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SEPARATION TECHNIQUE ON PROTEINS VIA A PH-PARAMETRIC PUMP: A THEORETICAL AND EXPERIMENTAL STUDY

New Jersey Institute of Technology D.SC.ENG. 1982

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SEPARATION TECHNIQUE ON PROTEINS VIA A pH-PARAMETRIC PUMP: A THEORETICAL AND EXPERIMENTAL STUDY

by
Ura Pancharoen

Dissertation submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Engineering Science
1982

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Title of Dissertation: Separation Technique on Proteins Via A pH-Parametric Pump: A Theoretical and Experimental Study

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Doctor of Engineering Science, 1982

Dissertation and Abstract Approved:

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Chen, H.T., W.T. Yang, U. Pancharoen, and R.J. Parisi, "Separation of Proteins Via Multi-Column pH-Parametric Pumping," AIChE. J., 26, 839 (1980)

Chen, H.T., U. Pancharoen, C.R. Huang and C.O. Kerobo, "Analytical and Graphical Solution for Separation of Proteins Via Multi-Column pH-Parametric Pumping," in preparation.

Chen, H.T., U. Pancharoen, C.R. Huang and C.O. Kerobo, "Separation of L-Dopa & D-Dopa Via Multi-Column Parametric Pumping," in preparation.

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ABSTRACT

Title of Dissertation: Separation Technique on Proteins Via A pH-Parametric Pump: A Theoretical and Experimental Study

Ura Pancharoen, Doctor of Engineering Science, 1982

Dissertation directed by: Dr. C.R. Huang, Professor and Assistant Chairman, Department of Chemical Engineering

The separation of protein mixtures via pH-parametric pumping was investigated both theoretically and experimentally. A simple system consisting of one column packed either with cation or anion exchanger was first considered. A system of parapumps was then developed with more columns which were connected in series and packed alternately with cation and anion exchangers. Various methods of operation of parapumps are discussed. Enrichment and spliting of protein mixtures were examined. In most cases, the separation factor was defined at a steady state condition and was improved by increasing the number of cycles and columns.

Computational methods for predicting both Batch and Semi-Continuous parametric pump performance, with equilibrium conditions described were developed. The physical system was characterized by means of interphase mass transfer rates. These methods were based on a set of exterior and interior material balances. Linear parameters were calculated for the

adsorption of the solute on the ion exchanger. A mathematical model based on elementary matrix algebra was developed as well as a graphical method. The properties of eigenvalues and eigenvectors of this formalism were studied. The subject was extended to more complex situations involving a multi-column, and the separation of multi-protein. There is good agreement between predicted and experimental results.

ACKNOWLEDGEMENT

This research was done under the direction and with the encouraging advice and guidance of Dr. H. T. Chen of the Chemical Engineering Department. After the tragic automobile accident which ended Dr. Chen's life on April 21, 1981; Dr. C. R. Huang was instrumental to see a successful completion of this work. Heartfelt appreciation is extended to Dr. H. T. Chen, thesis advisor, and to Dr. C. R. Huang for their invaluable assistance.

Greatful thanks are extended to the Doctoral Committee for their suggestion and encouragement, and to M.R. Idhinand Abhakara and to Dr. R. A. George who have offerred support and guidance over the years at this College.

Special thank you to Mr. D. F. Bravender, Vice President of The Chase Manhattan Bank, for prove reading this manuscript. Financial support from the Chemical Engineering Department at New Jersey Institute of Technology, where the author is currently employed, also the typing of initial drafts and the final manuscript by Y. Pihtayanukul, T. Nimboonchaj and M. Jampathom are appreciated.

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Chapter I

INTRODUCTION

A. DEFINITION

Parametric pumping is a separation technique that is based on the periodic movement of a fluid phase over a solid adsorbent bed and a coupled energy input into the system to induce the separation.

The term "Parametric Pumping" was applied to the separation process in 1966 by the late R.H. Wilhelm of Princeton University, inventor of the batch pump. This separation process was a recuperative mode, which dealt with a two-phase system by means of an interphase mass transfer with an oscillating direction of the fluid flow. The fluid was heated in a heat exchanger before flowing up through the column and cooled before the flow direction had been changed as shown in Figure 1.

B. OPERATION

The basic parametric pump handles a batch operation and illustrates the coupling action. Consider the removal of component A from a fluid mixture of A and B. Assume that component A is absorbed on solid (S), packed in the column as shown in Figure 1. The column will have some void volume to allow the fluid to flow into it by means of two piston-operated fluid reservoirs at each end, which are filled with

the fluid mixture at a known concentration of A, (y_0). Also assume that the condition of interphase equilibrium occurs at a high temperature. Allow the temperature of the two-phase system to change periodically through alternate heating and cooling by transferring heat energy through the media of appropriate temperature to the jacket of the column. Then, the concentration of A in the liquid (y) and the concentration of A on the solid (x), will periodically change to new values in response to the change in the thermodynamic state of the system. Normally, A will be adsorbed on S while the system is heated.

At this step, the direction of flow of the fluid will be changed periodically and synchronized with the system temperature; e.g., only upward flow will occur during heating only downward during cooling. The volume of fluid in the column interstitial space will be depleted in A by adsorption on S only while it moves downward and enriched in A by desorption from S while it moves upward. After the system has completed the cycle of hot upflow and cold downflow, fluid depleted in A will migrate to the bottom of the column; while fluid enriched in A migrates to the top. The result of this operation is a net displacement of component A to the top of the column after a number of synchronized temperature-flow cycles. Separation of the system fluid into a fraction relatively concentrated in A at the top of the column has been achieved while the bottom contains a fraction

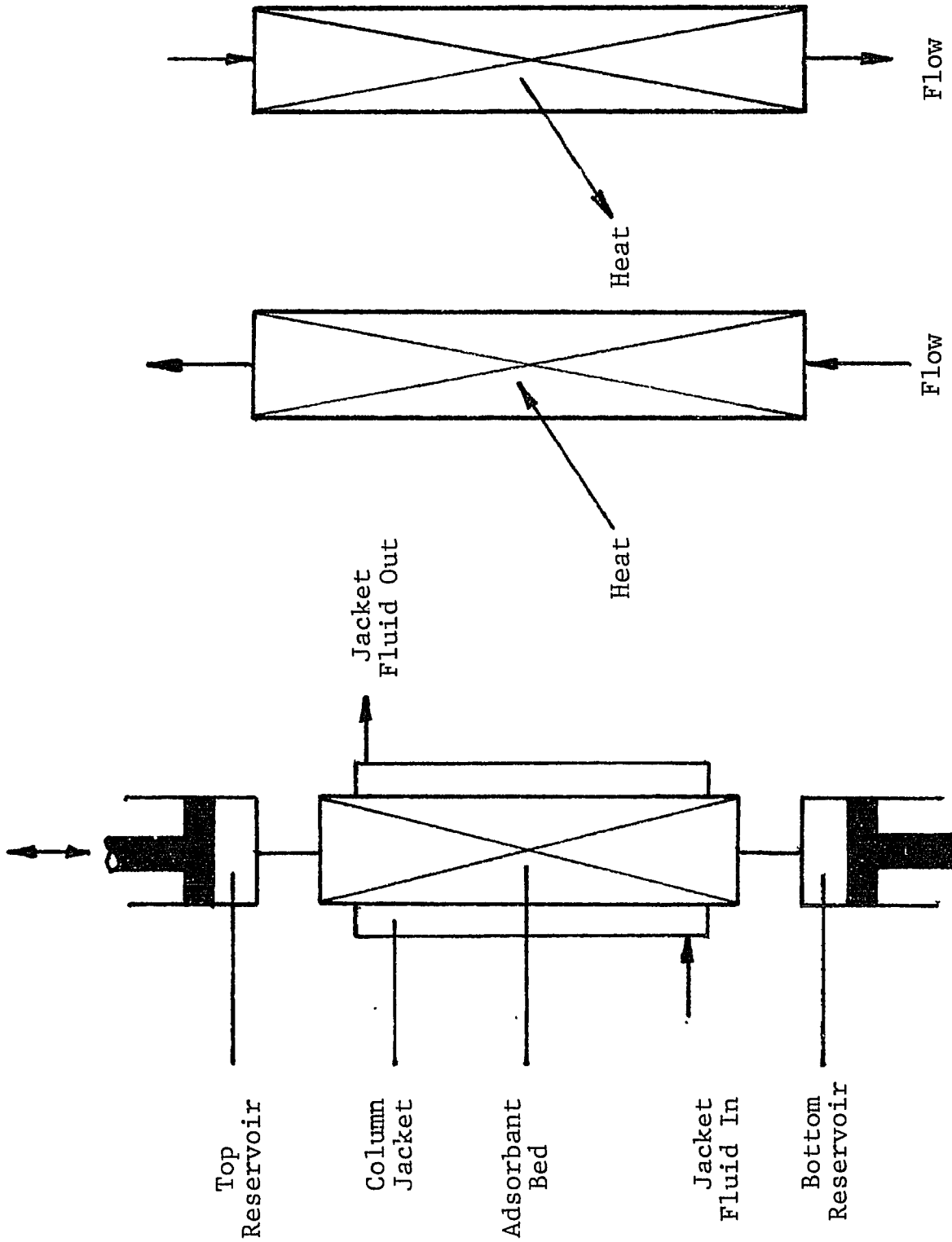


FIGURE 1 - THE BATCH PARAMETRIC PUMP (RAK, 1978)

relatively lean in A.

C. DEVELOPMENT

In 1966, Wilhelm and his co-worker had investigated the separation of NaCl from water using a mixed bed of ion-exchanger for a batch parametric pumping process in the recuperative mode. Wilhelm also expressed his idea of applying the parapump principle/processes with various driving forces such as magnetic, chemical potential, etc.

Wilhelm and Sweed (1968) had modified the system by using the direct mode as shown in Figure 1 on a batch system, to separate toluene from n-heptene, using silica gel as an absorbant. This direct mode is operated by a heating and cooling source which is supplied through the water jacket during upward and downward flow respectively. The stationary bed (column with jacket) is heated before the upward flow of liquid and is cooled before the downward flow by an external source. The parametric pumping process is not limited to only temperature induced liquid/solid mass transfer systems. Jenczewski and Myers (1970) separated the mixture of ethane and propane passing through activated carbon.

Sabadell and Sweed (1970) employed the recuperative mode to remove K^+ and Na^+ from water by changing pH levels. For this type of process the high pH end was opened while low pH end remained closed. HCl was introduced to the low end to

maintain the pH levels. The product was withdrawn while a fresh feed was supplied for every half cycle. During the process, the neutralization reaction occurred in the column and was claimed as an energy supplier for the separation. By means of this operation, the maximum separation factor (ratio of enriched product to depleted product) yield for total $K^+ + Na^+$ was obtained, although the deriving energy source by this method was not optimized.

Nor is parametric pumping limited to single adsorbant beds, one-solute systems, or oscillating flow patterns. In cycling zone extraction (see Figure 2), the mobile phase flows unidirectionally but enters successive zones of alternating, parameter values, e.g., hot and cold temperature, or high and low pH, both of which are utilized to separate glucose and fructose in an aqueous solution, which had been done by Busbice and Wankat in 1975. Chen, Jaferi, and Stokes (1972) had also used parametric pumping for sugar separation in aqueous media. The separation of Na^+ and K^+ in aqueous media has already been mentioned. In addition, toluene and analine can be separated from n-heptane using silica gel adsorbant in thermal parametric pumping.

In 1977, Chen and his co-workers had investigated the separation of proteins by use of a continuous pH parametric pump. Many system characteristics such as flow rate, buffer concentration, pH-level and reservoir displacement, which

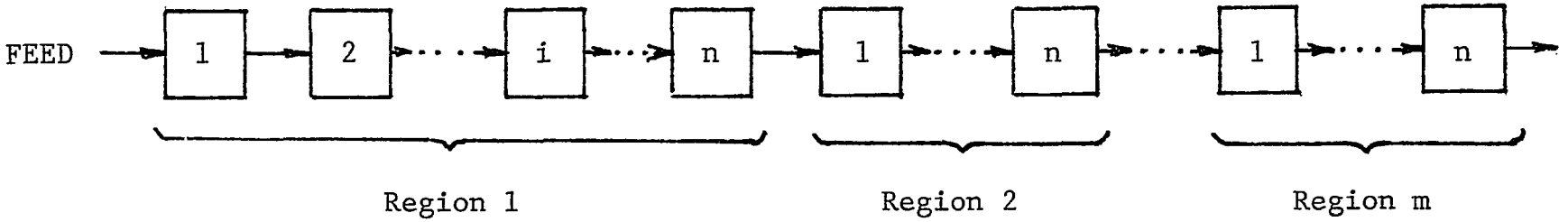


FIGURE 2 - STAGED CYCLING ZONE EXTRACTION SYSTEM (WANKAT, 1973)

would affect the separation were examined. Chen considered a process which consists of a one column system packed with S.P. Sephadex (C-50) exchange resin and two reservoirs, one having a high pH level the other having a low pH level. Both buffer solutions carry protein mixtures. Chen began the pH-parametric pump process operation using the batch system (before he developed the continuous process system) and investigated the separation of haemoglobin and albumin.

Chen, et al., (1979) developed the continuous separation process using a protein mixture (haemoglobin-albumin) via pH-parametric pumping. The exchange resin, CM Sepharose, was selected for this experiment. The protein mixture was fed alternately to the top and bottom of the column at different pH levels. The top and bottom products were removed conversely. Chen came to the conclusion that the continuous process reached the steady state faster than the batch parametric pump. Also, when the batch system reaches a peak (optimum of steady state value) the concentration of protein in the respective reservoirs will begin to decrease due to the dilution of acid/base titrations (to maintain the pH levels) while the continuous system can be maintained as the number of cycles increases.

The results of this experiment showed that haemoglobin, with an isoelectric point less than pH value, migrated to the higher pH end of the column while the albumin with an iso-

electric point greater than the pH value migrated to the low end of the column.

Chen and his co-workers (1979) also extended the parapump process to multi-column pH-parametric pumping. The system considered was a series of columns that were packed alternately with cation and anion exchangers. Many different types of methods and operations for single and multi-column parapump system were described.

D. DISSERTATION

This dissertation investigates both theoretical and experimental methods using a single or two-column parapump, operated on batch or semi-continuous systems, and also extending the method into a multi-column system. The emphasis of this work is divided into two parts the theoretical and the experimental. New methods of predicting performance are developed which include a method of mathematical approach and a graphical method. For experimental study, the separation of haemoglobin-albumin was selected. We established the necessary displacement, reservoir dead volume, buffer and Sodium Chloride concentration, circulation time and flow rate which would achieve the desired separation.

Chapter II

PROCESS DESCRIPTION

The One-Column Parametric Pumping System

The first system we will consider is shown in Figure 3. It consists of a column packed with an ion exchanger (cation or anion) and reservoirs attached to each end. The pump has dead volumes V_T and V_B for the top and bottom reservoirs, respectively. Initially, the mixture to be separated fills the column voids, the top reservoir and the bottom dead volume. The top reservoir is maintained at a low pH level (P_2) by an automatic titrator while a second titrator is used to keep the bottom reservoir at a high pH level (P_1). The ionic strengths of the buffer solutions in both top and bottom reservoirs are kept at IS_2 and IS_1, respectively by means of two hollow fiber dialyzers manufactured by Amicon.

Two constant pH fields (P_1 and P_2) are imposed periodically to the system. During the first half-cycle, the fluid with $pH = P_2$ in the top reservoir is pumped into the top of the column. At the same time, the solution that emerges from the column fills the bottom reservoir. On the next half-cycle, the solution with $pH = P_1$ in the bottom reservoir flows back into the column. At the end of this half-cycle, the top reservoir is filled with the solution that comes out of the top of

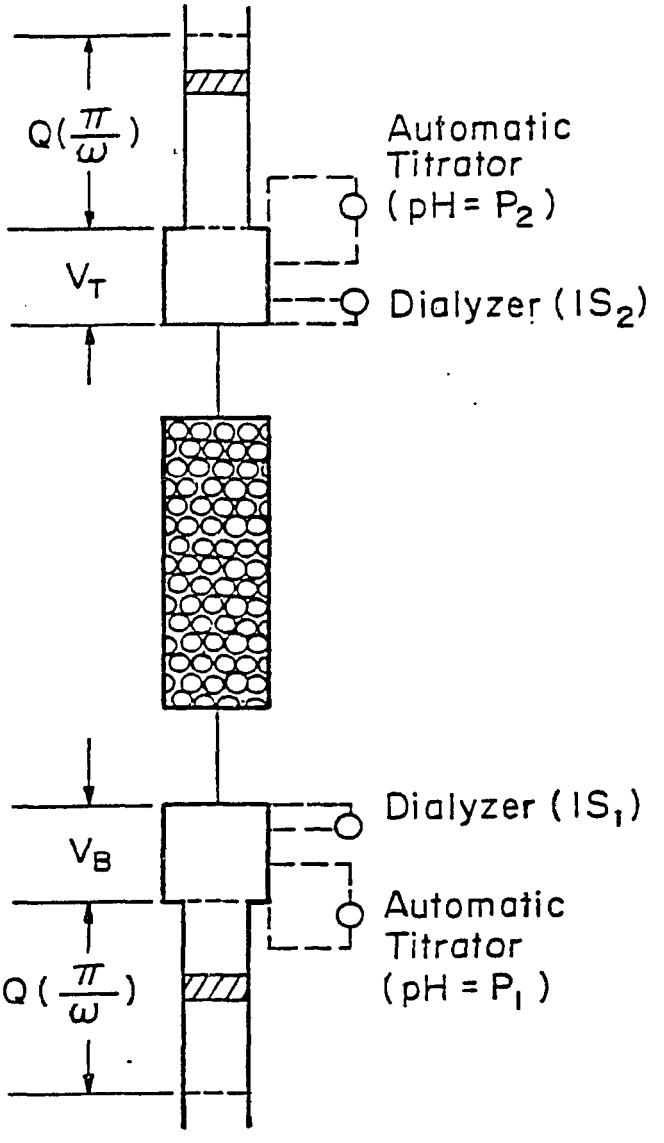


FIGURE 3 - COLUMN DIAGRAM FOR pH-PARAMETRIC PUMPING

the column, and one cycle is completed. This procedure is repeated in each of the succeeding cycles until the desired number of cycles are completed.

We deduce the characteristics of the pH-parametric pump described above via a simple discrete transfer equilibrium stage model (Jenczewski and Meyer, 1970; Wankat, 1974; Grevillot and Tondeur, 1976). Let us assume that the adsorbent bed is divided into N equal segments or cells (stages) of length Z/N, where Z is the length of the pump column. Each stage is represented as (I, J), where I is the stage number and J is the transfer step (Figure 4). The system is initially in equilibrium at J-1, and each cell has uniform concentrations in both fluid and solid phases. In the transfer step, each fluid segment is displaced exactly one step ahead. Thus, the fluid y(I,J-1) originally opposite the solid section I is now opposite I+1. After the transfer step, the phase equilibrium is immediately reestablished and the next transfer step J begins.

The mass balance for each component at I and J is,

$$Vy(I-1, J-1) + \overline{V}x(I, J-1) = Vy(I, J) + \overline{V}x(I, J) \quad (1.1)$$

where V and $\overline{V}$ are the volumes of the fluid and the solid phases per stage, respectively, and will be assumed to be constant. Furthermore, we will make the following assumptions:

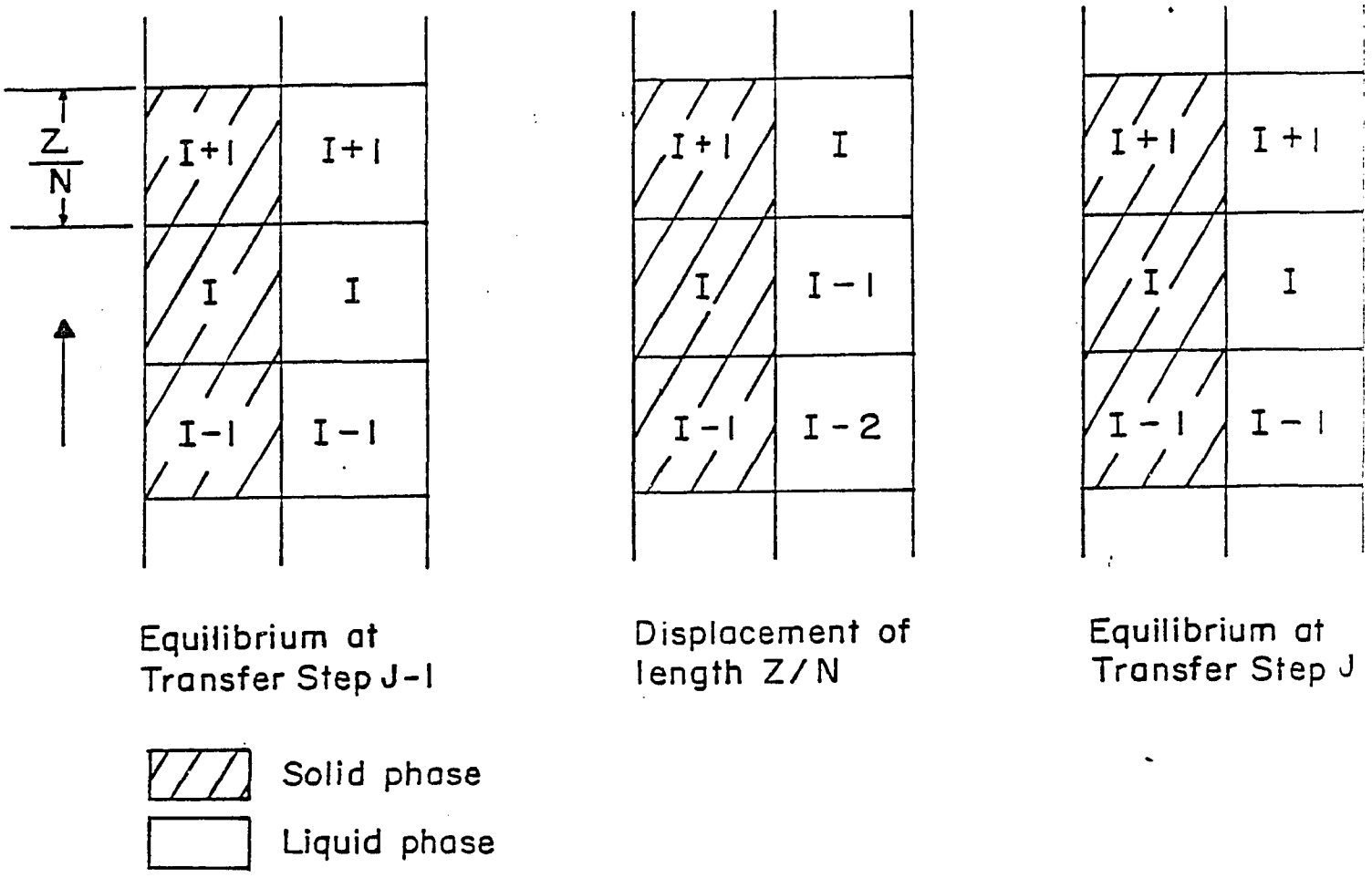


FIGURE 4 - SCHEMATIC FOR EQUILIBRIUM PLUG FLOW MODEL

(1) The solute will be distributed between the solid and fluid phases according to a linear form,

$$x = ky \tag{1.2}$$

where k is pH dependent only.

(2) The hydrogen ion does not exchange for the exchanger's counter ion, and therefore there is no lag of pH wave velocity behind the linear liquid velocity.

Proteins carry both negatively and positively charged groups of protein and can be bound to both anion and cation exchangers. Their net charge is dependent on pH. At low pH, the net charge is positive; at high pH it is negative. At the point of zero net charge, the isoelectric point, the substances are not bound to any type of ion exchanger.

