higher. Neither the formal nor the empirical theories explain this behavior as discussed in the previous reaction. In this case also, it was found that relaxation times were higher when K was larger.

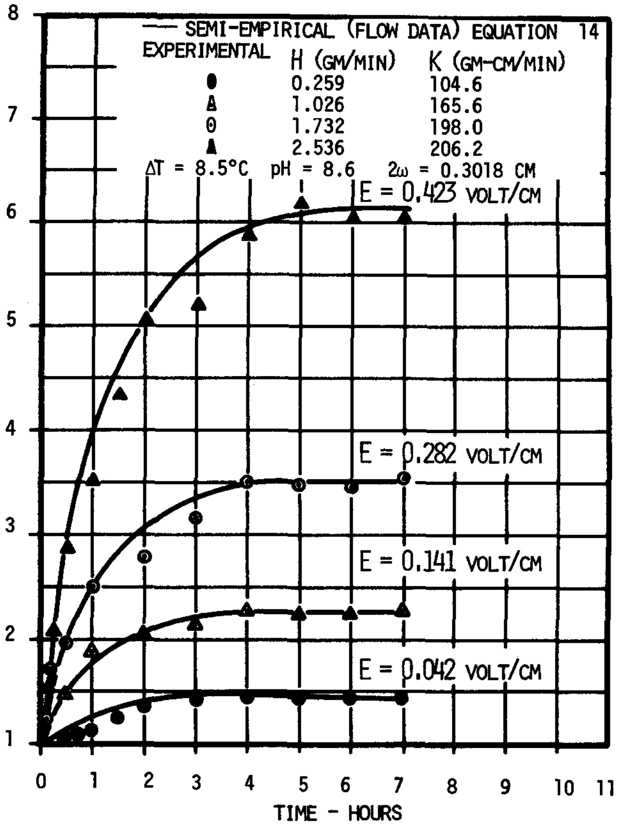

Fig. 2a. Transient separation factors for several field strengths.

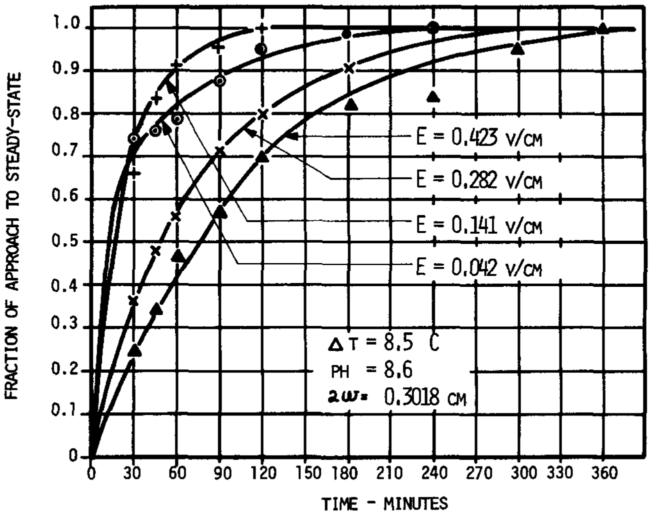

Fig. 2b. Approach to steady state in an electrophoresis column with field strength as a parameter.

Effect of Membrane Spacing

The theory predicts that *H* and *K*, and separation, depend strongly on membrane spacing. According to the theory, higher separation should result at lower spacings and the data with two membrane spacings presented in Figure 4a support this. The experimental transient separation data are in good agreement with the results calculated from theory using empirical column constants *H* and *K* derived from steady state flow data.

The approach to steady state shown in Figure 4b is faster with smaller spacing. Although this conflicts with the theory, it is consistent with the experimental trends established from flow rate data for the empirical values of *H* and *K*. It is possible that at the smaller spacing, the convective flow in the cell is stabilized quicker resulting in faster separation and a shorter time to reach steady state.