Suppose we are concerned with the separation of a protein A from a mixture or solution, and this protein has the isoelectric point I_A and $P_2 < I_A < P_1$. Thus, A will bear a negative charge at P_1 and a positive charge at P_2, whereupon A will be taken up by a suitable cation exchanger, R^- (with the counter ion S^+) at P_2 and released at P_1:

$$R^-S^+ + A^+ \longrightarrow R^-A^+ + S^+ \quad \text{at } P_2$$

$$R^-A^+ + S^+ \longrightarrow R^-S^+ + A^- \quad \text{at } P_1$$

Therefore, a parametric pump operating with levels of P_1 and P_2 should be capable of removing the solute A from the low

pH end of the column and concentrating it at the high pH end. The reverse effect will occur if an anion exchanger is selected.

Concentration transients calculated by means of Equations 1 and 2 are shown in Figure 5. The ordinate is the average reservoir concentration divided by the initial liquid phase concentrations. As long as $\alpha = 1$, the steady state concentrations in both top and bottom reservoirs are independent of N chosen. α is defined as the quotient of the reservoir displacement and the column void volume (i.e., number of transfer steps/number of stages). Note that when $\alpha = 1$, $N = Q(\pi/\omega)/V$.

Two-Column Parametric Pumping System

Mode 1: Three reservoirs with both cation and anion exchangers

The system has two columns and three reservoirs as shown in Figure 6. One column is packed with a cation exchanger (R^-) and the other with an anion exchanger (R^+). The pH level for the top and bottom reservoirs is maintained at $P_1(8)$ and that for the middle reservoir is kept at $P_2(6)$. Initially, the top reservoir and both columns are filled with a mixture of the concentration y_o. The R^- and R^+ columns are respectively in equilibrium at $P_1(=8)$ and $P_2(=6)$. One cycle of operation is described as follows:

(1) Transfer down:

Step 1. The fluid in TR (top reservoir) is trans-

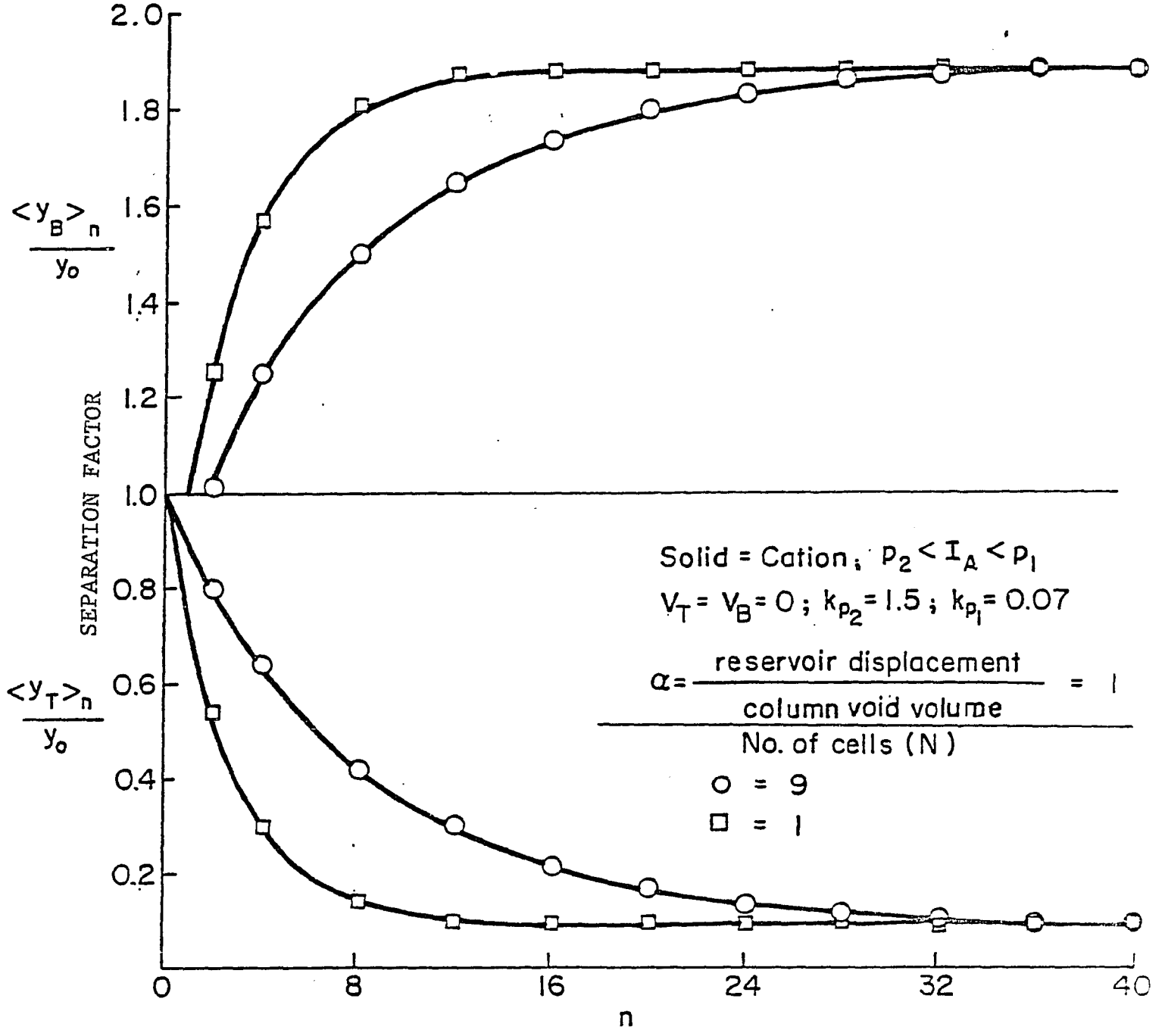


FIGURE 5 - EFFECT OF n ON CONCENTRATION TRANSEINTS (CHEN, 1980).

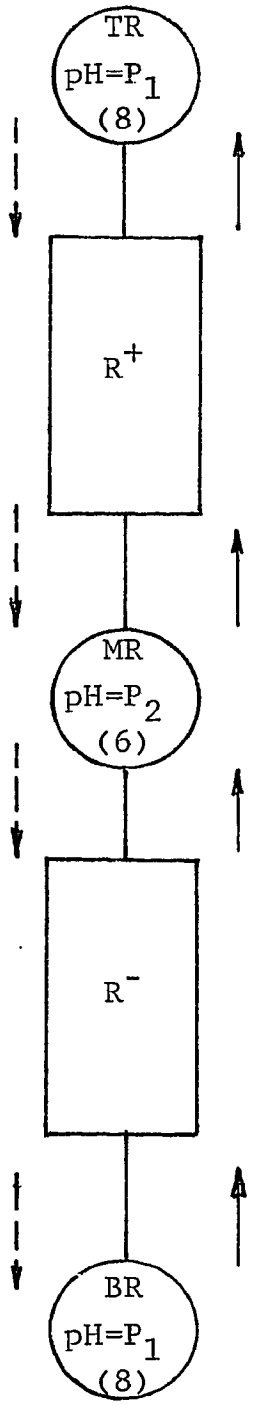


FIGURE 6 - TWO COLUMN DIAGRAM, PACKING WITH ANION AND CATION EXCHANGER FOR MODE 1

ferred to the R^+ column, and the content in the R^+ column goes to the MR (middle reservoir).

Step 2. The content of the R^- column goes to the BR (bottom reservoir) and the fluid in the MR is transferred to the R^- column.

(2) Equilibration: The pH in the R^+ column is changed from P_2 to P_1, and at the same time the pH in the R^- column is shifted from P_1 to P_2. Thus, re-equilibrium is allowed in both columns.

(3) Transfer up: The content in the BR is brought back to the R^- column. The content in the R^- is pushed through the MR and goes back to the R^+ column. The content in the R^+ column is transferred to the TR.

(4) Equilibration: The pH is switched from P_1 to P_2 for the R^+ column, and from P_2 to P_1 for the R^- column. The phase equilibrium is reestablished in both columns. Thus, one cycle is completed.

The procedures is repeated for each of the succeeding cycles.

Mode 2: Four reservoirs with both cation and anion exchangers

The system consists of two columns and four reservoirs as shown in Figure 7. One column is packed with a cation exchanger (R^-) and the other with anion exchanger (R^+). The flow rate within the column is always equal to the reservoir

displacement rate (Q). Thus for both down and up flow, the reservoirs have the same displacement. For this mode, the system requires three different levels of pH, P_1, P_2, and P_3 where $P_3 < P_2 < P_1$.

The pump has four reservoirs; one top (TR), two middle (ML and MR), and one bottom (BR), respectively, with pH = P_2, P_1, P_3 and P_2. The system starts with the top reservoir (TR), one middle reservoir (MR), and both R^+ and R^- columns filled with a mixture of concentration y_0. The R^+ and R^- columns are in equilibrium at P_1 and P_2 respectively. Flow sequences for one cycle are:

(1) Transfer down: Pump the fluid from the TR through the R^+ column to the ML (P_1), and at the same time, pump the fluid from the MR (P_3) through the R^- column to the BR.

(2) Equilibration: The pH in the R^+ column is changed from P_1 to P_2, and the pH in the R^- column is changed from P_2 to P_3. Then, the equilibration of both columns are reestablished

(3) Transfer up: The fluid from BR is pumped to the R^- column while the content in the R^- column goes through the middle reservoir (MR) to the R^+ column. At the same time, the content from the R^+ column is pumped into the TR.

(4) Equilibration: The pH in the R^+ and the R^- columns are changed from P_2 to P_3 and P_3 to P_2 respectively.

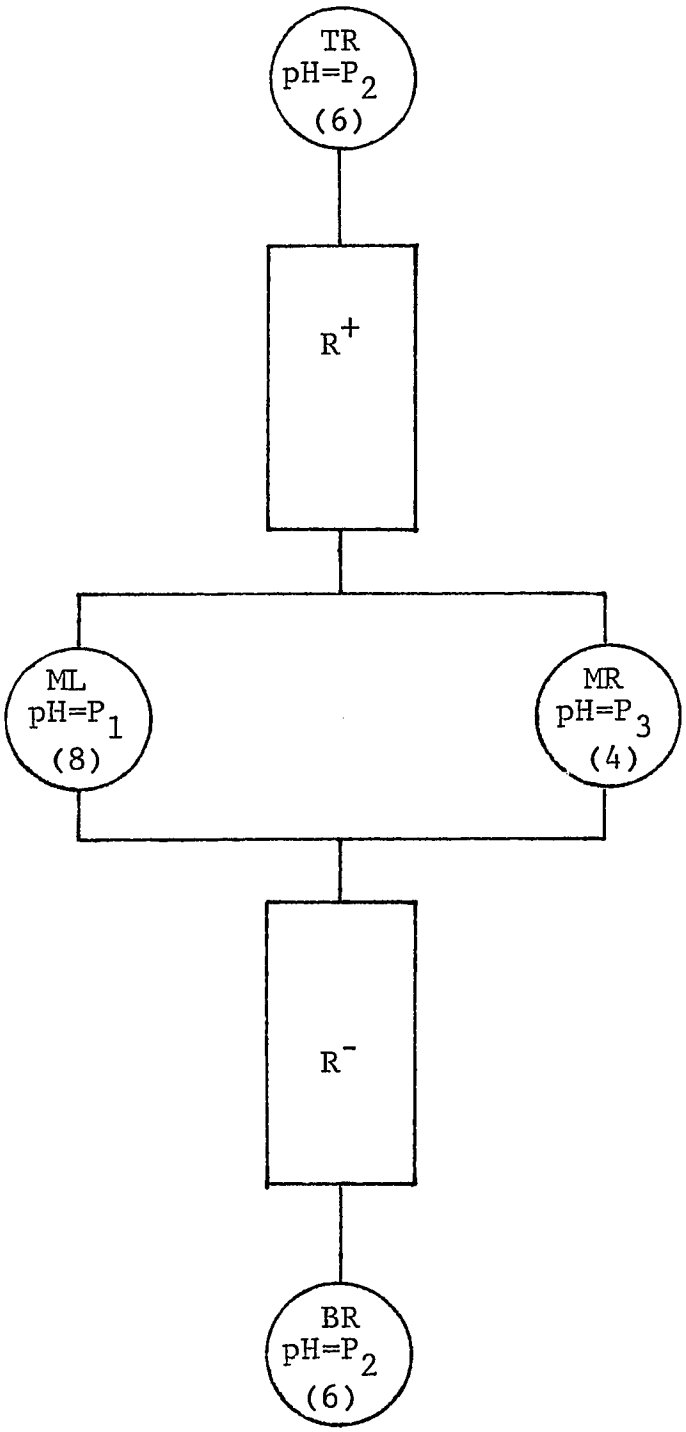


FIGURE 7 - TWO COLUMN DIAGRAM, PACKING WITH ANION AND CATION EXCHANGER FOR MODE 2

Thus, reequilibrium is allowed in both columns.

(5) Transfer down: The content in the R^+ place is taken by the fluid from the TR, and goes into the MR while the content in the R^- column is displaced by the fluid from the ML and transferred to the BR.

(6) Equilibration: The pH in the R^+ and the R^- columns are changed respectively from P_3 to P_2 and P_2 to P_1. Both columns are allowed to re-equilibrate.

(7) Transfer up: The liquid in the R^- column is pumped through the ML to the R^+ column while the fluid from the BR is transferred to the R^- column as well as the content from the R^+ column goes to the TR.

(8) Equilibration: The pH in the R^+ and the R^- columns again are changed from P_2 to P_1 and P_1 to P_2 respectively. Re-equilibration of both columns are allowed to re-establish.

Mode 3: Five reservoirs with both anion and cation exchangers

This process is consistent with two columns and five reservoirs. Both columns are packed; one with a cation exchanger (R^-) and the other with an anion exchanger (R^+). The system requires two top reservoirs, one middle reservoir and two bottom reservoirs and they are connected by the columns as shown in Figure 8. One top and one bottom reservoirs are maintained at $P_1(8)$ and the other top and bottom reservoirs are at $P_3(4)$ while the middle reservoir is kept at $P_2(6)$. Both

the R^+ column and the R^- column are in equilibrium at P_2(=6) and P_1(=8) respectively. The two top reservoirs are filled with a mixture of the concentration y_0 at P_1(8) and P_3(4). Also one of the bottom reservoirs is filled with the same concentration y_0 of a mixture at P_3(=4). The operation for the complete cycle is described as follows:

(1) Transfer down: The fluid from the TR(P_1) is transferred to the R^+ column, while the content in the R^+ column (P_2) is transferred through the MR to the R^- column. At the same time, the fluid in the R^- column (P_1) is transferred into the BR.

(2) Equilibration: The pH in the R^+ column is changed from P_2 to P_1 while the R^- column is changed from P_1 to P_2. Thus, re-equilibrium is allowed in both columns.

(3) Transfer up: The content in the BR(P_3) is pumped into the R^- column and at the same time, the fluid in the R^- column (P_2) is transferred through the MR to the R^+ column, while the content in the R^+ column (P_1) is transferred into the TR.

(4) Equilibration: The re-equilibration in both the R^+ and R^- columns are allowed due to their changing in pH respectively from P_1 to P_2 and P_2 to P_3.

(5) Transfer down: The fluid in TR (P_3) is transferred to the R^+ column. The content in the R^+ column (P_2) is pushed down through the MR to the R^- column while

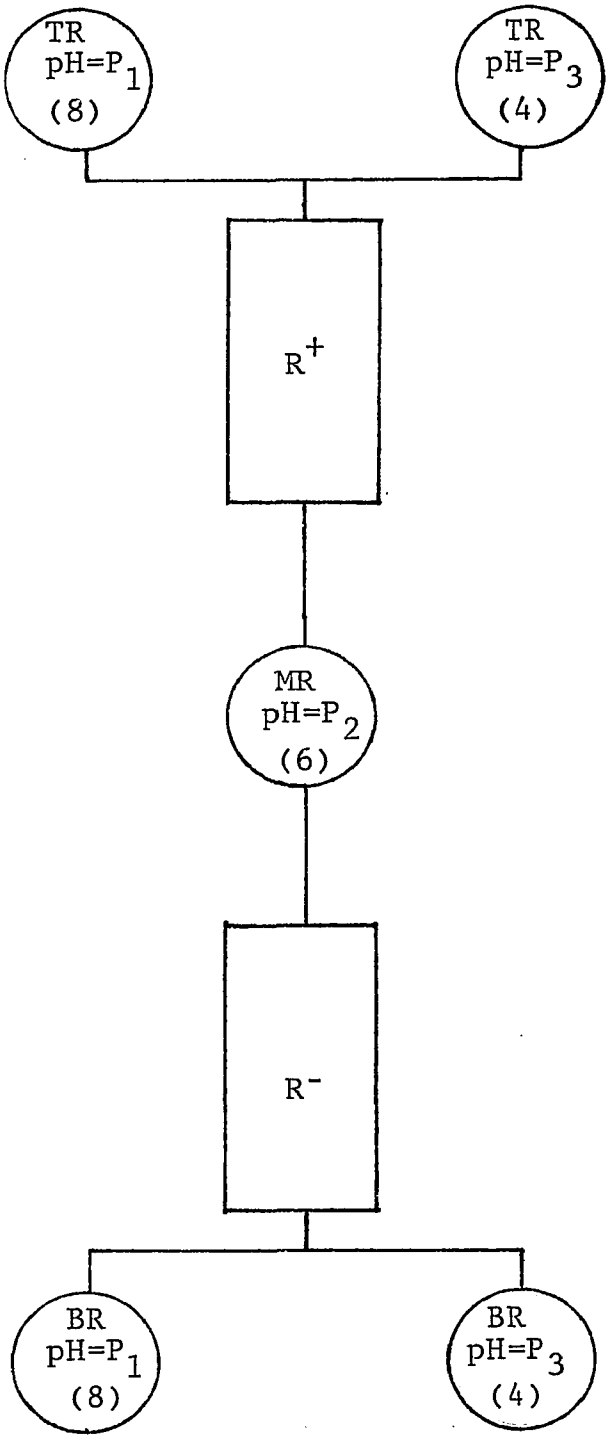


FIGURE 8 - TWO COLUMN DIAGRAM, PACKING WITH ANION AND CATION EXCHANGER FOR MODE 3

the fluid in the R^- column (P_3) is transferred to the BR.

(6) Equilibration: The equilibration in both the R^+ and R^- are re-established due to the pH change from P_2 to P_3 and P_3 to P_2 respectively.

(7) Transfer up: The content from the BR(P_1) is pumped to the R^- column, while the fluid in the R^- column (P_2) is transferred through MR to the R^+ column. Meanwhile, the fluid in the R^+(P_3) is transferred to the TR.

(8) Equilibration: Again, there are some changes in pH from P_3 to P_2 and P_2 to P_1 for the R^+ and R^- columns respectively. So the re-equilibration is allowed.

Mode 4: <u>Two reservoirs with an ion exchanger (cation or anion)</u>

This mode is different from the other three modes as explained on the previous pages. The system has two columns, two reservoirs and two pH-converters (dialyzers and titrators) as shown in Figure 9. Both columns are packed with an ion exchanger (either cation or anion). The pH levels for the top and bottom reservoirs are maintained respectively at P_1(8) and P_2(6). Between the top and the bottom columns are two pH-converters, for automatic changing the pH from P_1 to P_2 or from P_2 to P_1 and also maintained the ionic strength constant. Initially, the top reservoir and both columns are filled with a mixture of the concentration y_0. The equilibration of both

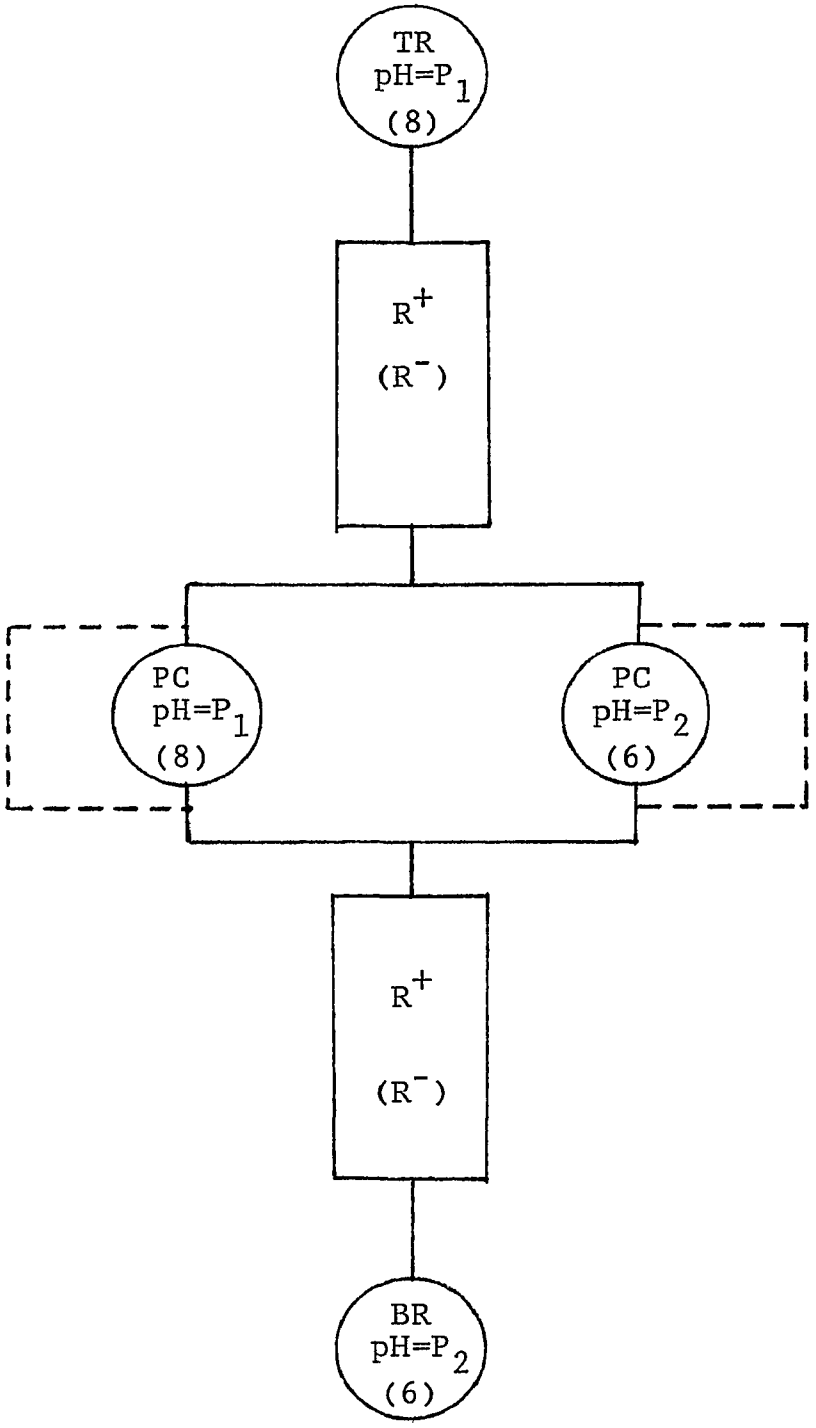


FIGURE 9 - TWO COLUMN DIAGRAM, PACKING EITHER WITH ANION OR CATION EXCHANGER FOR MODE 4

columns are at P_2(=6). A complete cycle of operation is described as follows:

(1) Transfer down: The fluid in TR (P_1) is transferred to the top column (M = 1), and the content in the top column (P_2) goes through the pH-converter (P_1) and transfers to the bottom column (M = 2). At the same time, the content in the bottom column (P_2) is transferred to the BR.

(2) Equilibration: The pH in both columns are changed from P_2 to P_1. Thus, the equilibration is allowed in both columns.

(3) Transfer up: The content in the BR (P_2) is transferred back to the bottom column (M = 2), while the content in the column goes through the pH-converter (P_2) to the top column (M = 1). The fluid in the top column (P_1) is transferred to the TR.

(4) Equilibration: Again, the re-equilibration in both columns are re-established due to the changing in pH from P_1 to P_2. Thus, one cycle of operation is completed.

Note: There is a minor change with a Semi-Continuous process which will be discussed in the next Chapter.

Chapter III

THEORY

Equilibrium Theory - Graphical Solution

1. One-Column System

The first system we will consider is shown in Figure 10. It consists of a column packed with an ion exchanger (cation or anion) and reservoirs attached to each end. The pump has dead volumes V_T and V_B for the top and bottom reservoirs respectively. Initially, the mixture to be separated fills the column voids, the top reservoir, and the bottom dead volume. The top reservoir is maintained at a low pH level (P_2) by an automatic titrator, while a second titrator is used to keep the bottom reservoir at a high pH level (P_1). The ionic strengths of the buffer solutions in both top and bottom reservoirs are kept at IS_2 and IS_1, respectively, by means of two hollow fiber dialyzers. The flow system has four distinct stages in each cycle:

(I) The low pH (P_2) fluid from the top reservoir enters the top of the column, while the solution emerging from the other end enters the bottom reservoir. The displacement Qt_I is set to be the void volume of the column V_ϵ ; that is $Qt_I = V_\epsilon$.

(II) Circulation between the top reservoir and the column: This will ensure a complete shift of the pH and ionic strength in the column to P_2 and IS_2, respectively. Also, at the end of the stage, the concentrations in both the top reservoir and column will be identical.

(III) The high pH (P_1) fluid from the bottom reservoir enters the bottom of the column, and the solution emerging from the other end enters the top reservoir, with the displacement $Qt_{III} = V_\epsilon$ and

(IV) Circulation between the column and the bottom reservoir: This will allow the pH and ionic strength to shift back to P_1 and IS_1, respectively, and at the end of the stage the concentration in the column will be the same as that in the bottom reservoir.

Note that the flow rate within the column is always equal to the reservoir displacement rate Q. The duration of circulation t_{II} or t_{IV}, which can be determined experimentally, depends on V_ϵ and V_B (or V_T), and the pH and ionic strength in both the column and reservoir.

Figure 11 is a graphical solution for one-column system. The assumption made here are:

1. The solute will be distributed between the solid and fluid phases according to a linear form from Equation 1.2,

$$x = ky$$

where k is a function of pH and ionic strength.

2. The duration of curculation t_{II} or t_{IV}, is long enough so that at the end of the stage II or IV a phase equilibrium is established.

In Figure 10, the pump consists of a column packed with cation exchanger and reservoirs attached to each end. The pH values of the top and bottom reservoirs are maintained at given levels P_2(=6) and P_1(=8), respectively. The operation begins with the column filled with a mixture of concentration y_0, every where at equilibrium with solid. The initial pH in the column is high (P_1 = 8). Also, there is fluid of the same initial concentration in the top reservoir. The first fluid motion is downward, and $V_T = V_B$. Let x and y be the concentrations of A in the solid and fluid phases, respectively. Using Equation 1.2, we draw two equilibrium lines (with slopes equal to k_{P_1} and k_{P_2}) on an x - y diagram. The initial concentration in the column (y_0;x_0) is represented by the point 0. One cycle of the operation includes four steps, and the effect of the operation in the first cycle is as follows:

(1) Transfer down: The fluid in the TR (top reservoir) is transferred to the column, and the fluid in the column is transferred to the BR (bottom reservoir). Therefore, the bottom reservoir concentration for the first cycle is y_0.

(2) Circulation and Equilibrium at P_2: The column pH

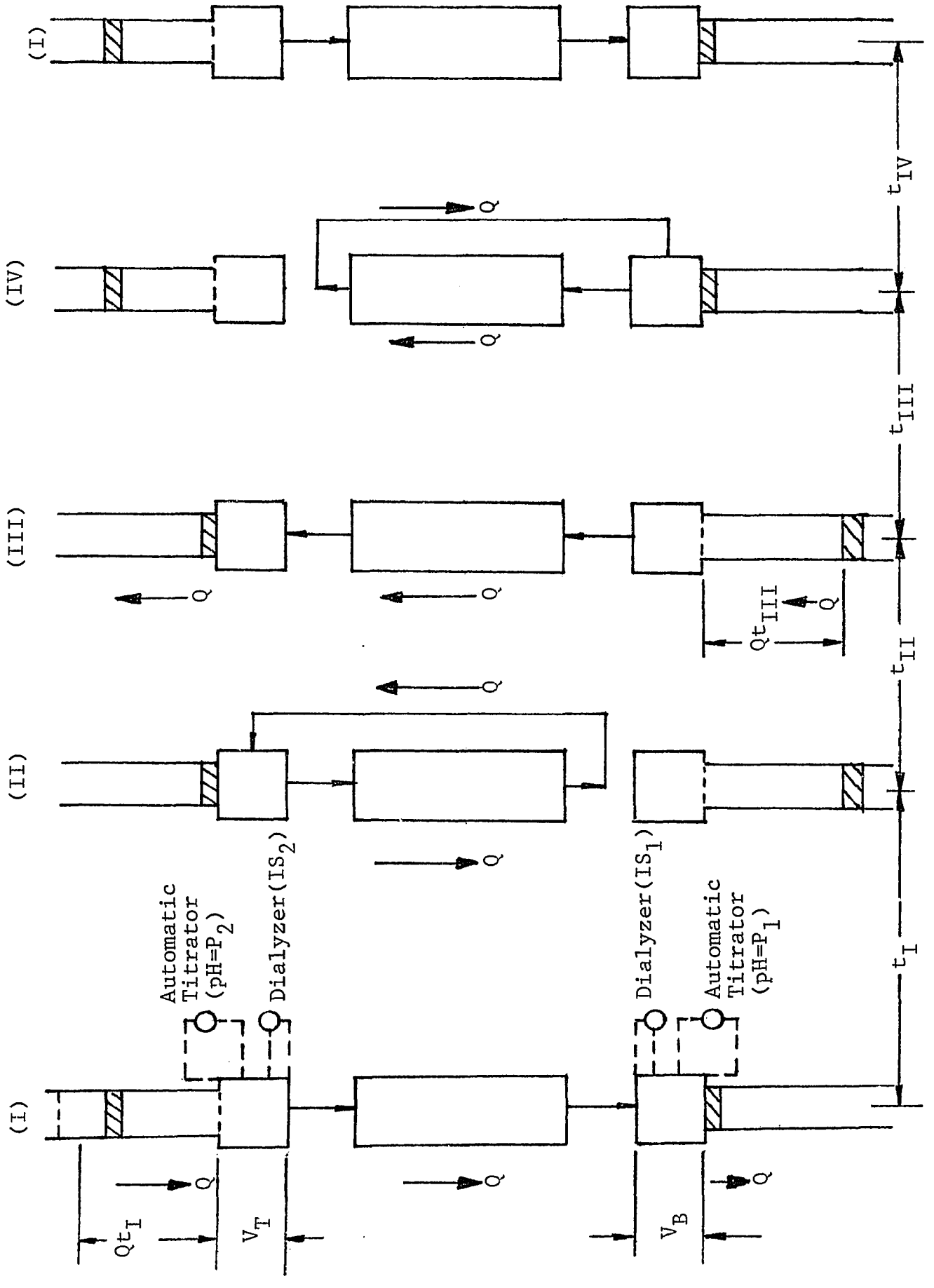


FIGURE 10 - SCHEMATIC OF ONE COLUMN SYSTEM

is changed from P_1 to P_2. The two phases are then allowed to equilibrate at P_2. This leads to a new composition in the column $(y_{T1};x_{T1})$, represented by the point T1. The point is located at the intersection of equilibrium line k_{P_2} and of the operating line passing through $(y_0;x_0)$. The slope of the operating line is $-(V_T + V)/\bar{V}$, and is obtained by the mass balance constraint, i.e.,

$$(V_T + V)y_{T0} + \bar{V}x_{B1} = (V_T + V)y_{T1} + \bar{V}x_{T1} \qquad (2.1)$$

or

$$(V_T + V)y_{T(n-1)} + \bar{V}x_{Bn} = (V_T + V)y_{Tn} + \bar{V}x_{Tn} \qquad (2.2)$$

where n = 1, 2, ...

Also $y_{T0} = y_0$, $x_{B1} = x_0$, and $V = V_\epsilon$.

(3) Transfer up: The solution in the column is brought to the TR and the solution in the BR is returned to the column. The composition in the column is now $(y_{B1};x_{T1})$.

(4) Circulation and Equilibration at P_1: The column pH is shifted back to P_1. A phase equilibrium is re-established. The new equilibrium point $(y_{B2}; x_{B2})$, represented by the point B2, is located at the intersection of the equilibrium line k_{P_1} and of the operating line passing through $(y_{B1};x_{B1})$ and having a slope of $(V_B + V)/\bar{V}$, and is obtained from the following mass balance,

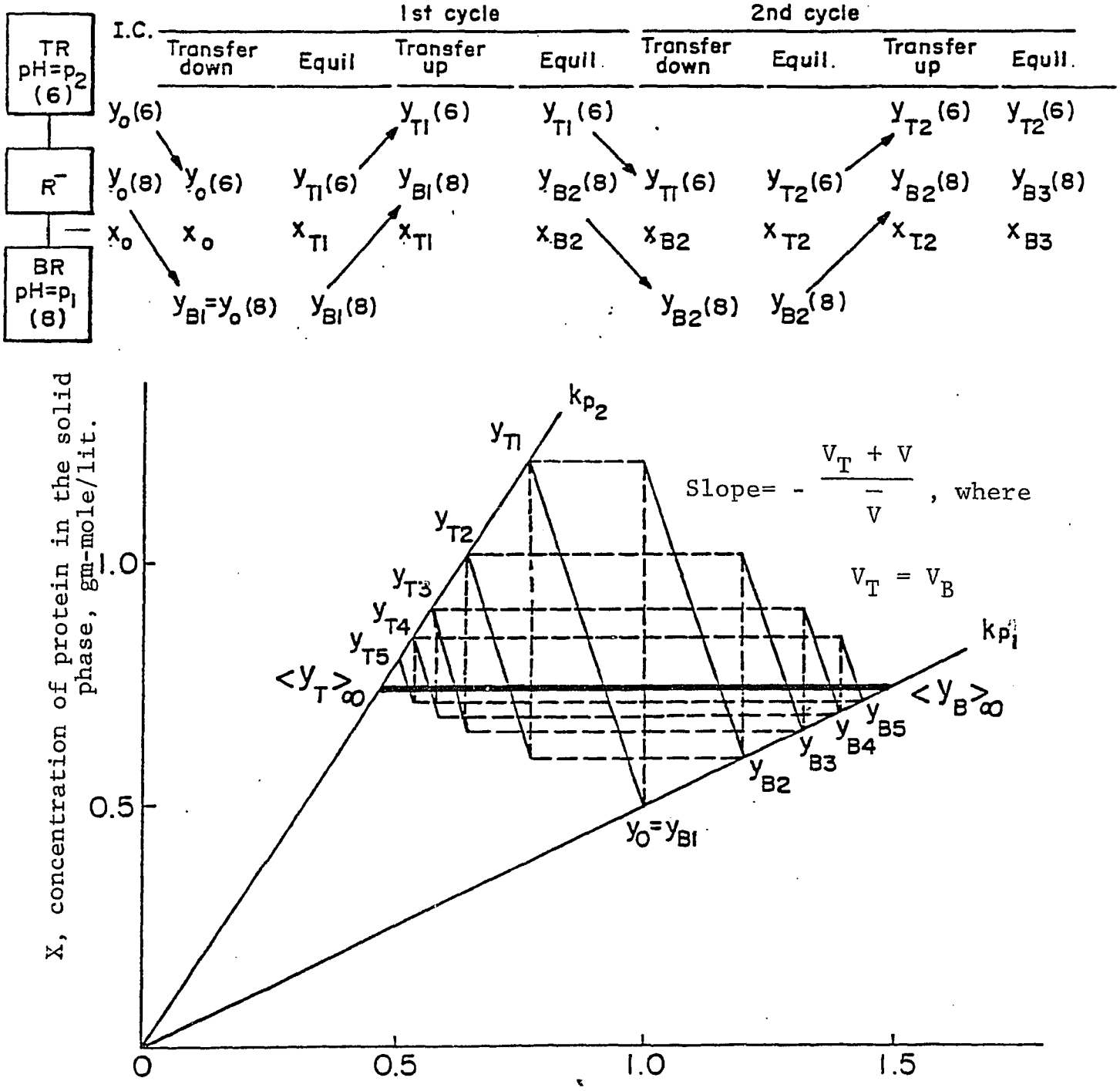


FIGURE 11 - GRAPHICAL SOLUTION FOR ONE COLUMN SYSTEM

$$(V_B + V)y_{B(n-1)} = (V_B + V)y_{Bn} + \bar{V}x_{Bn} \qquad (2.3)$$

where n = 2, 3, ..., and $V = V_\epsilon$.

This completes the first cycle. The second cycle will start from a transfer of the fraction y_{T1} from the TR to the column and the fraction y_{B2} to the BR. We then follow the steps described above. (See Figure 11). If the procedure is repeated in each of the succeeding cycles, one can see that as n becomes large, the top and bottom reservoir concentrations will approach steady values, i.e., $\langle y_T \rangle_\infty$ and $\langle y_B \rangle_\infty$, respectively. At steady state, the solid phase has a constant composition which is in equilibrium with both $\langle y_T \rangle_\infty$ and $\langle y_B \rangle_\infty$, i.e.,

$$x_\infty = k_{p_1} \langle y_B \rangle_\infty = k_{p_2} \langle y_T \rangle_\infty \qquad (2.4)$$

and therefore, the line $\overline{T_s B_s}$ must be paralleled to the y axis.

Note that the graphical method described above is based on a simple discrete transfer equilibrium model (Pigford et al., 1969; Jenczewski and Myers, 1970; Wankat, 1974b; Grevillot and Tondeur, 1976; 1977).

2. Two-Column Systems

We will consider four modes of two column system shown in Figures 6, 7, 8, and 9. For all systems but one, Mode 4,

one column is packed with a cation exchanger (R^-) and the other with an anion exchanger (R^+). The flow rate within the column is always equal to the reservoir displacement rate Q. For both down and up flow the reservoirs have the same displacement. It is assumed that we are concerned with the separation of a two-protein system. The two proteins, A and B, have the isoelectric points I_A and I_B, respectively, and $P_3 < I_B < P_2 < I_A < P_1$, where P_1, P_2, and P_3 are the pH levels in the reservoirs. Thus, both A and B will bear negative charges at P_1, and positive charges at P_3, while A and B will carry a positive and negative charge, respectively at P_2. Therefore, A will be taken up by a suitable cationic exchanger at P_2 or P_3 and released at P_1, The reverse effect will occur if an anion exchanger is selected. The steady state concentrations in the reservoirs are graphically shown in Figures 13, 15, and 17. For the purpose of simplification, it is assumed that for protein A, $k^-_{p_2} = k^-_{p_3}$ and $k^+_{p_2} = k^+_{p_3}$, and for protein B, $k^+_{p_1} = k^+_{p_2}$ and $k^-_{p_1} = k^-_{p_2}$. However, other conditions are conceivable. Two basic separation problems will be considered, i.e., enrichment and splitting:

A. Enrichment

The model to be discussed below is for an enrichment process. Our aim is to obtain a product in which the concentration of a component is larger than the corresponding concentration in the feed.

Mode 1: The system has three reservoirs as shown in Figure 12. The pH level for the top and bottom reservoirs is maintained at P_1 and that for the middle reservoir is kept at P_2. Initially, the top reservoir, the dead volumes for the middle and bottom reservoirs, and both columns are filled with a mixture of the concentration y_0. The R^- and R^+ columns are respectively in equilibrium at P_1 and P_2. One cycle of operation is:

(I) Pump the fluid from the top reservoir (TR) through the R^+ column, the middle reservoir (MR) and the R^- column to the bottom reservoir (BR), for time t_I.

(II) Circulate the fluid between the TR and the R^+ column, and between the MR and the R^- column, for time t_{II}.

(III) Pump the fluid from the bottom reservoir through the R^- column, MR and R^+ column to the top reservoir, for time t_{III}, and

(IV) Circulate the fluid between the MR and the R^+ column, and between BR and the R^- column.

The graphical construction for the concentration profile (Figures13a and13b) can be made in the same way as described for the one-column parametric pump. After a certain number of cycles, the concentrations of protein A in the solid phase for both R^+ and R^- columns converge to two limits, x_{R^+} and