Effects of Electric Mobility

The mobility appears in the equations in the same way as electric field strength. Therefore, higher mobilities should result in larger equilibrium separations achieved faster. The data presented in Figures 5a and 5b at two different mobilities with other parameter constants are in agreement with this.

Summary

In summary, empirical values for the column constants *H* and *K* describe excellently the transient and steady state flow separations for the thermoelectrogravitational column without reservoirs.

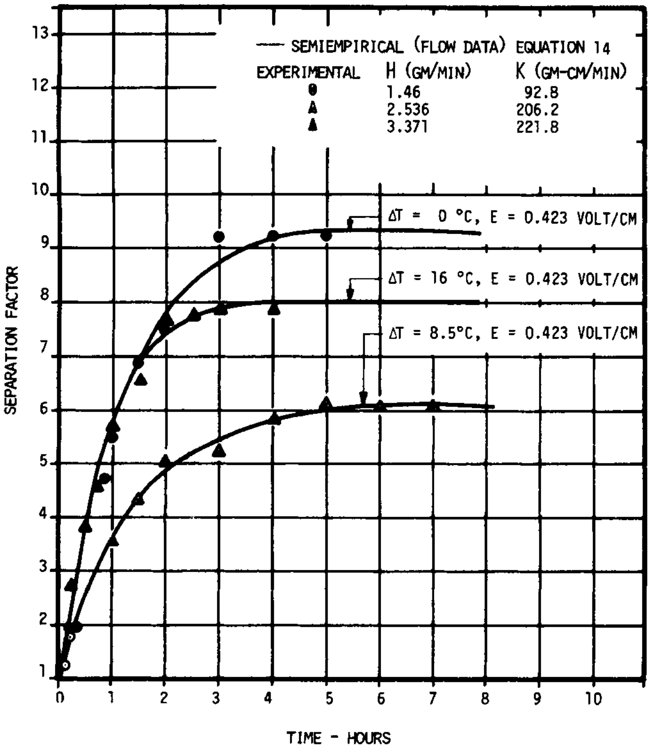

Fig. 3a. Transient separation factors at several temperature differences.

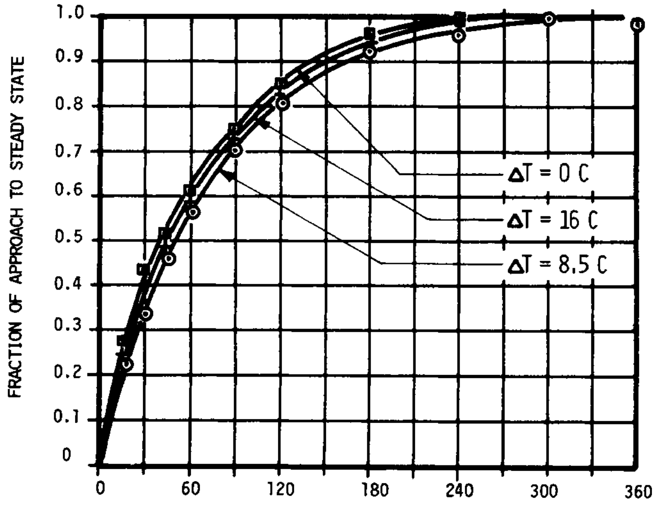

Fig. 3b. Approach to steady state in an electrophoresis column for several temperature differences.