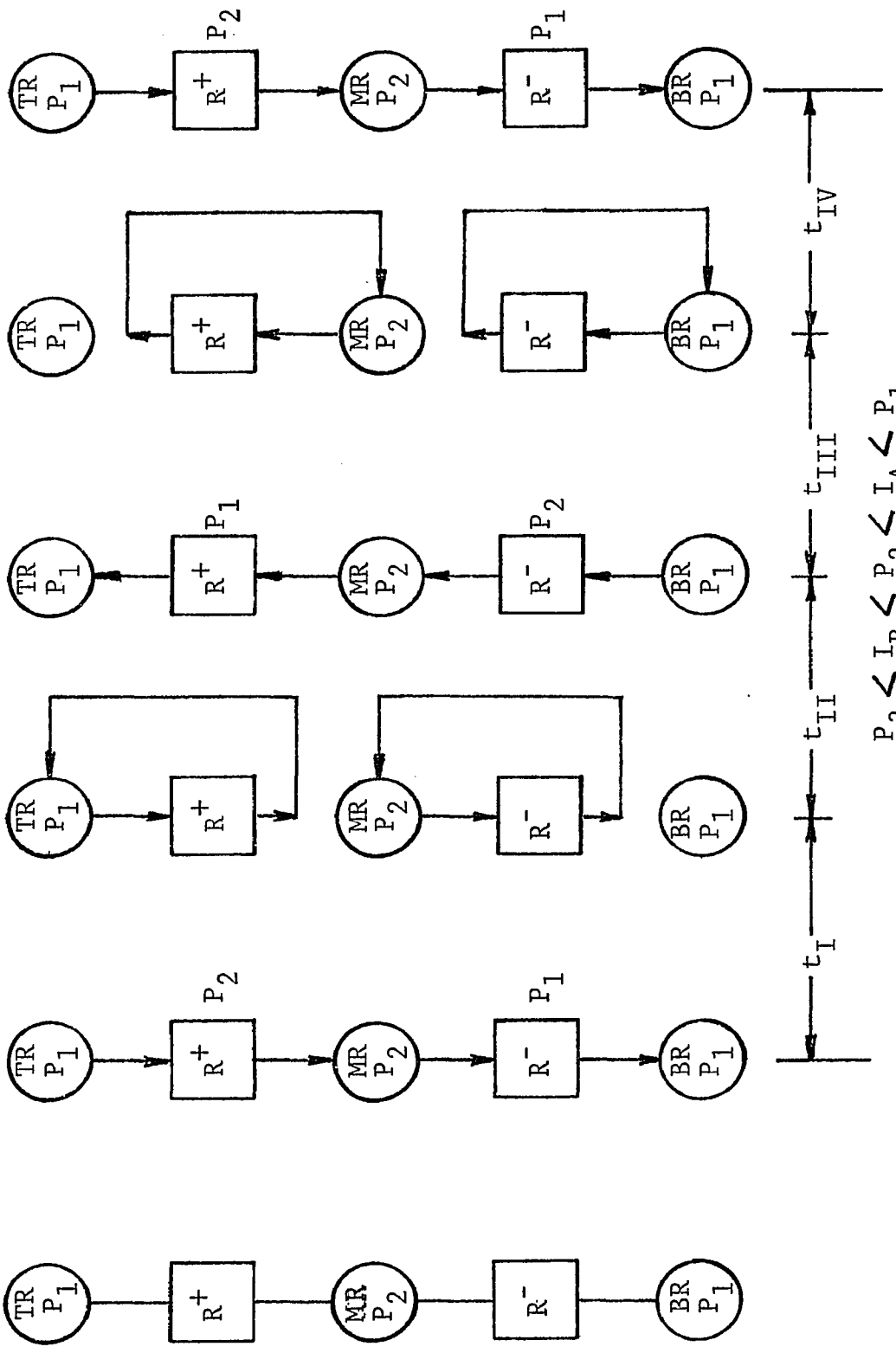


FIGURE 12 - SCHEMATIC OF TWO COLUMN SYSTEM: MODE 1

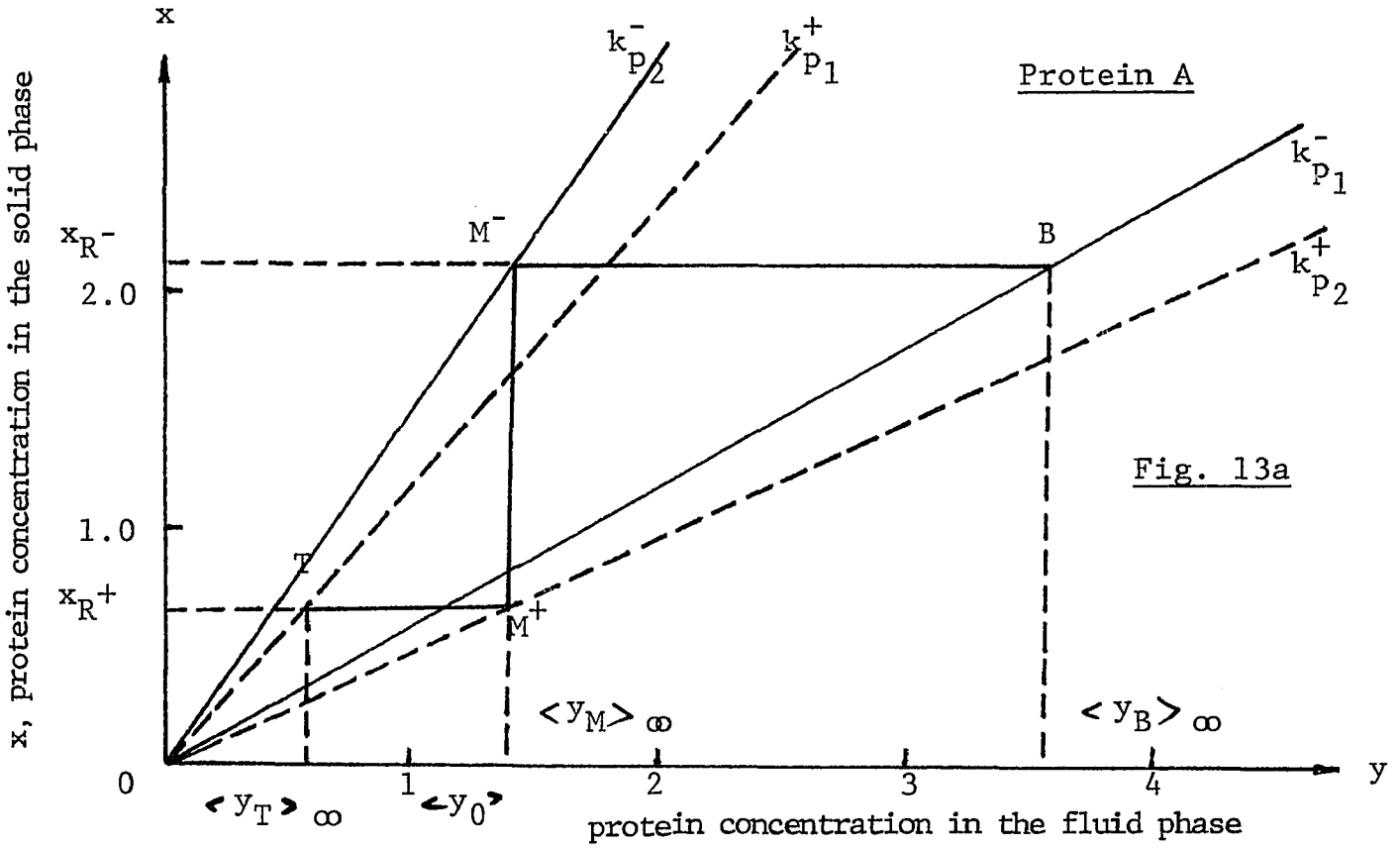


Fig. 13a

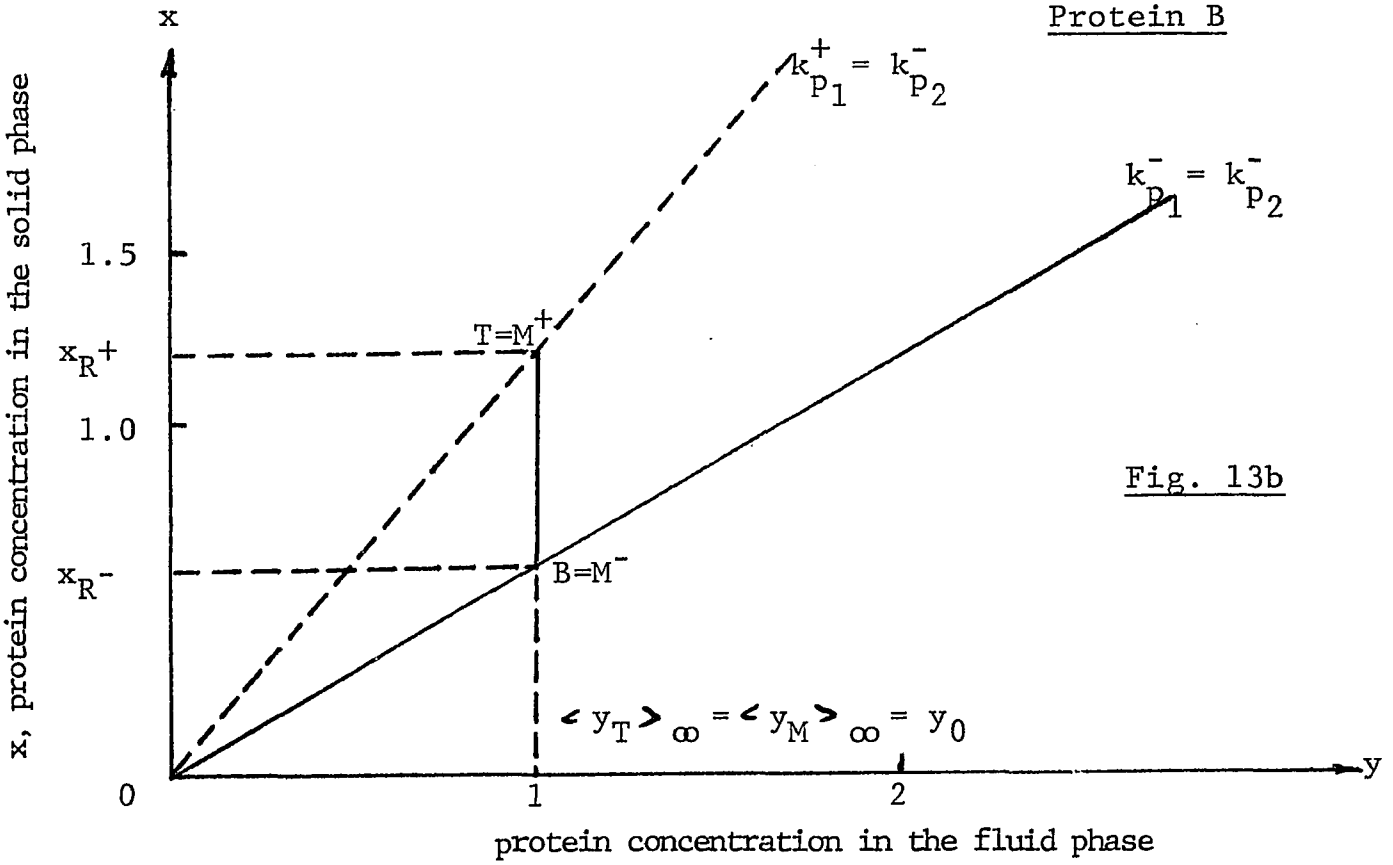


Fig. 13b

FIGURE 13 - GRAPHICAL SOLUTION FOR TWO COLUMN SYSTEM: MODE 1

x_{R^-}, respectively. Thus, a two step staircase is formed. Note that at steady state, the concentration in the middle reservoir is such that it is in equilibrium with both cation and anion exchangers at P_2, i.e.,

$$x_{R^+} = k^+_{P_1} \langle y_T \rangle_\infty = k^+_{P_2} \langle y_M \rangle_\infty$$

and

$$x_{R^-} = k^-_{P_1} \langle y_B \rangle_\infty = k^-_{P_2} \langle y_M \rangle_\infty \qquad (2.5)$$

In the R^+ column, the protein A migrates from the high pH end (P_1) toward the low pH end (P_2), whereas in the R^- column, it moves in the opposite direction. Thus, we accumulate protein A at the high pH end of the R^- column, i.e., the bottom reservoir. By comparing Figures 11 and 13, one can see that the separation factor ($\langle y_B \rangle_\infty / \langle y_T \rangle_\infty$) for the two column system is much higher than that for the one column system. Also, from the diagram on Figure 13, no separation occurs for protein B, i.e., ($\langle y_B \rangle_\infty / \langle y_T \rangle_\infty$) = 1. It should be pointed out that, though B carries the same charge at P_1 and P_2, there may be a difference in the k values at these pH levels (depending on the ionic strength, and some amount of separation may occur on B.

B. Splitting

Modes 2 and 3, shown in Figures 14 and 16 respectively,

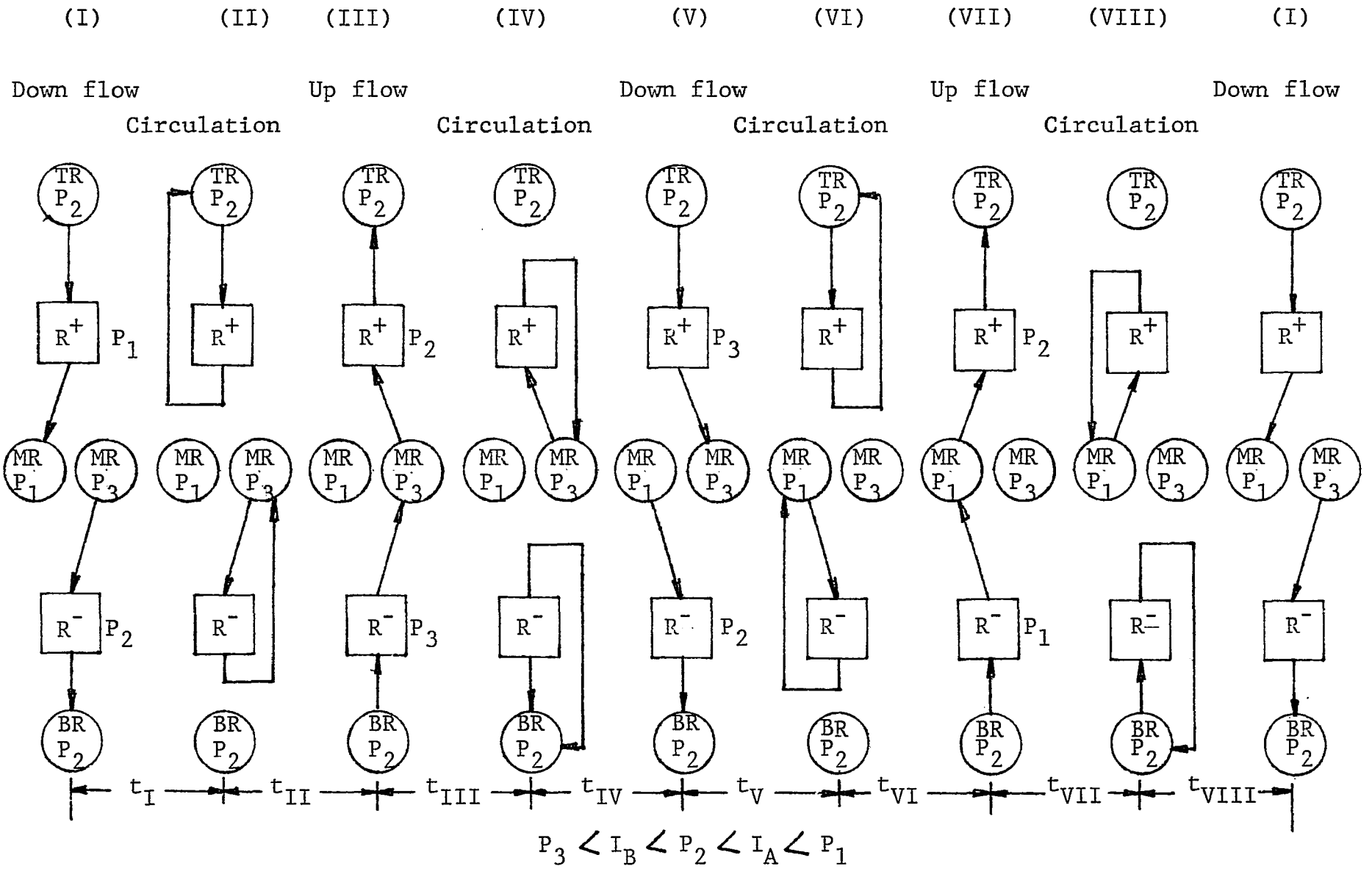


FIGURE 14 - SCHEMATIC OF TWO COLUMN SYSTEM: MODE 2

are for split processes. The purpose of these modes of operation is to separate the desired proteins from each other.

Mode 2: The pump has four reservoirs; one top, two middle, and one bottom reservoirs, respectively with pH = P_2, P_1, P_3, and P_2. Flow sequences (Figure 14) for one cycle are:

(I) Pump the fluid from TR through the R^+ column to the MR (P_1), and, at the same time pump the fluid from the MR (P_3) through the R^- column to the BR, for time t_I.

(II) Circulate the fluid between the TR and the R^+ column, and between the MR (P_3) and the R^- column, for time t_{II}.

(III) Pump the fluid from the BR through the R^- column, the MR (P_3) and the R^+ column to the TR, for time t_{III}.

(IV) Circulate the fluid between the BR and the R^- column, and between the MR (P_3) and the R^+ column, for time t_{IV}.

(V) Pump the fluid from TR through the R^+ column to the MR (P_3), and at the same time pump the fluid from the MR (P_1) through the R^- column to the BR, for time t_V.

(VI) Circulate the fluid between the TR and the R^+ column, and between the MR (P_1) and the R^- column, for time t_{VI}.

(VII) Pump the fluid from the BR through the column, MR (P_1), and R^+ column to the TR, for time t_{VII}, and

(VIII) Circulate the fluid between the BR and the R^- column, and between the MR (P_1) and the R^+ column, for time t_{VIII}.

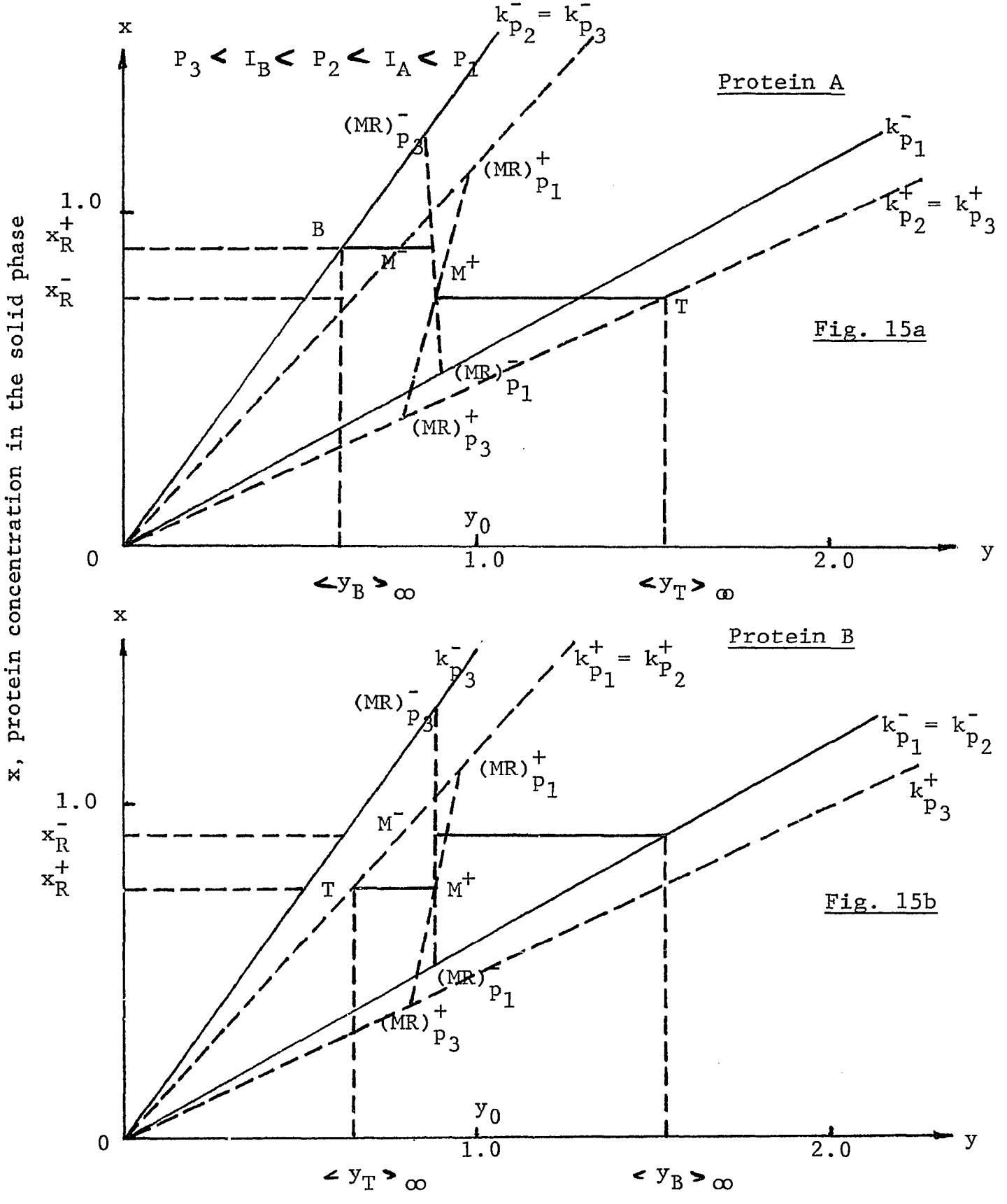
Figures 15a and 15b show the steady state concentrations in the reservoirs. At steady state, the average concentration of the middle reservoirs (MR (P_1) and MR (P_3)) is such that it is in equilibrium with both cation and anion exchangers, i.e.,

$$x_{R^+} = k^+_{p_2} \langle y_T \rangle_\infty = \bar{k}^+ \langle y_{M^+} \rangle_\infty$$
$$x_{R^-} = k^-_{p_2} \langle y_B \rangle_\infty = \bar{k}^- \langle y_{M^-} \rangle_\infty \qquad (2.6)$$

and

$$\langle y_{M^+} \rangle_\infty = 0.5(y_{MR^+_{p_1}} + y_{MR^+_{p_3}}) = \langle y_{M^-} \rangle_\infty$$
$$= 0.5(y_{MR^-_{p_1}} + y_{MR^-_{p_3}}) \qquad (2.7)$$

where $y_{MR^+_{p_1}}$ and $y_{MR^+_{p_3}}$ are the steady state solute concentrations from the R^+ column to the MR (P_1) and MR (P_3) respectively, whereas $y_{MR^-_{p_1}}$ and $y_{MR^-_{p_3}}$ are those from the R^- column to the MR (P_1) and MR (P_3), respectively.



x, protein concentration in the solid phase

y, protein concentration in the fluid phase

FIGURE 15 - GRAPHICAL SOLUTION OF TWO COLUMN SYSTEM: MODE 2

The results in Figures 15a and 15b show that, A and B move in the opposite directions, but in this case, protein A migrates upward to the top reservoir (pH = P_2), while B moves downward to the bottom reservoir (pH = P_2).

Mode 3: The system contains five reservoirs, tow top, one middle, and two bottom reservoirs, respectively with pH = P_1, P_3, P_2, P_1 and P_3 (Figure 16). The flow system has eight distinct steps in each cycle:

(I) Pump the fluid from the TR (P_1) through the R^+ column to the MR and the R^- column to the BR (P_1), for time t_1.

(II) Circulate the fluid between the TR (P_1) and R^+ column, and between the MR and the R^- column, for time t_{II}.

(III) Pump the fluid from the BR (P_3) through the R^- column to the MR and the R^+ column to the TR (P_1), for time t_{III}.

(IV) Circulate the fluid between the BR (P_3) and the R^- column, and between the MR and the R^+ column, for time t_{IV}.

(V) Pump the fluid from the TR (P_3) through the R^+ column, MR and R^- column to the BR (P_3), for time t_V.

(VI) Circulate the fluid between the TR (P_3) and the R^+ column, and between the MR and the R^- column, for time t_{VI}.

(VII) Pump the fluid from the BR (P_1) through the R^- column, MR and R^+ column to the TR (P_3), for time t_{VII}.

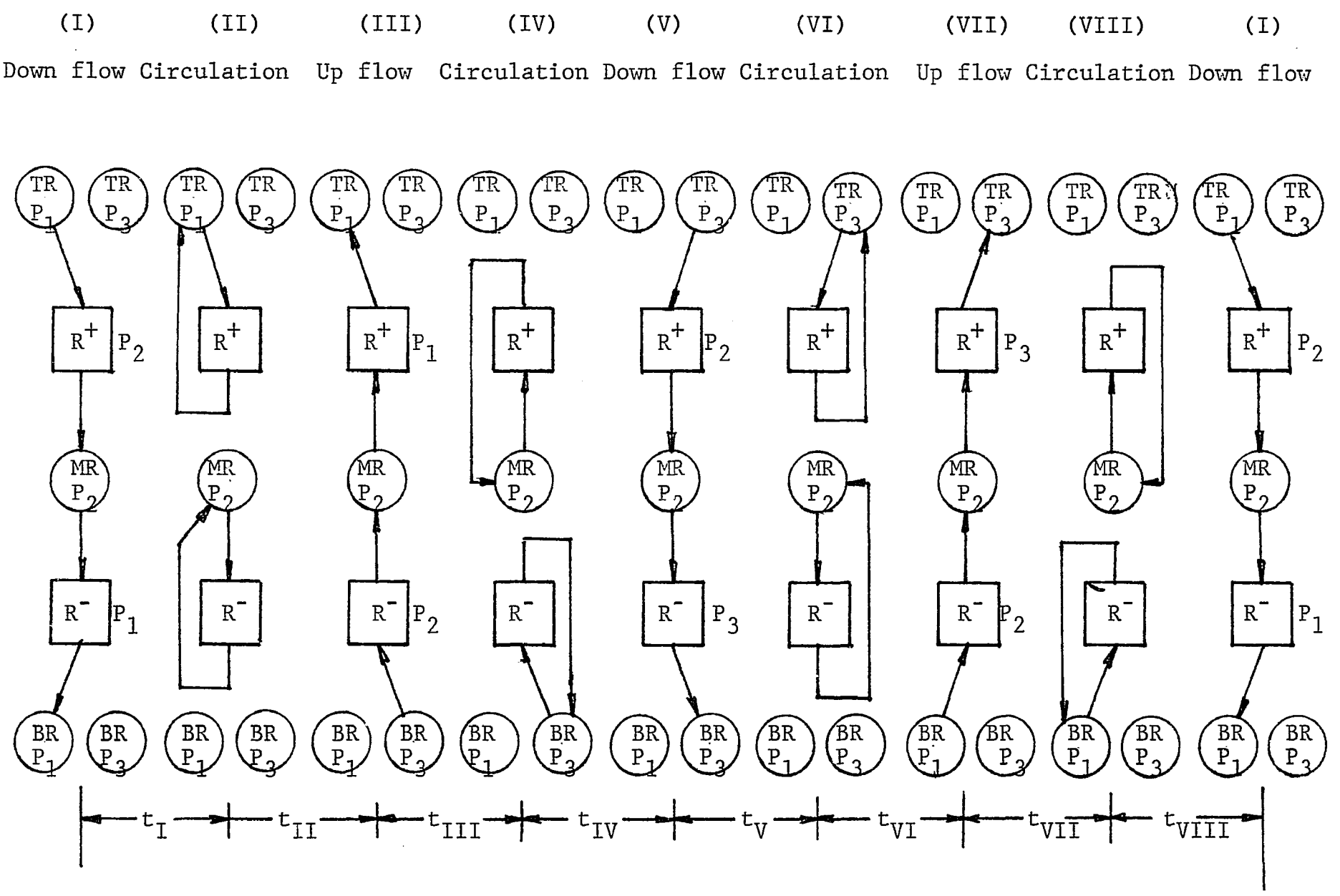


FIGURE 16 - SCHEMATIC OF TWO COLUMN SYSTEM: MODE 3

(VIII) Circulate the fluid between the BR (P_1) and the R^- column, and between the MR and the R^+ column for time t_{VIII}.

The steady state concentrations in the reservoirs are graphically presented in Figures 17a and 17b. At steady state ($n \rightarrow \infty$)

$$x_{R^+} = k^+_{p_1} \left[\langle y_T \rangle_\infty \right]_{P_1} = k^+_{p_3} \left[\langle y_T \rangle_\infty \right]_{P_3} = k^+_{p_2} \left[\langle y_M \rangle_\infty \right]$$

and

$$x_{R^-} = k^-_{p_1} \left[\langle y_B \rangle_\infty \right]_{P_1} = k^-_{p_3} \left[\langle y_B \rangle_\infty \right]_{P_3} = k^-_{p_2} \left[\langle y_M \rangle_\infty \right] \qquad (2.8)$$

By connecting the points T_{P_1}, T_{P_3}, $M^+_{P_2}$, $M^-_{P_2}$, B_{P_3} and B_{P_1}, a two step staircase is formed for both proteins, A and B. However, the concentration of A in the bottom reservoir BR (P_1) ($\left[\langle y_B \rangle_\infty \right]_{P_1}$) is much higher than that in the TR (P_1) ($\left[\langle y_T \rangle_\infty \right]_{P_1}$), while the concentration of B in the top reservoir TR (P_3) ($\left[\langle y_T \rangle_\infty \right]_{P_3}$) is much greater than that in the BR (P_3) ($\left[\langle y_B \rangle_\infty \right]_{P_3}$). This separation phenomena can be explained as follows: The pH levels, P_1 and P_2, and P_2 and P_3, respectively bracket the isoelectric points of A and B, i.e., $P_2 < I_A < P_1$ and $P_3 < I_B < P_2$. Thus, in the R^- column,

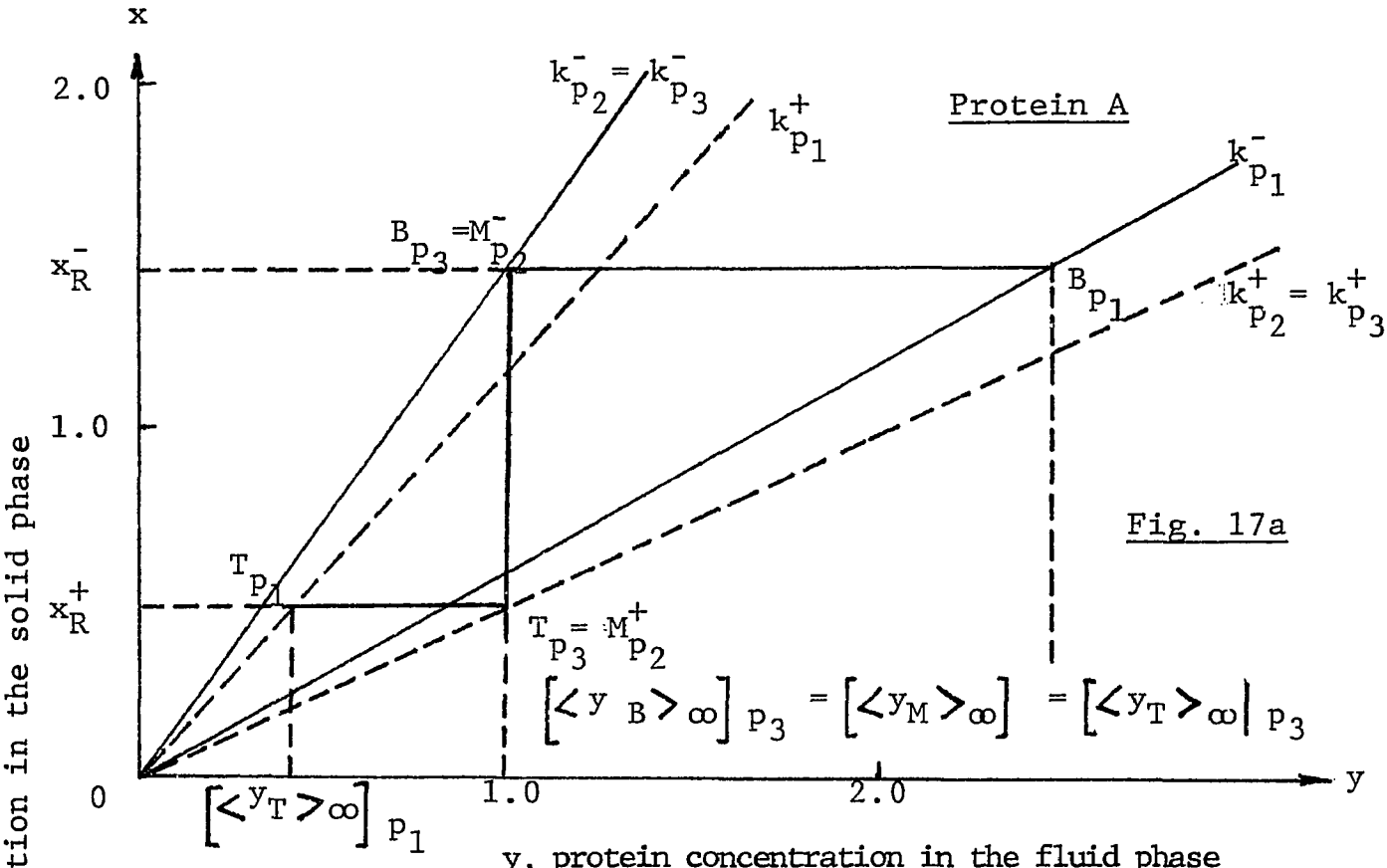


Fig. 17a

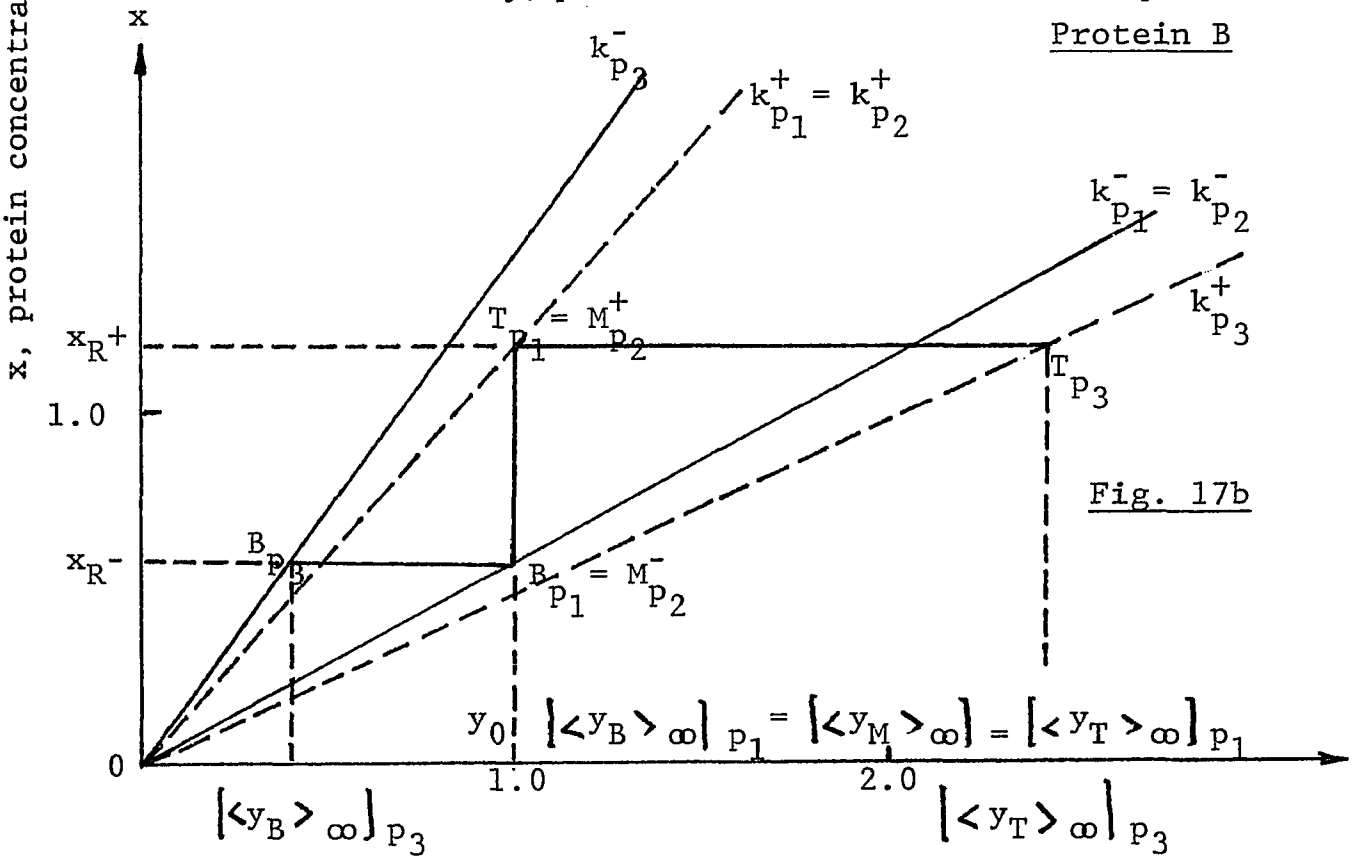


Fig. 17b

FIGURE 17 - GRAPHICAL SOLUTION OF TWO COLUMN SYSTEM: MODE 3

A and B respectively migrate toward the BR (P_1) and the MR (P_2), whereas in the R^+ column, A and B respectively, move toward the MR (P_2) and TR (P_3). In the other words, A and B migrate in opposite directions and concentrate respectively in BR (P_1) and TR (P_3).

The results shown in Figures17a and17b are very similar to those for Mode 2. Also, by comparing Figures15a and 15b, and Figures 17a and17b one can see that separations by Mode 3 are better than those by Mode 2, i.e.,

$$\text{For protein A: } \left[\frac{[\langle y_B\rangle_\infty]_{P_1}}{[\langle y_T\rangle_\infty]_{P_1}}\right]_{\text{Mode 3}} > \left[\frac{\langle y_T\rangle_\infty}{\langle y_B\rangle_\infty}\right]_{\text{Mode 2}}$$

$$\text{For protein B: } \left[\frac{[\langle y_T\rangle_\infty]_{P_3}}{[\langle y_B\rangle_\infty]_{P_3}}\right]_{\text{Mode 3}} > \left[\frac{\langle y_B\rangle_\infty}{\langle y_T\rangle_\infty}\right]_{\text{Mode 2}} \qquad (2.9)$$

Mode 4: The system has two reservoirs; one top and one bottom reservoirs, with pH = P_1 and P_2 respectively. Also the pH-converters are connected to the columns as shown in Figure 18. Flow sequences for one completed cycle are:

(I) Pump the fluid from TR (P_1) to the first column (R^-) while the content from the R^- column (P_2) is transferred through the pH-converter (P_1) to the second column (R^-). At the same time, the fluid

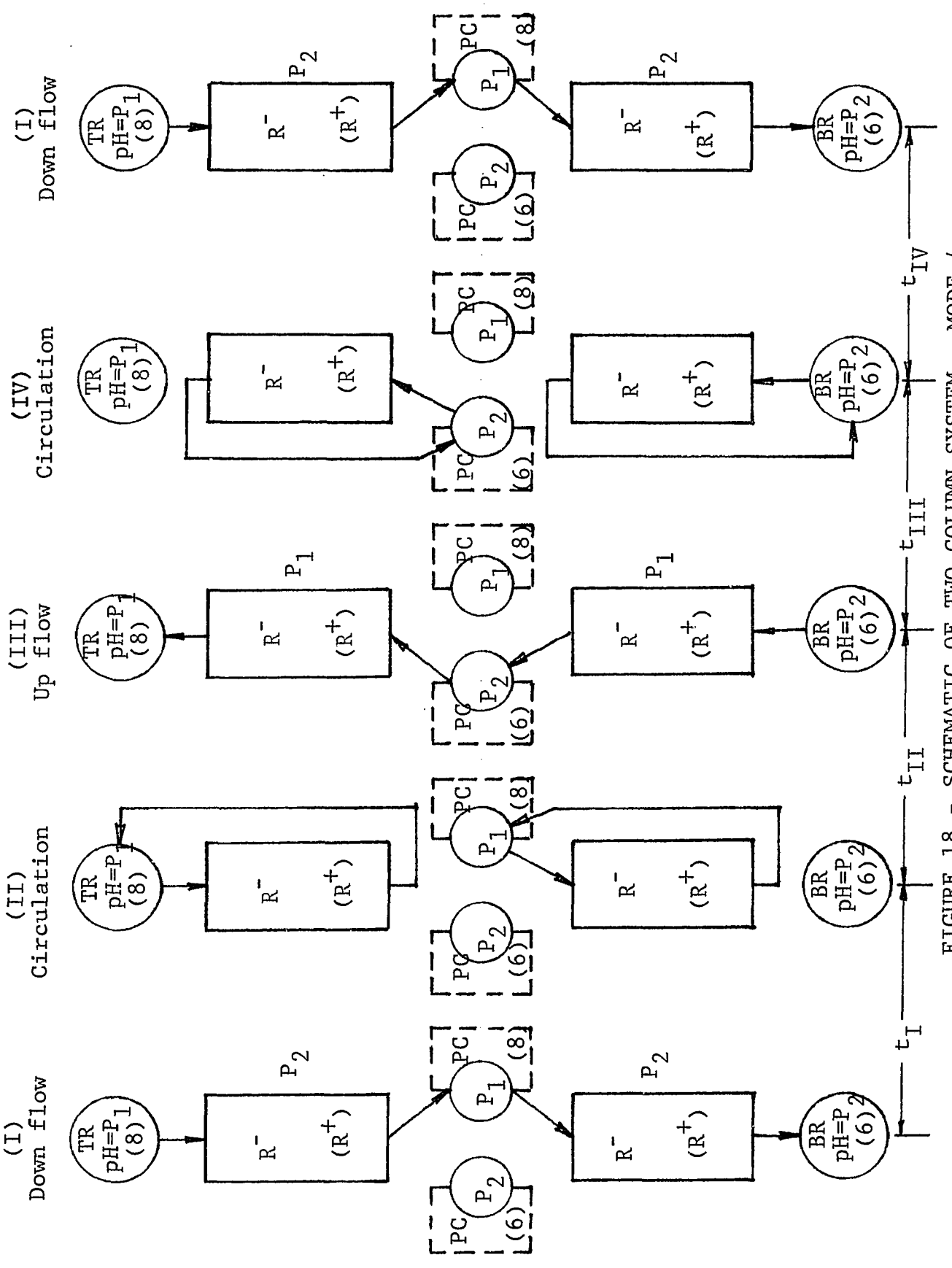


FIGURE 18 - SCHEMATIC OF TWO COLUMN SYSTEM: MODE 4

from the second column (P_2) is transferred to BR, for time t_I.

(II) Circulate the fluid between the TR and the first column and between the pH-converter and the second column, for time t_{II}.

(III) The fluid from the BR (P_2) is pumped back to the second column. Meanwhile, the fluid in the second column (P_1) is transferred through the pH-converter (P_2) to the first column. Also, the fluid from the first column transfers to the TR, for time t_{III} and

(IV) Circulate the fluid between the first column and the pH-converter, and between the second column and the BR.

Figures 19a and 19b show the steady state concentration in the reservoirs. After a certain number of cycles, the concentrations of protein A in the solid phase for both R^- columns converge to two limits, x_{R^-} (at M = 1) and x_{R^-} (at M = 2). Thus, a two step staircase is formed, i.e.,

$$x_{R^-}{}_{(M=1)} = k^-_{p_1}\left[\langle y_T\rangle_\infty\right]_{P_1} = k^-_{p_2}\left[\langle y_M\rangle\right]$$

and

$$x_{R^-}{}_{(M=2)} = k^-_{p_2}\left[\langle y_B\rangle_\infty\right]_{P_2} = k^-_{p_1}\left[\langle y_M\rangle\right] \qquad (2.10)$$

The results of this system is similar to Mode 1. The

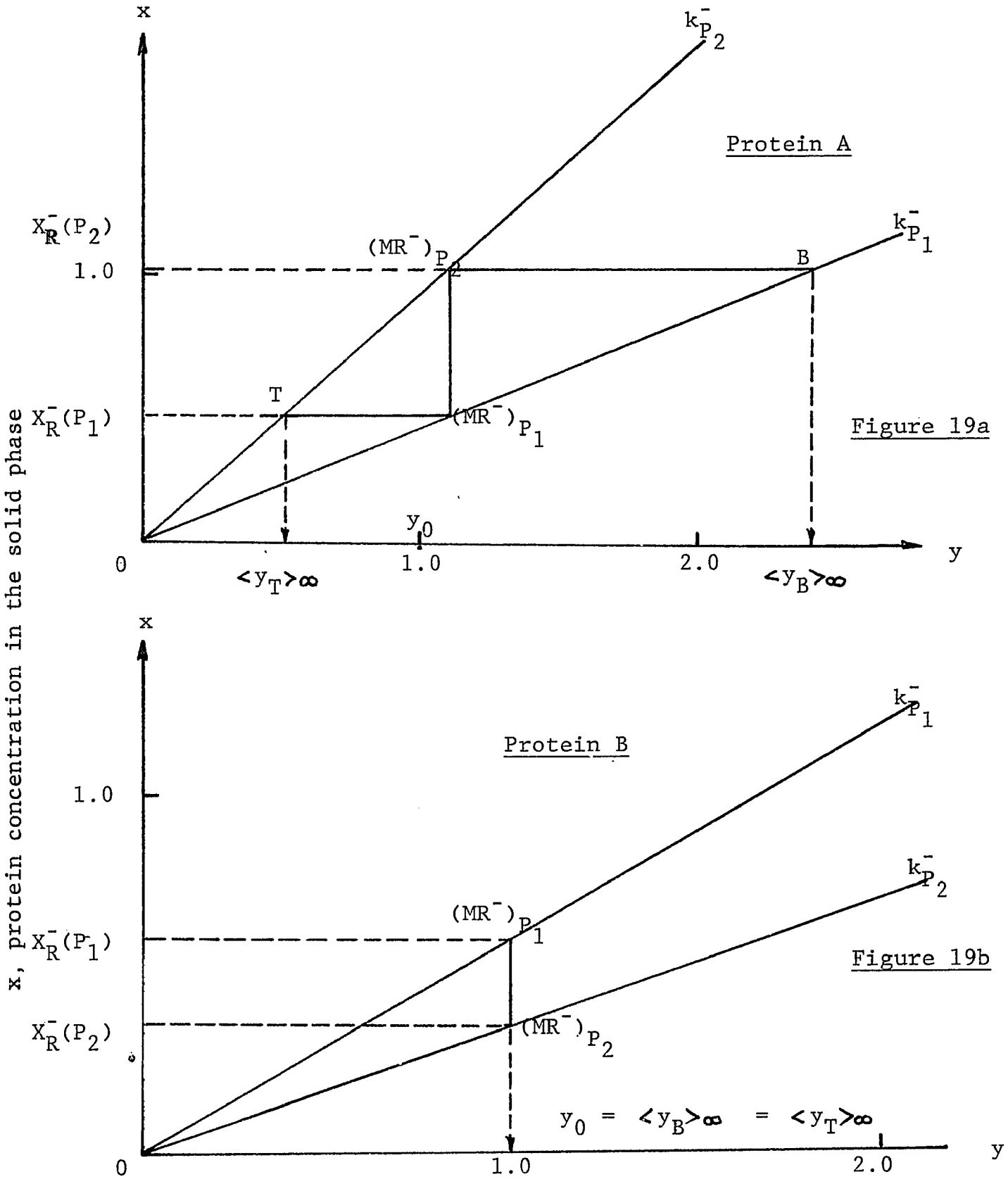


Figure 19a

Figure 19b

x, protein concentration in the solid phase

y, protein concentration in the fluid phase

FIGURE 19 - GRAPHICAL SOLUTION OF TWO-COLUMN SYSTEM: MODE 4.

protein A migrates from the low pH end (P_2) toward the high pH end (P_1), also Figure 19b shows no separation occurs for protein B.

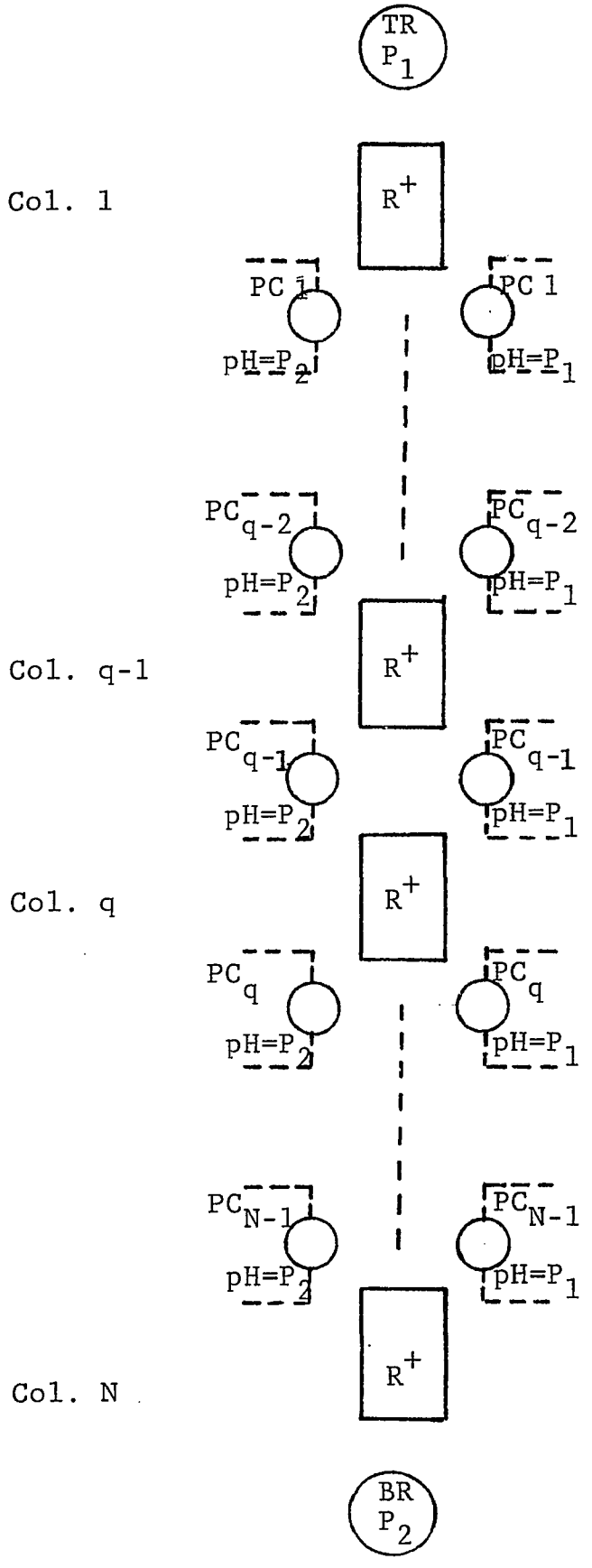
Chapter IV

ANALYTICAL SOLUTION

Formal Mathematical Solution

Figure 20 shows the principle of the discrete transfers and equilibrations for a total reflux parapump, with a single transfer per half-cycle. The system consists of N columns, which are packed with an ion exchanger (either cation or anion); and two reservoirs, top and bottom, where maintained at a high pH (P_1) and a low pH (P_2) respectively. Between the columns, the pH-converters are connected as shown in Figure 9.

Let $y_j^1(n)$ designate the protein concentration, in grams per liter, for the fluid phase in the column (fraction stage) number j (j = 0, 1, ... N, and where j = 0 is a reservoir), at the low pH level, during cycle n. In the same manner, $x_q^h(n)$ designates the protein concentration in the solid phase, at the high pH level, in the column or stage q (q = 1, 2, ... N), during cycle n. N is defined as the total number of columns or stages; the number of protein fraction is thus N + 1. With these notations, at the beginning of cycle n (See Figure 20), $y_0^1(n)$ is the concentration in the high pH product reservoir; and in stage q, $y_q^1(n)$ is in equilibrium at low pH with $x_q^1(n)$. Let V be the volume of the fluid phase in each



Col. = Column #
PC = pH-Converter

FIGURE 20 - THE pH-PARAMETRIC PUMPS DIAGRAM FOR MATHEMATICAL APPROACH.

column while $\bar{v}$ is the volume of the solid phase in each column. It is also assumed both are constant.