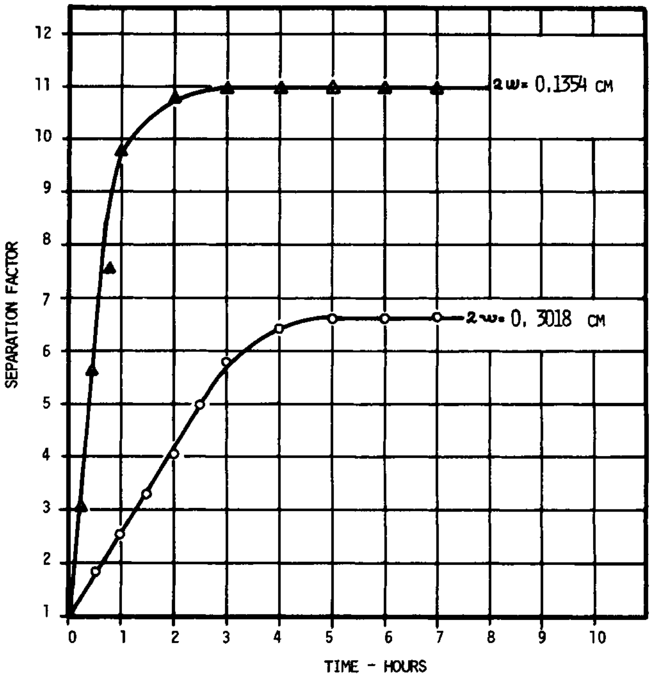

Fig. 4a. Transient separation factors for two-membrane spacings.

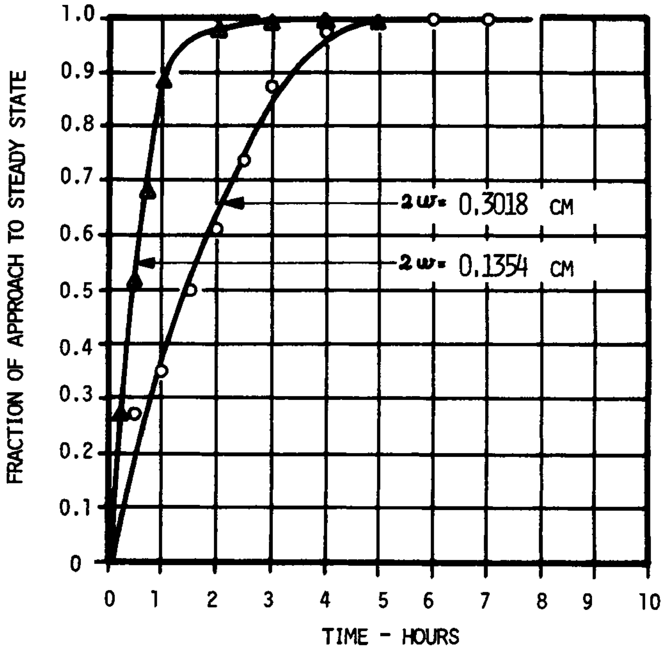

Fig. 4b. Approach to steady state in an electrophoresis column for two-membrane spacings.

The experimental behavior of *H* follows its theoretical prediction as a function of field strength, mobility, and temperature difference, but *H* depends much less strongly on plate spacing than predicted. Experimental values for *K* increase to a plateau, following the theory qualitatively until the plateau is reached. Empirical values for *H* and *K* permit calculation of separation factors for the entire range of experimental field strength, mobility, temperature difference, and plate spacing.

The use of the Furry, Jones, Onsager (transport equation) procedure with values of *H* and *K* determined empirically is established by the excellent agreement between the empirical column constants for the transient and steady state flow experiments.

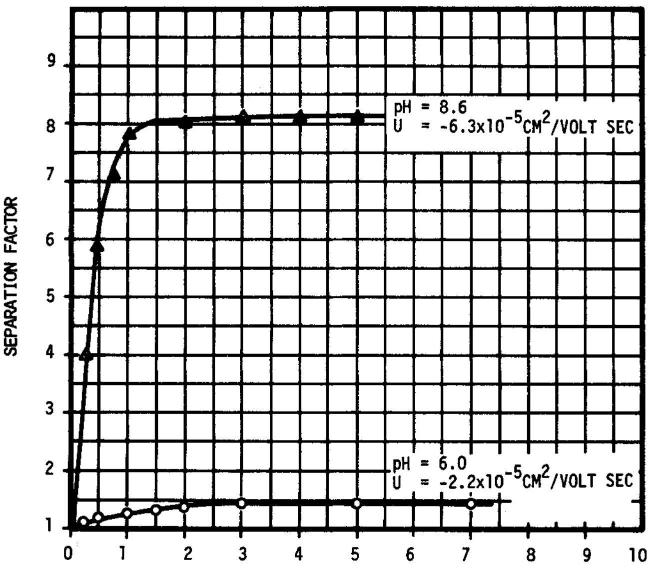

Fig. 5a. Transient separation factors for two values of electric mobility.