We shall define a relation between the concentrations in cycle n and in cycle n + 1. By using the material balances and the equilibrium relation within cycle n, the index (n) of the cycle being omitted for simplicity. After a forward transfer, and before any equilibration, stage q contains V liter of liquid phase at concentration y^1_{q-1} and $\bar{V}$ liter of solid phase at concentration s^1_q. After re-equilibration at high pH (P_1), these concentrations become respectively y^h_{q-1} and x^h_q. Conservation of protein implies:

$$x^h_q + \varrho y^h_{q-1} = x^1_q + \varrho y^1_{q-1}; \qquad (3.1)$$

$$(q = 1, 2, \ldots N)$$

where ϱ is the ratio of fluid to solid phase volumes:

$$\varrho = V/\bar{V} \qquad (3.2)$$

The low pH equilibration at the start of the cycle and the high pH equilibration after transfer are expressed by:

$$x^1_q = k^1 y^1_q ; \quad (q = 1, 2, \ldots N) \qquad (3.3)$$

$$x^h_q = k^h y^h_{q-1} ; \quad (q = 1, 2, \ldots N) \qquad (3.4)$$

where k^1 and k^h are the linear (See Figure 21) equilibrium constants for low pH and high pH respectively. Equations 3.3 and 3.4 are substituted into Equation 3.1 and simplify it. Then we shall obtain:

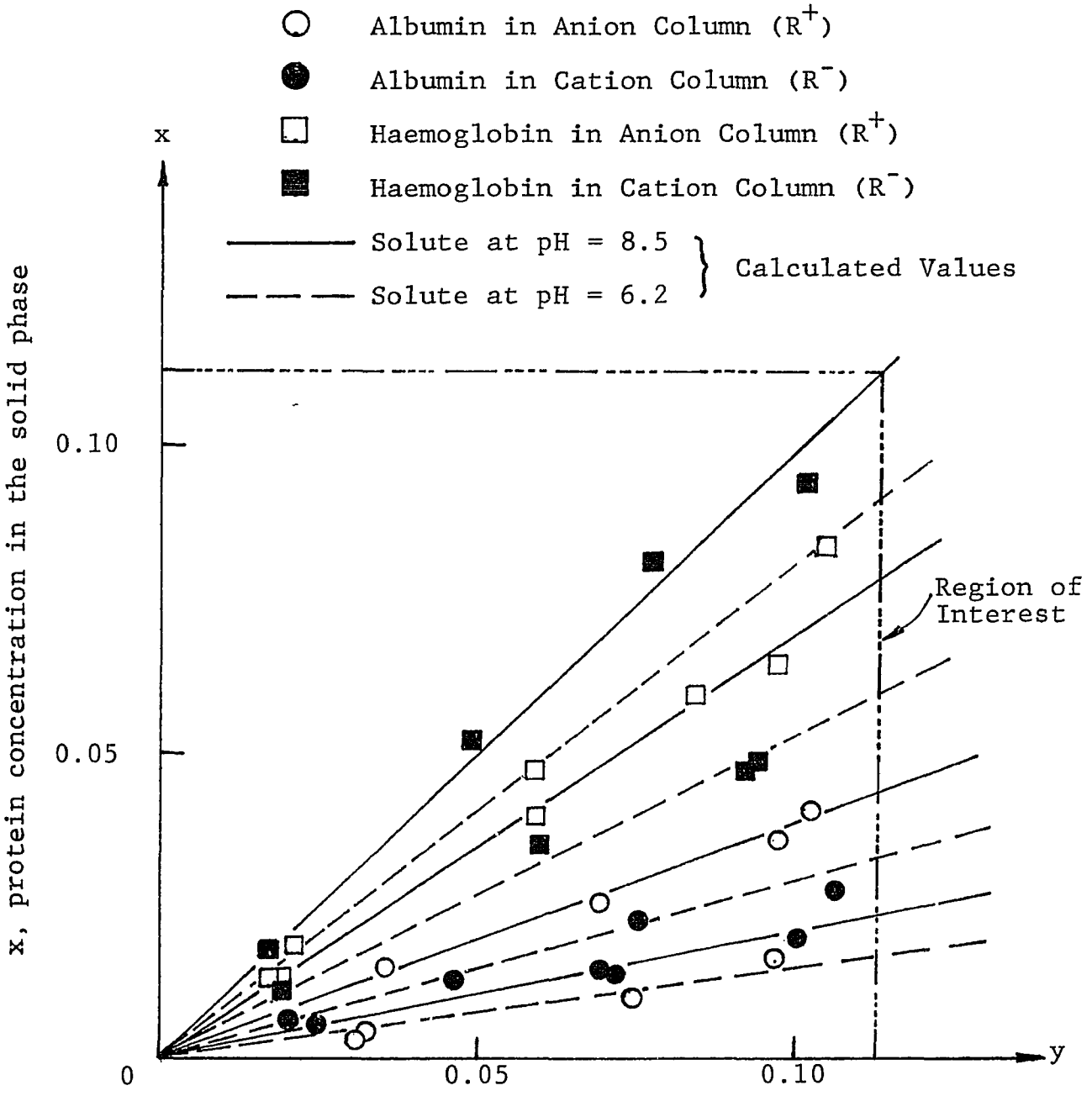


FIGURE 21 - EQUILIBRIUM CONSTANTS FOR THE HAEMOGLOBIN-ALBUMIN SYSTEM: SEPHADEX ION EXCHANGER (REFERENCE 28).

$$y^h_{q-1} = \frac{\varsigma}{\varsigma + k^h}.y^l_{q-1} + \frac{k^l}{\varsigma + k^h}.y^l_q \; ; \qquad (3.5)$$

$$(q = 1, 2, \text{---- } N)$$

Since the last protein fraction is in the low pH reservoir during the high pH equilibration, it undergoes no exchange, and also no concentration change; thus:

$$y^h_N = y^l_N \qquad (3.6)$$

Equation 3.5 and 3.6 from a system of N+1 linear difference equations relating the y^h's to the y^l's in the matrix form and be expressed by:

$$\underset{\sim}{Y}^h(n) = [\underset{\sim}{\Theta}_h].\underset{\sim}{Y}^l(n) \qquad (3.7)$$

where $\underset{\sim}{Y}^h$ and $\underset{\sim}{Y}^l$ are the column vectors of the protein fraction concentrations, and $[\underset{\sim}{\Theta}_h]$ is the N+1 dimensional bi-diagonal matrix:

$$[\underset{\sim}{\Theta}_h] = \frac{1}{\varsigma + k^h}\begin{bmatrix} \varsigma & k^l & 0 & \cdots & 0 \\ 0 & \ddots & \ddots & & \vdots \\ \vdots & & \ddots & \ddots & \\ & & & \ddots & k^l \\ 0 & \cdots & & & (\varsigma + k^h) \end{bmatrix} \qquad (3.8)$$

In the same manner, the analysis for the backward half-cycle

leads to a symmetrical relationship between $Y^h(n)$ and the concentration vector $Y^1(n+1)$ which represents the conditions after the low pH re-equilibration, thus at the beginning of cycle n+1 :

$$\underset{\sim}{Y}^1(n+1) = [\underset{\sim}{\Theta}_1] \cdot \underset{\sim}{Y}^h(n) \qquad (3.9)$$

where:

$$[\underset{\sim}{\Theta}_1] = \frac{1}{\rho + k^1} \cdot \begin{vmatrix} (\rho + k^1) & 0 & . & . & 0 \\ k^h & \ddots & & & \\ 0 & \ddots & \rho & & \\ . & & \ddots & \ddots & \\ 0 & . & . & k^h & \rho \end{vmatrix} \qquad (3.10)$$

Then, we combine both Equations 3.7 and 3.9 to yield a complete cycle:

$$\underset{\sim}{Y}^1(n+1) = [\underset{\sim}{M}] \cdot \underset{\sim}{Y}^1(n) \qquad (3.11)$$

where $[\underset{\sim}{M}]$ is the tridiagonal Jacobi matrix of dimension N+1:

$$[\underset{\sim}{M}] = [\underset{\sim}{\Theta}_1][\Theta_h] = \frac{1}{p} \cdot \begin{vmatrix} d & e & 0 & 0 & . & . & 0 \\ a & b & c & 0 & . & & . \\ 0 & a & b & c & 0 & . & . \\ . & \ddots & \ddots & \ddots & \ddots & \ddots & \\ 0 & . & . & & & a & a+b \end{vmatrix} \qquad (3.12)$$

where: $a = \varrho k^h$; $b = \varrho^2 + k^1 k^h$; $c = \varrho k^1$

$$d = \varrho(\varrho + k^1); \quad e = k^1(\varrho + k^1) \quad \text{and} \qquad (3.13)$$

$$p = (\varrho + k^1)(\varrho + k^h) = a + b + c$$

Equation 3.11 is in a form of a linear first order difference equation, so we can solve this equation by recursion

$$\underset{\sim}{Y}^1(n) = [\underset{\sim}{M}]\underset{\sim}{Y}^1(n-1) = [\underset{\sim}{M}]^2\underset{\sim}{Y}^1(n-2) = \ldots\ldots\ldots$$

$$= [\underset{\sim}{M}]^n\underset{\sim}{Y}^1(0) \qquad (3.14)$$

From Equation 3.14 the concentration vector for any cycle can be obtained in terms of the initial concentration vector $\underset{\sim}{Y}^1(0)$. Thus, it can be called "A Solution of the Conservation Equations", and this formal solution is very simple. However, the calculation of the n^{th} power of the matrix $[\underset{\sim}{M}]$ is not a trivial matter if its dimension and n are large. Therefore, the next section covers the calculation of this matter which will lead us into the physical problem.

Calculation of $[\underset{\sim}{M}]^n$

The calculation of the n^{th} power of a matrix by successive multiplication is numerically straight forward, although it may require much time and give little qualitative information. So another method is introduced by the calculation of

the eigenvalues λ of $[\underset{\sim}{M}]$ for which standard numerical methods exist. In this case, much more information can be obtained on the eigenvalues by algebraic means, and a simple rapidly converging numerical method can be used, (See Appendix A) owing to the fact that the matrix is of the Jacobi type (tridiagonal matrix). Once the eigenvalues (λ) are known, the elements of the corresponding eigenvectors are calculated directly by:

$$
\begin{aligned}
y_{1q} &= -\frac{1}{e}(d - p\,\lambda_q)\, y_{0q} \\
y_{2q} &= -\frac{1}{c}(b - p\,\lambda_q)\, y_{1q} + a y_{0q} \\
&\text{----------} \\
y_{jq} &= -\frac{1}{c}(b - p\,\lambda_q)\, y_{j-1,q} + a y_{j-2,q} \qquad (3.15) \\
&\text{----------} \\
y_{Nq} &= -\frac{1}{c}(b - p\,\lambda_q)\, y_{N-1,q} + a y_{N-2,q} \\
q &= 0,\ 1,\ \text{-------}\ N
\end{aligned}
$$

where a, b, c, d, e and p are given by Equation 3.13. As usual, the elements of the eigenvectors are defined up to a multiplicative factor, y_{0q}. Designating, by $[\underset{\sim}{S}]$ the matrix of column eigenvectors of elements y_{jq}, the matrix $[\underset{\sim}{M}]$ may be re-written in a diagonalized form:

$$[\underset{\sim}{M}] = [\underset{\sim}{S}]\,[\underset{\sim}{D}]\,[\underset{\sim}{S}]^{-1} \qquad (3.16)$$

where $[\underset{\sim}{D}]$ is the diagonal matrix of the eigenvalues. Then:

$$[\underset{\sim}{M}]^n = [\underset{\sim}{S}][\underset{\sim}{D}]^n \cdot [\underset{\sim}{S}]^{-1} \tag{3.17}$$

where

$$[\underset{\sim}{D}]^n = \begin{vmatrix} \lambda_0^n & 0 & 0 & 0 & \cdots & 0 \\ o & \lambda_1^n & 0 & 0 & \cdots\cdots & . \\ . & . & . & . & & . \\ . & . & . & & & . \\ & & & & & \\ 0 & . & . & & & \lambda_N^n \end{vmatrix} \tag{3.18}$$

An equivalent approach is the use of Sylvester's Theorem Expression and gives:

$$[\underset{\sim}{M}]^n = \sum_{j=0}^{N} \lambda_j^n \, [\underset{\sim}{A}_j] \tag{3.19}$$

where:

$$[\underset{\sim}{A}_j] = \frac{\text{adj} \; (\lambda_j \underset{\sim}{I} - \underset{\sim}{M})}{\prod_{i \neq j} (\lambda_j - \lambda_i)} \tag{3.20}$$

and adj $(\lambda_j \underset{\sim}{I} - \underset{\sim}{M})$ is the transposed matrix of cofactors of $[\lambda_j \underset{\sim}{I} - \underset{\sim}{M}]$, independent of n. It is seen that the number of cycles (n) appears only as the powers of the eigenvalues, and this allows a quick qualitative look on how the system converges toward its steady state. Here, we shall first try to characterize this steady state.

The Cyclic Steady State

The behavior of the system when the number of cycles (n) becomes large, can be deduced from a close examination of Equations 3.16 to 3.20 and of the eigenvalues. It also can be deduced by physical reasoning. In Appendix A, we demonstrate that all eigenvalues of $[\underset{\sim}{M}]$ are real, positive and smaller than or equal to 1. These conclusions may also be reached by the following considerations:

1. Any negative eigenvalue would bring a contribution to $[\underset{\sim}{M}]^n$, that changes sign every cycle, leading to an oscillatory behavior of certain concentrations.

2. Any eigenvalue larger than one, would lead to an ever increasing contribution to $[\underset{\sim}{M}]^n$, and to infinite concentrations for certain initial conditions.

3. Any positive eigenvalue, smaller than one, has an ever decreasing contribution as n becomes large; if there were no eigenvalue equal to one, $[\underset{\sim}{M}]^n$ would tend toward the zero matrix, and all final concentrations would be zero.

We thus have:

$$0 \leq \lambda_0 \leq \lambda_1 \leq \lambda_2 \leq \text{-----} \leq \lambda_N = 1 \qquad (3.21)$$

From Equations 3.14, 3.19, and 3.20, when n becomes large, the contribution of all eigenvalues different from one disappear and the cyclic steady state is given by:

$$Y^1(\infty) = [\underset{\sim}{M}]^{\infty}\, \underset{\sim}{Y}^1(0) = \frac{[\text{adj } (\underset{\sim}{I} - \underset{\sim}{M})]}{\prod_{i \neq N} (1 - \lambda_i)}\, \underset{\sim}{Y}^1(0) \qquad (3.22)$$

More explicit information is obtained by noting that, in the steady state, we will have:

$$\underset{\sim}{Y}^1(n+1) = \underset{\sim}{Y}^1(n) = \underset{\sim}{Y}^1(\infty) \qquad (3.23)$$

and that this equality is compatible with Equation 3.11 only if $\underset{\sim}{Y}^1(\infty)$ is an eigenvector of matrix $[\underset{\sim}{M}]$. From the discussion above, it must be the eigenvector corresponding to $\lambda_N = 1$. Thus, the components y^*_i of $\underset{\sim}{Y}^1(\infty)$ are calculated by letting $\lambda_q = \lambda_N = 1$ in the set of Equation 3.15. It may easily be verified that the following relations hold between the concentrations y* thus calculate:

$$\frac{y^*_0}{y^*_1} = \frac{y^*_1}{y^*_2} = \text{---} \quad \frac{y^*_j}{y^*_{j+1}} = \text{---} \quad \frac{y^*_{N-1}}{y^*_N} = \frac{k^1}{k^h} = \beta \qquad (3.24)$$

which implies:

$$\frac{y^*_0}{y^*_N} = \beta^N \qquad (3.25)$$

This is the equivalent of Fenske's equation. The steady state composition vector, then may be written in terms of y_0^*, for example:

$$\underset{\sim}{Y}^1(\infty) = \begin{vmatrix} y_0^* \\ y_1^* \\ y_2^* \\ . \\ y_N^* \end{vmatrix} = y_0^* \begin{vmatrix} 1 \\ \beta^{-1} \\ \beta^{-2} \\ . \\ \beta^{-N} \end{vmatrix} \qquad (3.26)$$

An interesting property of this vector is that, it is invariant upon multiplication on the left by $[\underset{\sim}{\Theta}_h]$, which from Equation 3.7, implies that:

$$\underset{\sim}{Y}^h(\infty) = \underset{\sim}{Y}^1(\infty) \qquad (3.27)$$

This means that the compositions of the protein fractions are the same after an equilibration at high pH and low pH. In other words, in the cyclic steady state, all compositions are constant, and no protein transfer occurs between phases.

The geometric interpretation from Equations 3.24 to 3.27 is like the relations in the McCabe-Thiele diagram which is a staircase construction between two straight lines as shown in Figure 22, and is consistent with the graphical method from the previous chapter. The steady state composition vector, in Equation 3.26, is defined up to the value of y_0^*. This

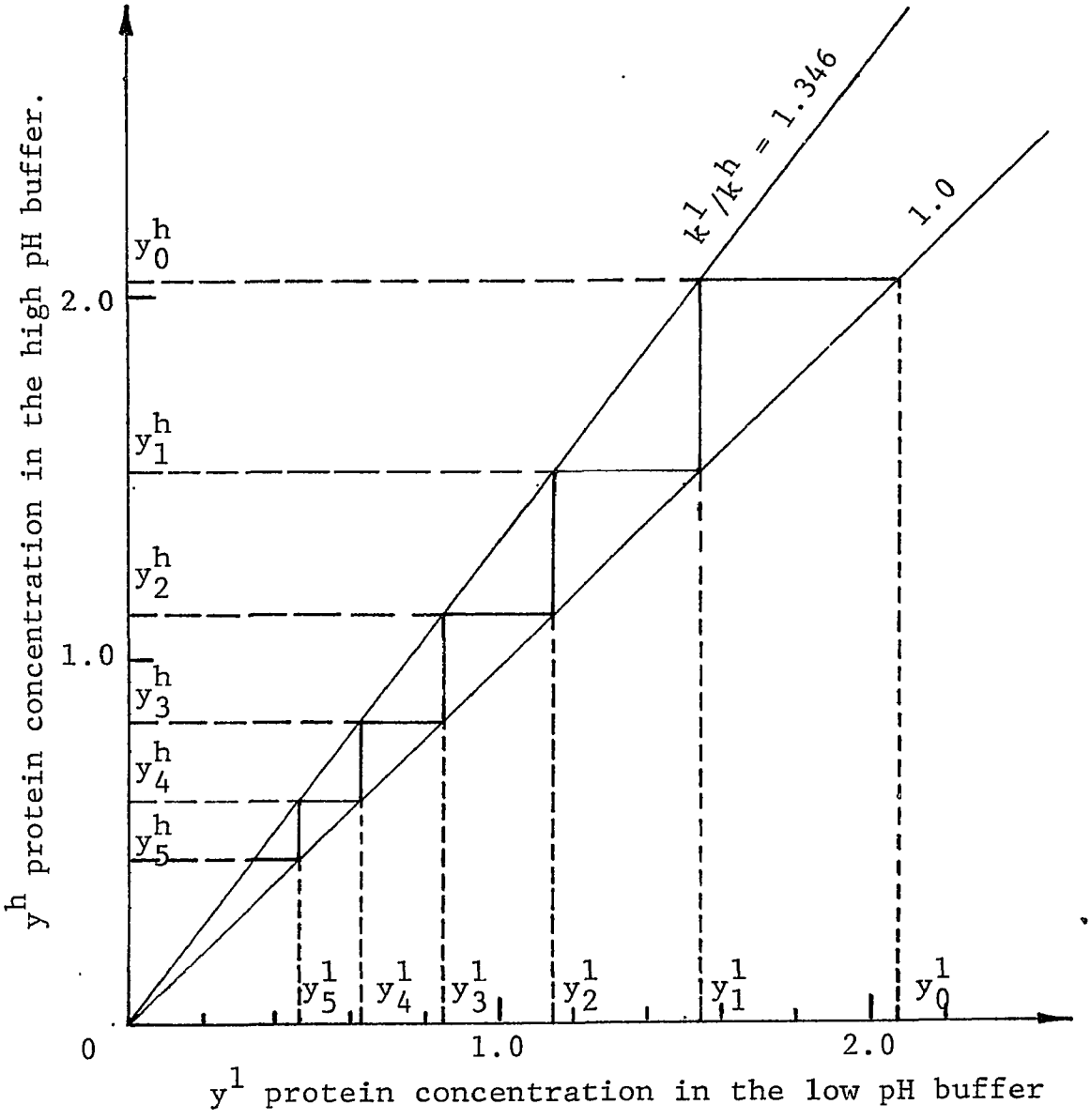


FIGURE 22 - GRAPHICAL SOLUTION BASED ON McCABE-THIELE DIAGRAM.

parameter is calculated from an overall material balance over the system, and give (see Appendix A)

$$y_0^* = \frac{\bar{W/V}}{\rho + (\rho + k^1) \sum_{i=1}^{N} \beta^{-i}} \qquad (3.28)$$

This result is seen to be an independent of the initial distribution, but to depend only on W, the total mass of protein present in the system.

The knowledge of this steady state allows us in turn to determine the structure of $[\underset{\sim}{M}]^n$ when n becomes large, as illustrated in Appendix A.

Chapter V

EXPERMENTAL METHOD

A. The Experimental System

The system of separation is considered for removing either Hemoglobin or Albumin from its aqueous solution or their mixture by pumping through a suitable solid phase (absorbant bed) i.e., an ion exchanger (anion or cation). The ion exchanger will attract a protein (absorption) which carries an opposite charge to one in a pH buffer solution, and the protein will be released (desorption) when its charge is changed back (to the same as an ion exchanger) in the other pH buffer solution, in such a way that both fluid and solid phases, in the absorbant bed, are in equilibrium.

Equation 1.1 shows the material balance of protein in both fluid and solid phases. The relationship ratio of the concentration of the solute in the solid to a fluid phase is a function of solute concentration, pH buffer and ionic strength and also expressed in Equation 1.2:

$$x = ky \qquad (1.2)$$

where k is called as "an equilibrium constant". The experimental and calculated the equilibrium constant are pictured on Figure 21, where the specific region of interest treated

in this work is marked. The linearlity of the adsorption equilibrium constant in this region is apparent.

B. Description of Apparatus

The system consists of one or two chromatographic columns manufactured by Phamarcia Fine Chemicals. The column(s) was packed either with DEAE-Sepharose (anion exchanger) or with CM-Sepharose (cation exchanger) for a one column system otherwise the top and the bottom columns were packed alternately with anion and cation exchangers. The reservoirs (50 cc Pyrex beakers) were connected to the column(s) as the system required. Reciprocating flow was introduced into the system by a four channel Multi-Staltic Pump # 2-6200 manufactured by Buchler Instruments. The tubing used in the pump was 1/16" I.D. Tygon tubing manufactured by Norton Plastics. The pump was set up according to manufacturer's specifications and the flow rates of all four channels were adjusted to 1 cc/minute.

The pH levels in the reservoirs were maintained by using three PHM61 meters and two TTT60 automatic titrators, all manufactured by Radiometer/Copenhagen. The pH 8.5 (P_1) and the pH 4.0 (P_3) reservoirs were monitored by the automatic titrating the solution manually. The acid and base solution used were Hydrochloric acid (0.5N) and Sodium Hydroxide (0.5N). Magnetic stirrers were used to ensure perfect mixing in the reservoirs. The reservoirs were placed in the jacketed Pyrex

beakers. Both the reservoirs and the columns were maintained at 5°C by the use of circulation bath. An electrically controlled timer was connected to the system for the purpose of starting and ending the process automatically. To maintain the ionic strength, a hollow fiber dialyzer manufactured by Harvard Apparatus Company, was introduced. The experimental apparatus for the three reservoirs batch system is shown in Figure 23.

C. Solutions and Buffers

Three types of buffers were used. For pH value of 8.5, a buffer of tris (Hydroxy-Methyl) Aminomethane and HCl was used. For pH value of 6.2, the buffer was tris (Hydroxy-Methyl) Aminomethane, Malaic Acid and NaOH was used. For pH value of 4.0, a buffer of Acetic Acid and Sodium was used.

The concentration of the above buffer solutions was 0.2 M. Sodium Chloride in calculated amounts was added to each of them. Dilution of the buffers were made according to the experimental parameters.

The two proteins were selected to investigate the separation were Haemoglobin and Albumin; both are manufactured by Worthington Biochemicals.

Component	Protein	Molecular Weight	Isoelectric Point
A	Haemo.	63,000	6.7
B	Alb.	69,000	4.7

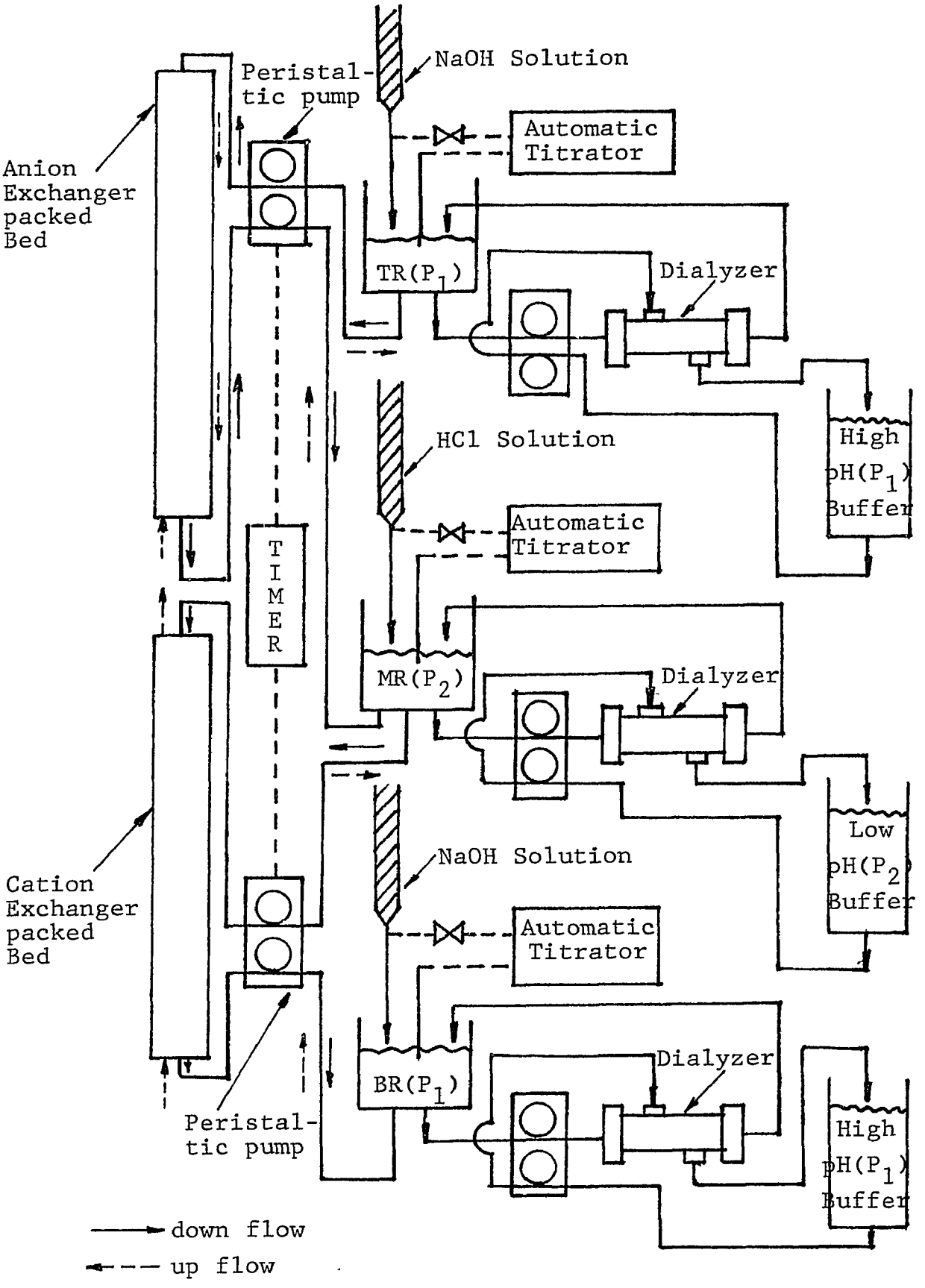


FIGURE 23 - THE EXPERIMENTAL APPARATUS DESCRIBES THE THREE RESERVOIRS BATCH SYSTEM.

The concentration of protein in the feed solution was 0.02 weight percent (i.e.,0.02 gram protein/100 cc. buffer).

D. Gel Preparation and Packing

Both the CM and the DEAE-Sepharose exchangers were washed and stored for 24 hours in their respective initial buffer solutions. This ensured that the gels would be at their respected pH. The gels were then loaded into the vertical columns and were allowed to settle to a volume of 12 cc.

The columns were next connected to the pump. The next step involves the saturating of gel with the specific feed or buffer solution. This step is a function of the system under investigation. The solutions were pumped into the respective columns for a period of 90 minutes to ensure that solid and the liquid phases were in equilibrium. (NOTE: Pump flow rate = 1.0 cc/min).

E. Measurements

A sample was taken at the end of each cycle from each reservoir, with the Batch System, or at the end of each step, with the Semi-Continuous System. The sample was analyzed on a Bausch and Lomb Spectrophotometer. Past work shows that the absorbance obtained from the spectrophotometer can be related to the relative amounts of protein in the solution. The wave lengths used, were 430μ, 560μ, 576μ, 595μ and

630μ.

A dye reagent was used to measure the total amount of protein at 595μ. The dye is manufactured by Bio-Rad Laboratories and was prepared and stored according to the manufacturer's specification. The sample/dye ratio varied, but remained constant for any given experiment. The ratio was sometimes changed to acquire more accurate readings. The reaction time for all samples which required the use of the dye reagent was 5 minutes. This procedure is described in the analysis of the experimental data (see Appendix B for sample of calculation).

F. Operation Processes

One-Column: Semi-Continuous

We first consider two types of systems. In one the column was packed with an anion where the other packed with a cation exchanger. The equipment and the column preparation for the two reservoirs, Semi-Continuous single column (anion) is already explained in Section B and D, respectively. This system consists of an anion exchanger and two reservoirs; 6.2 top (P_2) and 8.5 bottom (P_1). The pumping flow diagram is shown in Figure 24.

Procedure. The reservoirs's specifications were:

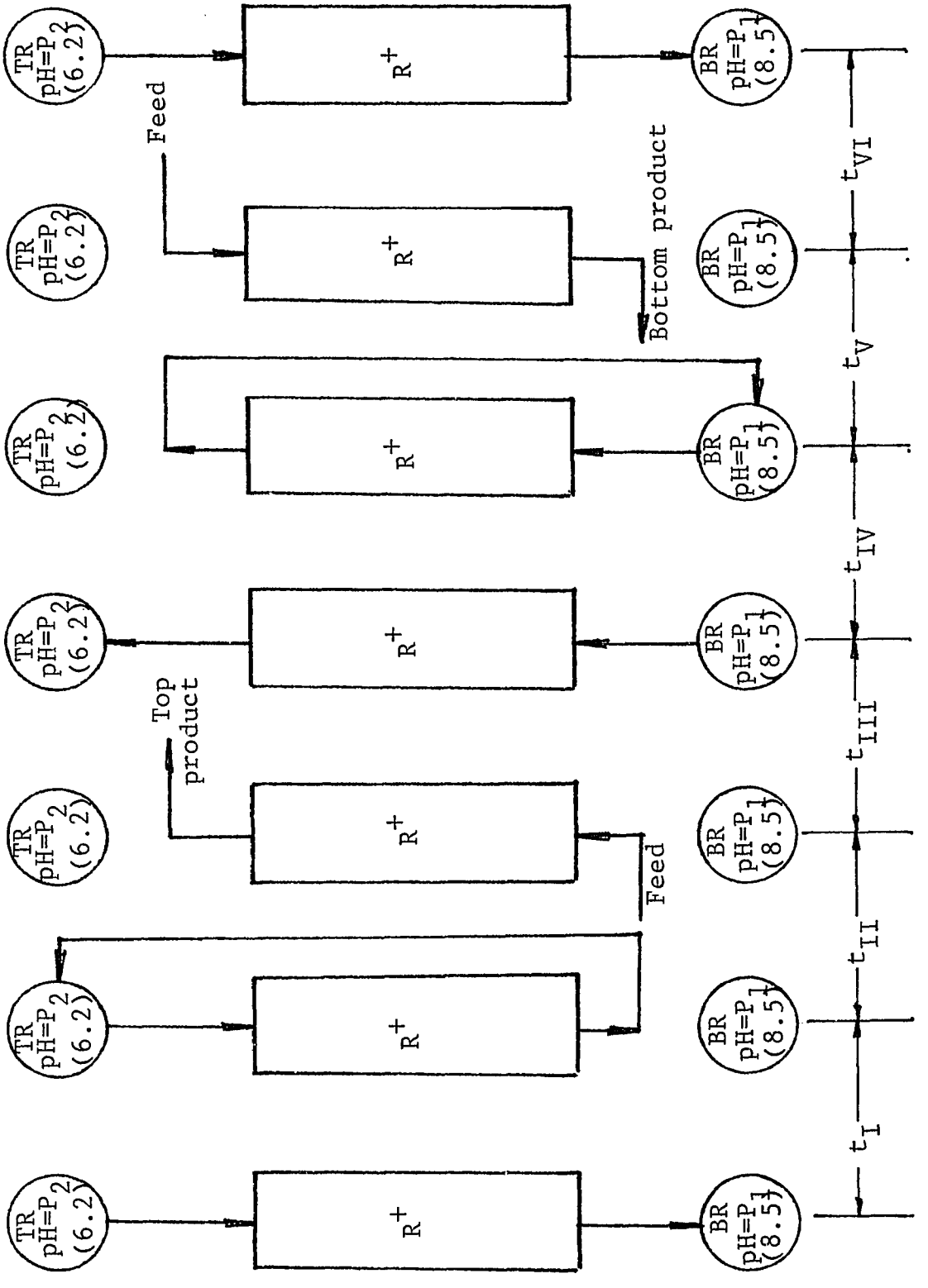


FIGURE 24 - SCHEMATIC OF SINGLE COLUMN, SEMI-CONTINUOUS SYSTEM.

	6.2 TR(P_2)	8.5 BR(P_1)
Dead Volume	30 cc	30 cc
Displacement	12 cc	0 cc

(NOTE: In some experiments the dead volume was either increased or decreased).

(I) Pump the fluid from 6.2 TR through the R^+ column to the 8.5 BR, for a time period t_I (t_I = 12 min).

(II) Circulate the fluid between the 6.2 TR and the R^+ column, for a period of t_{II} (t_{II} = 24 min).

(III) Fresh 6.2 (P_2) feed enters the R^+ column from the bottom, for a time period of t_{III} (t_{III} = 8 min). At the same time, the top product was withdrawn for analysis.

(IV) The fluid from the 8.5 BR is pumped through the R^+ column to the 6.2 TR for a period of t_{IV} (t_{IV} = 12 min).

(V) Circulate the fluid between the 8.5 BR and the R^+ column, for a period of t_V (t_V = 24 min).

(VI) Fresh 8.5 (P_1) feed enters the R^+ column from the top, for a period of t_{VI} (t_{VI} = 8 min). The bottom product was withdrawn and analyzed.

The completion of step VI is the end of one cycle. All of the products were analyzed on a Bausch and Lomb Spectrophotometer (BLS) at 403μ, 595μ, 560μ, 576μ and 630μ.

The purpose of using 560μ, 576μ and 630μ is to determine the concentration of haemoglobin. It was determined that the type of buffer will affect the absorbance, thus leading to an error in calculating the relative amount of protein. The 403μ reading was not versatile enough to detect the protein concentration. The procedure for analyzing the samples is described in Section E.

NOTE: For the cation column, the equipment and the operation steps are exactly the same as the operation on an anion column as explained above. Also the Batch System operation for the experiment was described earlier in Chapter II.

Two-Column System

Mode 1: Three Reservoirs Batch System

The apparatus and the preparation of the columns for this system has already been explained previously in this chapter. The pump flow diagram can be seen on Figure 12.

Procedure. The reservoirs were started with the following dead volume:

	8.5 TR(=P_1)	6.2 MR(=P_2)	8.5 BR(=P_1)
Dead Volume	30 cc	30 cc	30 cc
Displacement	12 cc	0 cc	0 cc

NOTE: In some experiments the dead volume was either increased or decreased.

The four operational steps were explained in Chapter II, where $t_I = t_{III} = 12$ min and $t_{II} = t_{IV} = 24$ min.

At the end of each cycle, a 3.0 cc sample was removed from each reservoir, TR, MR and BR. The samples were analyzed on a BLS at 403 μ and 595 μ. The procedure for sample analysis, is described in the earlier pages of this chapter.

NOTE: For the Semi-Continuous or Continuous Process, the results were not consistent and omitted from discussion (see Reference 48 for detail).

Mode 2: Four reservoirs Semi-Continuous System

The equipment and the column preparation in the operation of the four reservoirs Semi-Continuous process is the same as described at the beginning of this chapter. This system requires the introduction of fresh feed after each circulation step. The pump flow diagram is shown in Figure 25.

Procedure. The reservoirs were started with the following dead volume:

	6.2TR(P_2)	8.5MR(P_1)	4.0MR(P_3)	6.2BR(P_2)
Dead Volumes	30 cc	30 cc	30 cc	30 cc
Displacements	12 cc	0 cc	12 cc	0 cc

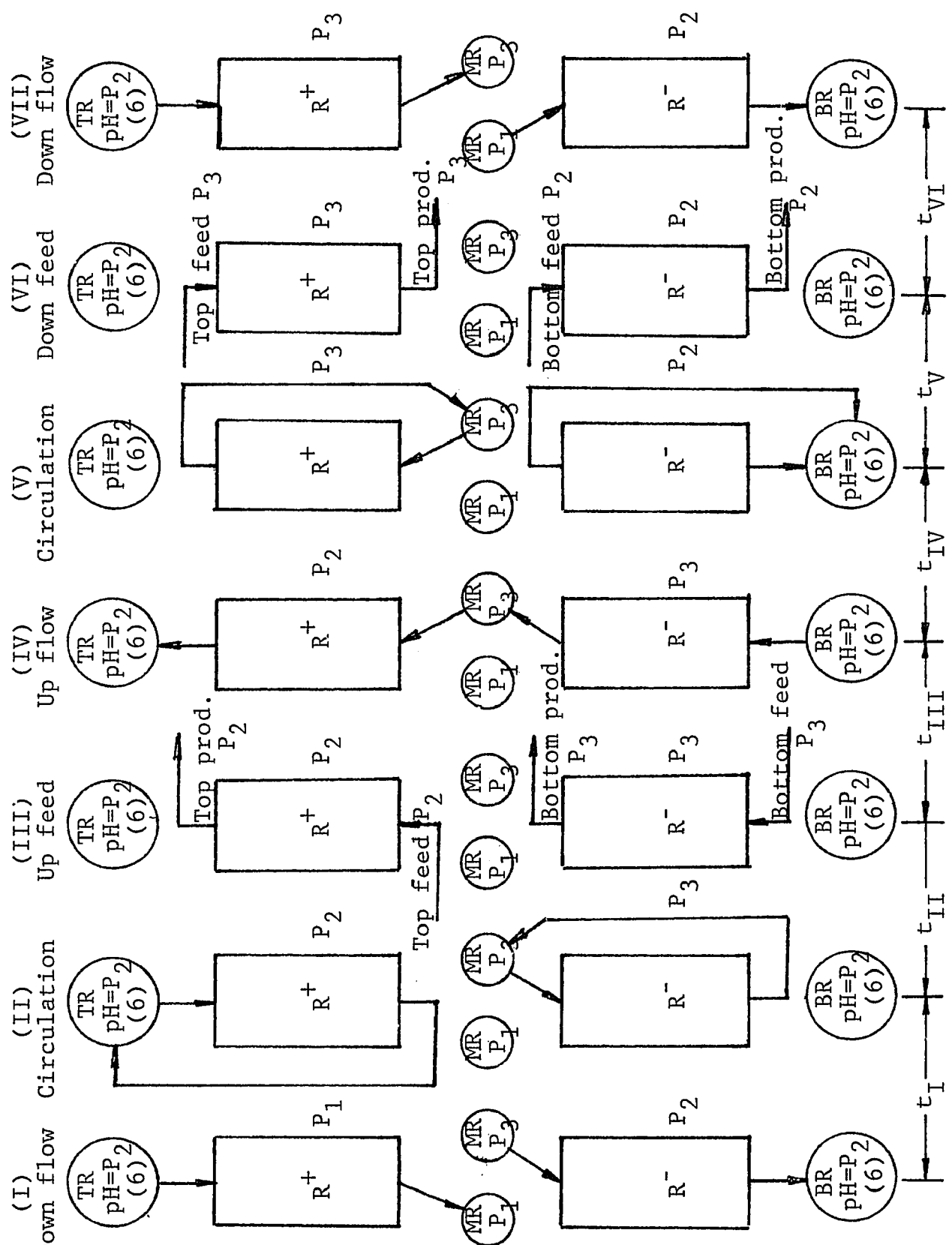


FIGURE 25 - SCHEMATIC OF TWO-COLUMN, SEMI-CONTINUOUS SYSTEM: MODE 2.

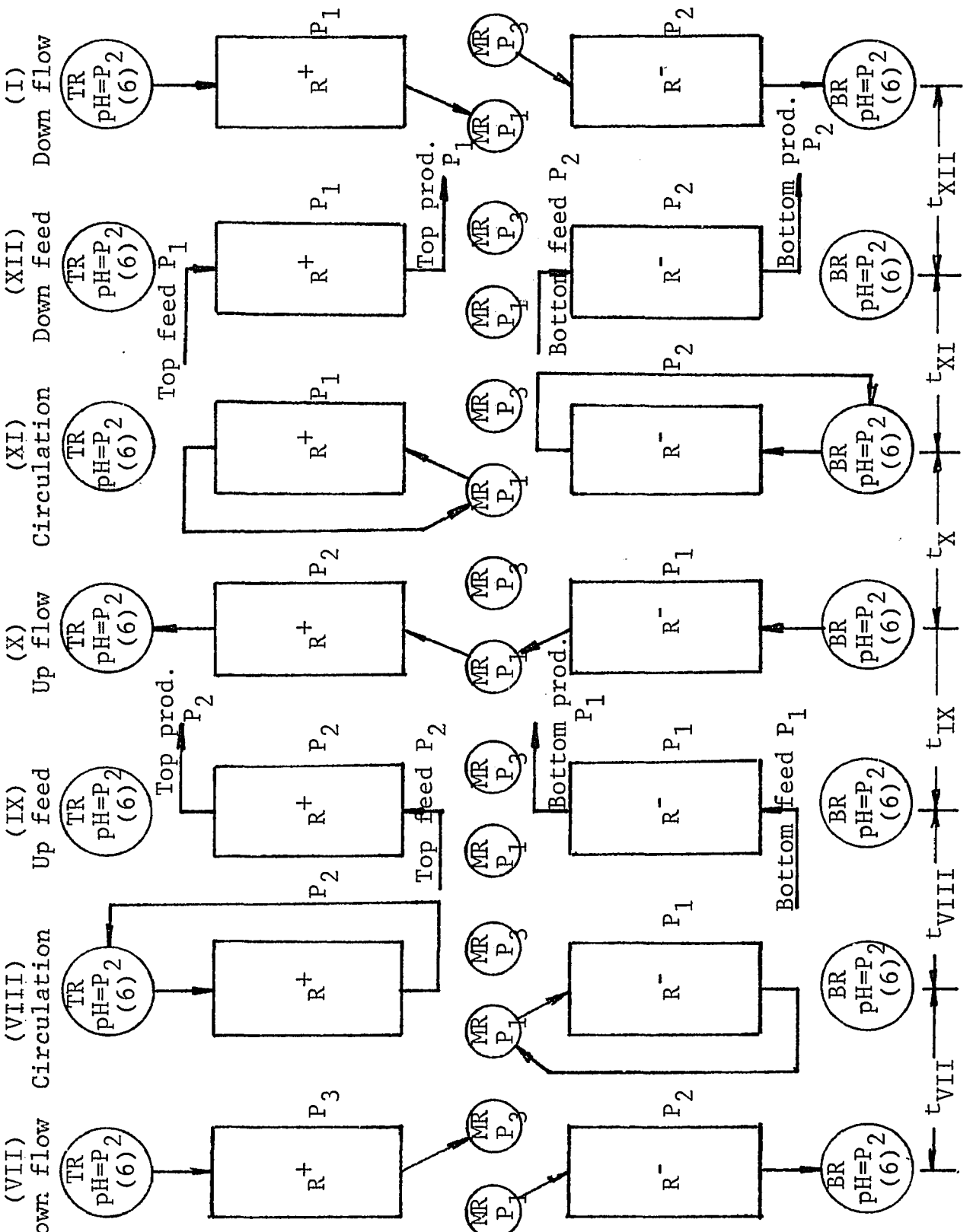


FIGURE 25 - CONTINUED.

(NOTE: In some experiments the dead volume was either increased or decreased).

(I) Pump the fluid from 6.2TR through the R^+ column to the 8.5MR, and at the same time pump the fluid from the 4.0MR through the R^- column to the 6.2BR, for a time period of t_I (t_I = 12 min)

(II) The fluid between the 6.2TR and the R^+ column, and the 4.0MR and the R^- column were circulated, for a period of t_{II} (NOTE: t_{II} depended on the experiment; see the experiment data for the correct value)

(III) Fresh 6.2(P_2) feed enters the R^+ column from the bottom and fresh 4.0(P_3) feed enters the R^- column from the bottom, for a perid of t_{III} (t_{III} = 8 min), Both top products were analyzed.

(IV) The fluid from the 6.2BR is pumped through the R^- column, the 4.0MR and the R^+ column to the 6.2TR, for a period of t_{IV} (t_{IV} = 12 min).

(V) The fluid from the 6.2BR and the R^- column, and the 4.0MR and R^+ column were circulated for a period of t_V.

(VI) Fresh 4.0(P_3) feed enters the R^+ column from the top and fresh 6.2(P_2) feed enters the R^- column from the top, for a period of t_{VI} (t_{VI} = 8 min), while the bottom product were withdrawn and analyzed.

(VII) The fluid from the 6.2TR is pumped through the R^+

column to the 4.0MR , and simultaneously, pump the fluid from the 8.5MR through the R^- column to the 6.2BR, for a period t_{VII} (t_{VII} = 12 min).

(VIII) The fluid between the 6.2TR and the R^+ column, and the 8.5MR and the R^- column were circulated, for a period of t_{VIII}.

(IX) Fresh 6.2(P_2) feed enters the R^+ column from the bottom and fresh 8.5(P_1) feed enters the R^- column from the bottom, for a period of t_{IX} (t_{IX} = 8 min). Both of the top products obtained were analyzed.

(X) The fluid from the 6.2BR is pumped through the R^- column, 8.5MR and R^+ column to the 6.2TR, for a period t_X (t_X = 12 min).

(XI) The fluids between the 6.2BR and the R^- column, and the 8.5MR and the R^+ column were circulated, for a a period of t_{XI}.

(XII) Fresh 6.2 (P_2) feed enters the R^- column from the top and fresh 8.5 (P_1) feed enters the R^+ column from the top, for a period of t_{XII} (t_{XII} = 8 min). Both of emerging bottom products were analyed.

The completion of step XII is the end of one cycle. All of the products were analyzed on a BLS at 403μ, 595μ, 560μ, 576μ and 630μ. As mentioned before that, the purpose of using 560μ, 576μ and 630μ is to determine the concentration of haemoglobin. It was determined that the type of buffer will affect the absorbance, thus leading to an error in cal-

culating the relative amount of protein. The procedure to analyze the sample is already described in the earlier pages of this chapter.

Mode 2: Four reservoirs Batch System

The apparatus and the preparation of the column for this system is the same as the Semi-Continuous System as explained in the above paragraph. Figure 14 is shown the flow diagram of the system.

Procedure. The reservoirs were started with the following dead volumes:

	6.2TR(P_2)	8.5MR(P_1)	4.0MR(P_3)	6.2BR(P_2)
Dead volumes	30 cc	30 cc	30 cc	30 cc
Displacements	12 cc	0 cc	12 cc	0 cc

(NOTE: In some experiments the dead volume was either increased or decreased).

The steps of operation were explained in the Chapter II, page where $t_I = t_{III} = t_V = t_{VII} = 12$ min and $t_{II} = t_{IV} = t_{VI} = t_{VIII} = 24$ min.

At the end of each cycle, a 3 cc sample was removed from each reservoir. The sample was then analyzed on a BLS (Bausch and Lomb Spectrophotometer) at 403 μ and 595 μ. The procedure for the analysis of each sample was described as the same as the other Modes from the previous section.

Mode 3: Five Reservoirs Batch System

The equipment and the column were prepared for a system of five reservoirs Batch Operation as was described in the earlier pages of this chapter. The flow diagram of this operation is shown in Figure 16.

Procedure. The reservoirs' dead volume and the displacements were as follows:

	$8.5TR(P_1)$	$4.0TR(P_3)$	$6.2MR(P_2)$	$8.5BR(P_1)$	$4.0BR(P_3)$
Dead Volumes	30 cc	30 cc	30 cc	30 cc	30 cc
Displacements	12 cc	12 cc	0 cc	0 cc	12 cc

NOTE: In some experiments the dead volume was either increased or decreased.

The operation steps of this type of system, can be found in Chapter II. Allow $t_I = t_{III} = t_V = t_{VII} = 12$ minutes while $t_{II} = t_{IV} = t_{VI} = t_{VIII} = 24$ minutes.

A complete cycle is ended by step VIII and 3.0 cc sample was taken from each reservoir. The samples were then analyzed according to the procedure described, and used, for other different mode(s) as mentioned before.

NOTE: For the Semi-Continuous process, the system had been investigated and the results were very poor. The detail of this explanation is in the Reference 48.

Mode 4: Two Reservoirs Semi-Continuous System

The apparatus and the column preparation for the system have been explained in the earlier pages of this chapter. First of all, the system was packed with anion exchanger in both columns. The system is supplied the fresh feed of both at P_1 = 8.5 and P_2 = 6.2 after each circulation step. Figure 26 shows the diagram of flow sequences.

Procedure. The reservoirs and pH-Converters (PC) were started with the following dead volumes:

	8.5TR(P_1)	8.5PC(P_1)	6.2PC(P_2)	6.2BR(P_2)
Dead Volume	30 cc	30 cc	30 cc	30 cc
Displacements	12 cc	0 cc	0 cc	0 cc

NOTE: In some case of the experimental, the dead volume was either increased or decreased.