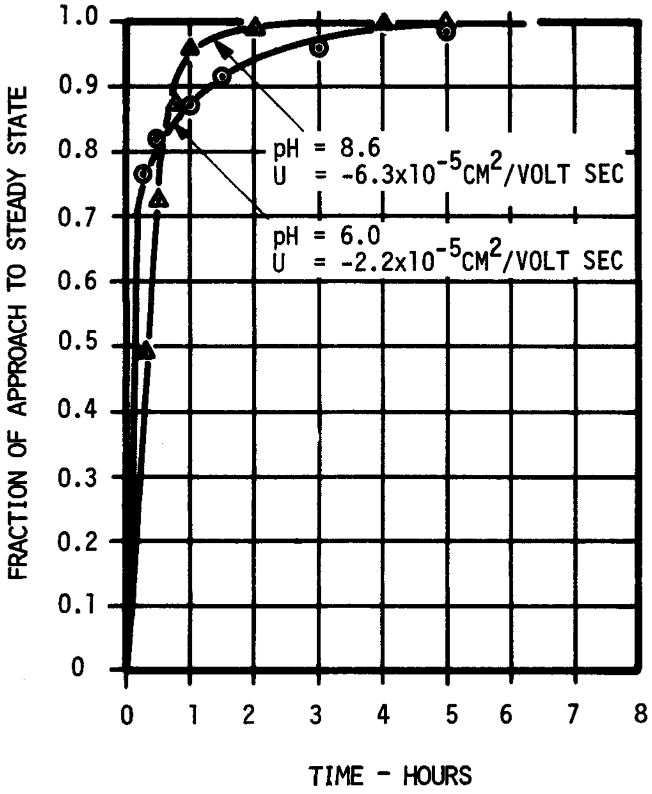

Fig. 5b. Approach to steady state in an electrophoresis column for two values of electric mobility.

CONCLUSIONS

The rate of approach to steady state in a thermoelectrogravitational electrophoresis column can be represented by Equation (10). Experimental values for the column constants *H* and *K* do not agree with theoretical expressions, but empirical values represent both transient and steady state flow data. The dependence of column constants upon design parameters follows the theory except that the influence of plate spacing is much less and that *K* plateaus for larger values of temperature difference. These developments should facilitate preliminary design estimates, and experimental results for transient or steady state flow separations can be used to predict experimental performance for a wide range of conditions.

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NOTATION

A = $HL/2K$, defined by Equation (7)
b_n = coefficient in series defined by Equation (9c)
B = subscript to identity streams leaving from bottom of column
C = mass fraction of mobile component
C_0 = mass fraction of mobile component in feed
E = electric field strength
H = column transport coefficient defined by Equation (32) of Part I
K = column transport coefficient defined by Equation (33) of Part I
L = total column length
m = amount of solution per unit length of column
n = index of summation
q_s = steady state batch separation factor
q_t = transient separation factor
S, S' = defined in Equations (9a) and (9b)
T = subscript for top section of column
ΔT = temperature difference between hot and cold membranes
U = electrophoretic mobility
y = axis parallel to membrane
α = mL^2/K, defined in Equation (6)
τ = amount of component 1 passing through a cross section of electrophoresis column normal to the membranes
Ψ = dimensionless length (y/L)
2ω = spacing between hot and cold semipermeable membranes

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