(I) Pump the fluid from 8.5TR through the first column and also through the 8.5PC to the second column, while the fluid from the second column is transferred the 6.2BR, for a time period of t_I (t_I = 12 min).

(II) The fluid between the 8.5TR and the first column, and the 8.5PC and the sedcond column were circulated, for a period of t_{II} (NOTE: t_{II} = 24 min, otherwise see the table for the correct value).

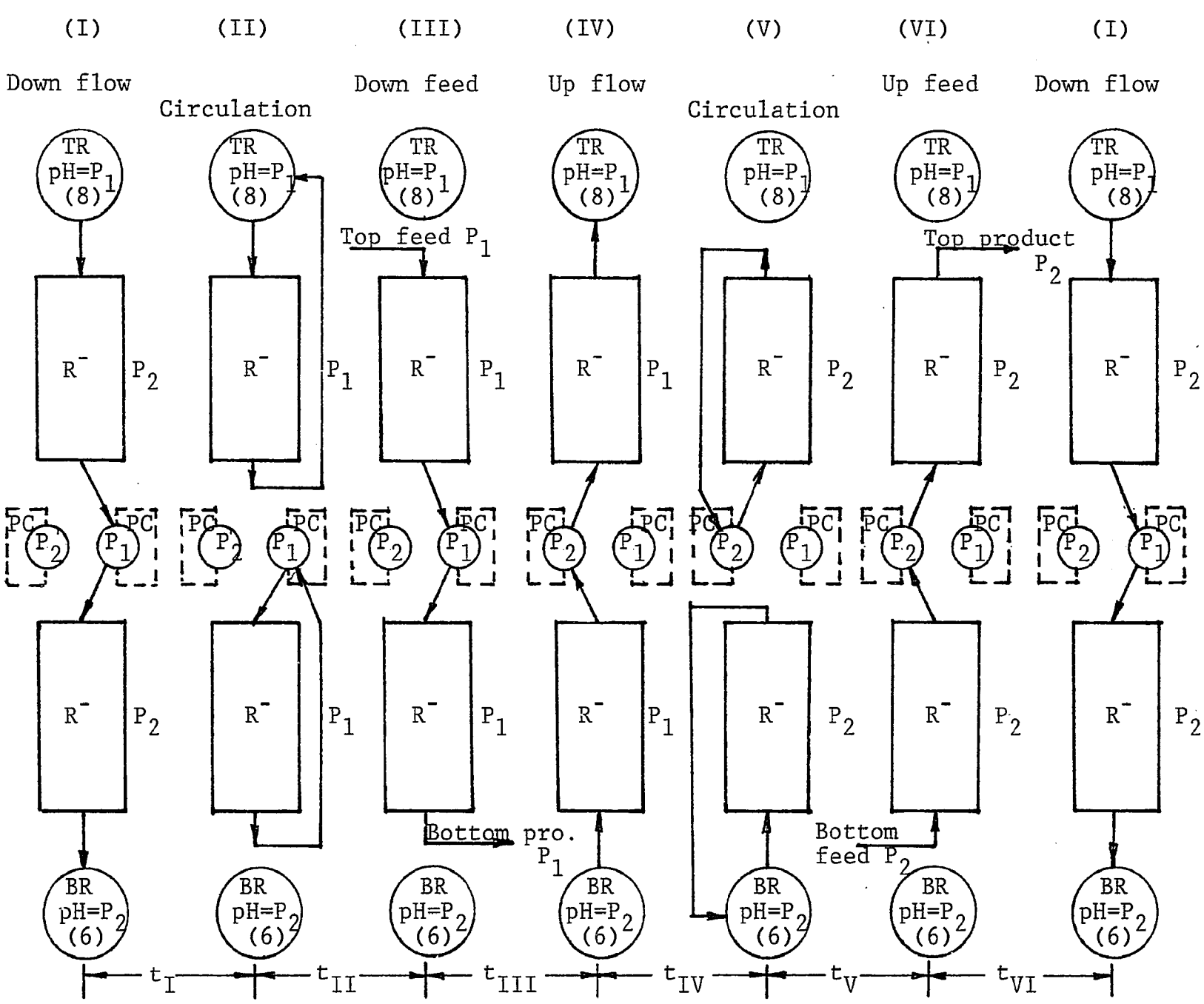


FIGURE 26 - SCHEMATIC OF TWO-COLUMN, SEMI-CONTINUOUS SYSTEM: MODE 4.

(III) Fresh 8.5TR is fed from the top of the first column through 8.5PC and through the second column, for a period of t_{III} (t_{III} = 8 min). The bottom product was collected at pH = 8.5 and analyzed.

(IV) Pump the fluid from 6.2BR through the second column and through the 6.2PC to the first column, while the content in the first column goes back to the 8.5TR, for a time t_{IV} (t_{IV} = 12 min).

(V) The fluid from the first column and 6.2PC, and the 6.2BR and the second column were circulated for a period of t_V (t_V = 24 min).

(VI) Fresh 6.2BR feed enters the bottom of the second column through 6.2PC and first column, and at the same time, the top product (pH = 6.2) was collected for a period of t_{VI} (t_{VI} = 8 min).

Each cycle is ended at step VI. Then, all the products (top and bottom) were analyzed on a Bausch and Lomb Spectrophotometer at each different wave length as mentioned in the previous section.

NOTE: We repeated the process by replacing the cation exchanger instead of an anion exchanger. The preparation of the apparatus, the operation steps and also the analysis of the sample are exactly the same as we operated on an anion exchanger system. Any minor changes either increasing or decreasing the time

period (t) are indicated in Appendix B.

Mode 4: Two Reservoirs Batch System

The apparatus and the preparation of the column are the same as the Semi-Continuous System. The flow diagram and the procedures were shown in Figure 18 and described in Chapter II. Any minor changes will be explained in Appendix B.

Chapter VI

SEPARATION OF PROTEINS VIA MULTI-COLUMN

This chapter extends the work theoretically from the separation of two proteins via two columns into multi-protein via multi-column. The system is developed and established base on Mode 2 as described in the Chapter I.

Examine any multi-unit system where M columns are considered. Each unit consists of two columns, one which is packed with a cation exchanger (R^-) and the other with an anion exchanger (R^+). For the first unit, we need four reservoirs, while each additional unit requires three reservoirs as shown in Figure 27. We will follow the operational steps as mention in Mode 2 Chapter I. Therefore, the steady state concentration of protein A will reach the top reservoir of the first unit, while the steady state concentration of protein B will be found in the bottom reservoir of the last unit. As we explained for the two columns system (see Chapters I, II and IV on Mode 2), proteins A and B will move in opposite directions. The movement of both proteins A and B is shown in Figure 28 and the operational steps, which are exactly the same as Mode 2 system, is shown in Figure 14.

Process Designing for Multi-Protein Separation.

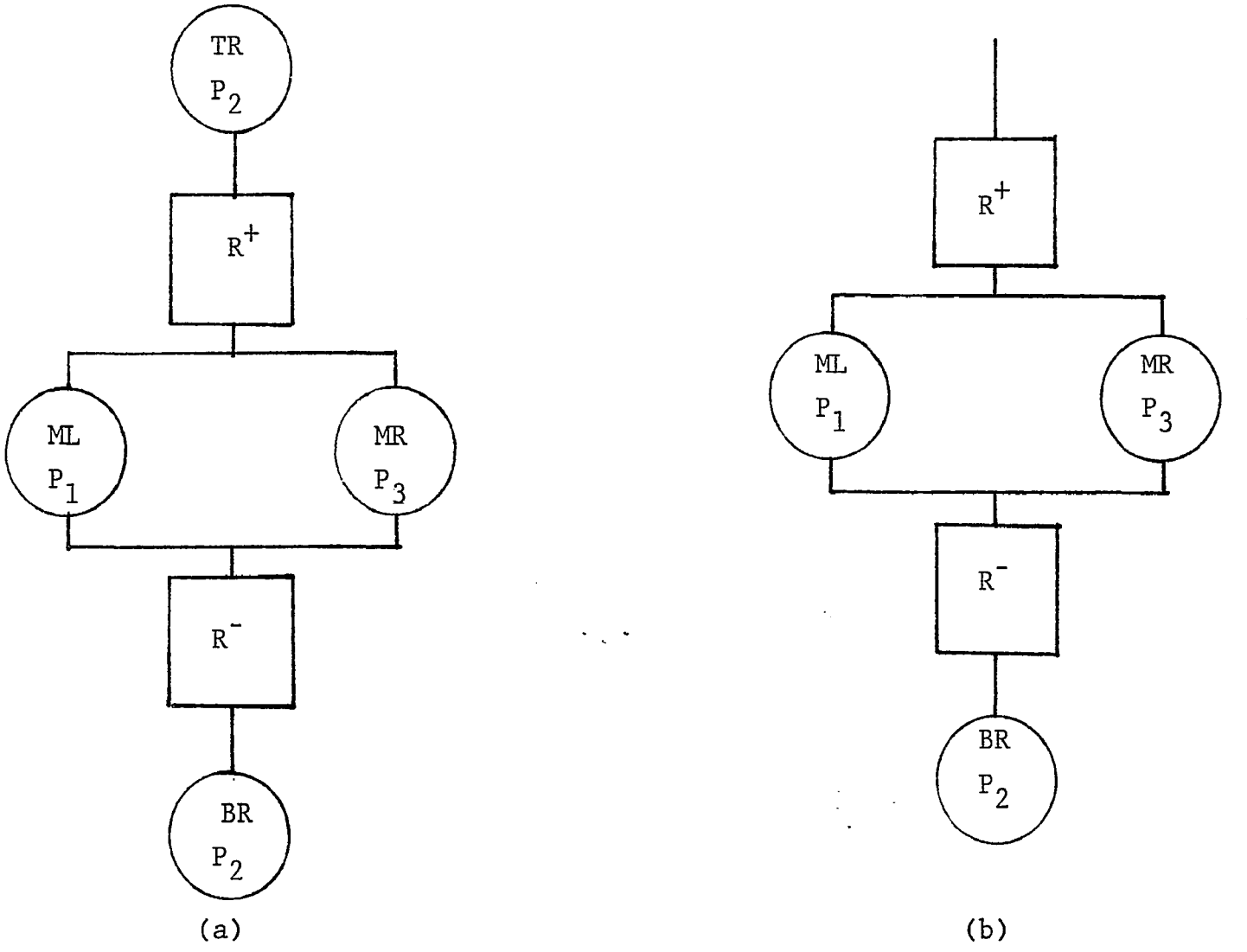
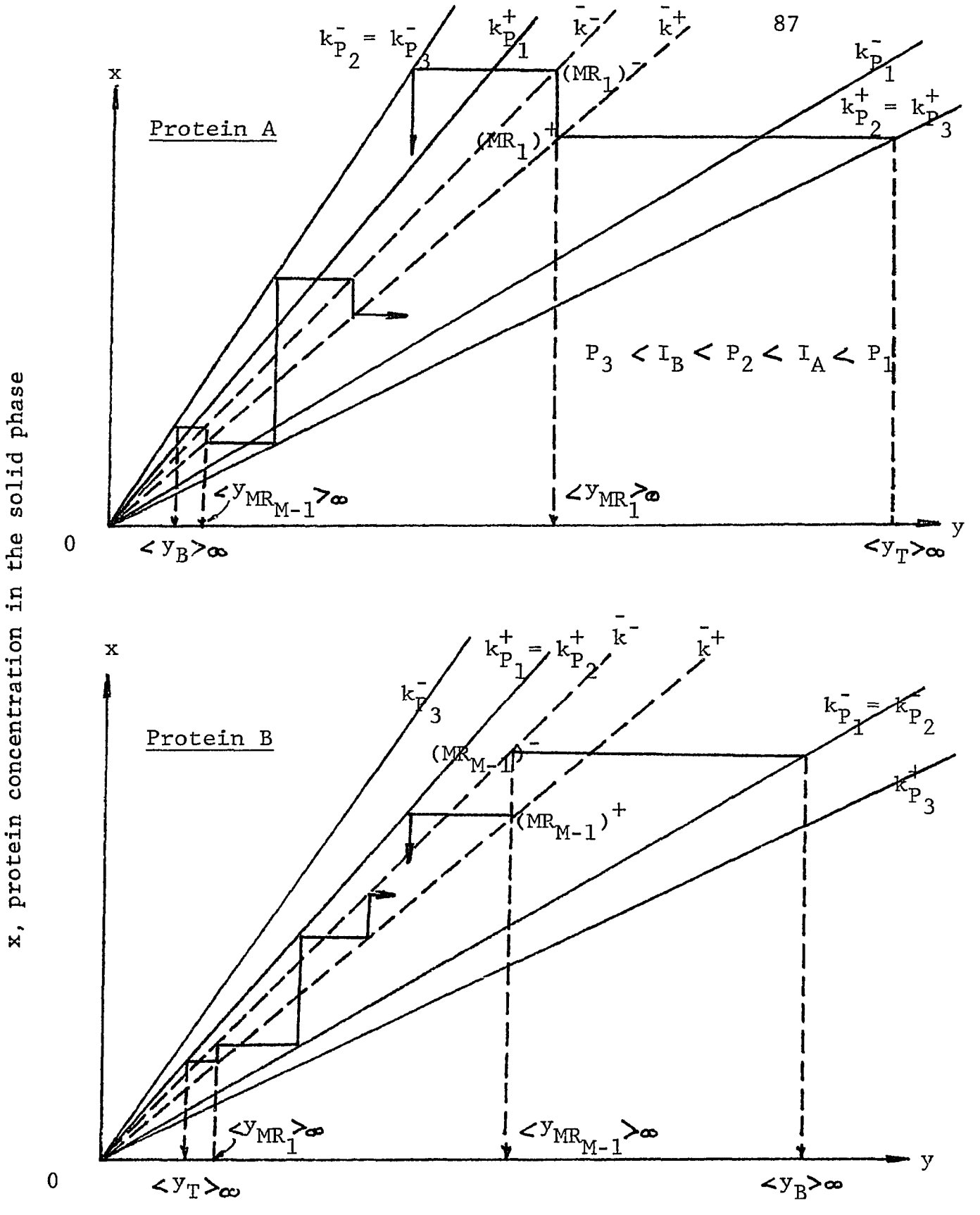


FIGURE 27 - SCHEMATIC OF PROCESS-DESIGNING FOR: (a) AN ORIGINAL FIRST UNIT AND (b) AN ADDITIONAL UNIT.



x, protein concentration in the solid phase

y, protein concentration in the fluid phase

FIGURE 28 - GRAPHICAL SOLUTION FOR PROCESS DESIGNING, M-COLUMN SYSTEM

This process is quite similar to the separation in the two proteins system; the differences which we have to considered are:

I. Selection of pH level in the reservoirs.

II. Adding the extension system(s) to the original system.

Selection of pH Level:

To commence we choose any four proteins A, B, C and D which have the isoelectric points I_A, I_B, I_C and I_D respectively and

$$P_5 < I_D < P_4 < I_C < P_3 < I_B < P_2 < I_A < P_1$$

where P_1, P_2, P_3, P_4 and P_5 are the pH levels in the reservoirs. Thus, all proteins (A, B, C and D) will bare a negative charge at P_1 and positive charge at P_5. Of course, at a different pH levels P_2, P_3 and P_4, proteins A, B, C and D will carry a different charge as shown in Table 1.

To select the pH level for the reservoirs, the first necessity is to choose three pH levels. One will be located in TR, R_2, ... R_{M-2} and BR, the other two will be located in ML_1, ML_3, ... ML_{M-1}, on the same level of pH, while MR_1, MR_3, ... MR_{M-1}, will be on the other level of pH.

Case I. If we consider P_2 for reservoirs R_1, R_2, ...

TABLE 1

An expression of protein charges, those which they would bear in the different pH level solutions.

$$P_5 < I_D < P_4 < I_C < P_3 < I_B < P_2 < I_A < P_1$$

pH Level	Protein A	Protein B	Protein C	Protein D
P_1	-	-	-	-
P_2	+	-	-	-
P_3	+	+	-	-
P_4	+	+	+	-
P_5	+	+	+	+

.... R_{M+1}, then proteins will be grouped where B, C and D are in the first group while A is in the other, thus

$$I_D,\ I_C \text{ and } I_B \prec P_2 \prec I_A$$

In the next step we have to evaluate the lowest and the highest pH levels for the middle reservoirs ML and MR. Rewritten, the isoelectric points of these four proteins, will reduce to:

$$P_5 \prec I_D,\ \ I_C \text{ and } I_B \prec P_2 \prec I_A \prec P_1$$

Then, ML_1, ML_3, ... ML_{M-1}, will have the pH level P_1 while MR_1, MR_3, ... MR_{M-1}, carry the pH level at P_5.

Case II. If P_3 be selected for reservoirs TR, R_2, ... R_{M-2} and BR, then groups of proteins will be as:

$$I_D \text{ and } I_C \prec P_3 \prec I_B \text{ and } I_A$$

Similar to Case I, P_1 and P_5 must be selected for ML_1, ML_3, ... ML_{M-1} and MR_1, MR_3, ... MR_{M-1}, respectively. The isoelectric point can be written as:

$$P_5 \prec I_D \text{ and } I_C \prec P_3 \prec I_B \text{ and } I_A \prec P_1$$

Case III. If P_4 be desired for TR, R_2, ... R_{M-2} and BR then groups of proteins will be different than the other two cases mentioned before. Also, we will have:

$$I_D \prec P_4 \prec I_C,\ I_B \text{ and } I_A$$

Then P_1 and P_5 are selected for ML and MR, the same as the first two cases. The isoelectric points now can be stated as

$$P_5 < I_D < P_4 < I_C,\ I_B \text{ and } I_A < P_1$$

An Addition to the Original System (Extension System):

The new system of multi-separation via multi-column will be developed into two type of systems, one called "SYMMETRICAL SYSTEM" and the other called "UNSYMMETRICAL SYSTEM". To decide which system will be used depends on the way we choose the pH level. If the pH level is selected as in Case I or Case III, then the system developes into an "UNSYMMETRICAL SYSTEM". If Case II is selected then the system will be the "SYMMETRICAL SYSTEM".

Symmetrical System:

We consider A and B which are in one group where isoelectric points (I_A and I_B) fall in between P_3 and P_1. Where as in the other two, proteins C and D, are in the other group where isoelectric points (I_C and I_D) fall in between P_5 and P_3. At the outset, we will separate these two groups of proteins by using the same system as shown in Figure 14. We select pH level P_3 in TR, R_2, ... R_{M-2} and BR reservoirs and take P_1 in the middle reservoirs, ML_1, ML_3, ... ML_{M-1} while P_5 will be in the reservoirs MR_1, MR_3, ... MR_{M-1}.

Then both proteins A and B will move upward together, and the steady state value of both concentrations, A and B, will locate together at the top reservoir (pH = P_3), while the other two proteins C and D will move in the opposite direction into the bottom reservoir (as the same pH level P_3).

The next step is to separate A from B and C from D. We need two more extension system to connect with the top reservoir and the bottom reservoir.

Let us consider the separation of A and B first, the isoelectric points of A and B can be written as:

$$P_3 < I_B < P_2 < I_A < P_1$$

So we select P_2 as a pH level in TR, R_2, ... R_{M-2} and BR of the next extension system. However we choose P_3 for the left hand side middle reservoirs ML_1, ML_3, ... ML_{M-1} and P_1 for the right hand side middle reservoirs MR_1, MR_3, ... MR_{M-1} to be used in the new extension system. Protein A will move to the top of the extension system which is on the right of the original system, while protein B will migrate to the bottom of the extension system as shown in Figure 29. The steady state concentrations of A and B will be collected at the top and bottom reservoir respectively on this extension system and locate to the right of the original system.

Separation of protein C and D is similar to the

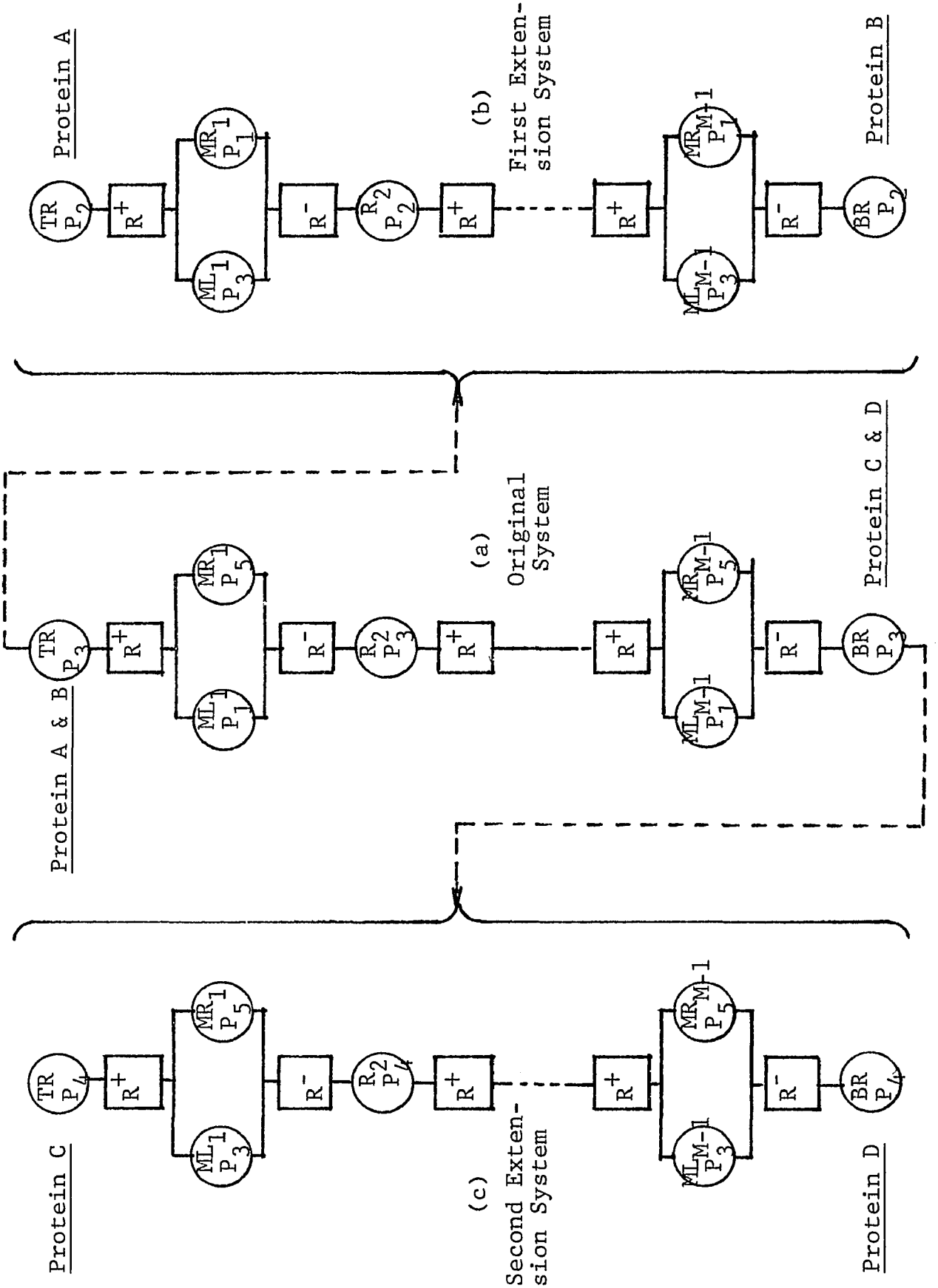


FIGURE 29 - SCHEMATIC OF PROCESS DESIGNING FOR: A SYMMETRICAL MULTI-SEPARATION SYSTEM.

separation of A and B. Thus the isoelectric points of proteins C and D are stated as:

$$P_5 < I_D < P_4 < I_C < P_3$$

So we need pH level for reservoir TR, R_2, ... R_{M-2} and BR in the second extension system at P_4, while the middle reservoirs ML_1, ML_3, ... ML_{M-1}, are P_3 and MR_1, MR_3, ... MR_{M-1}, are P_5, for the second extension system. Protein C will migrate to the top reservoir on this second extension system which locate to the left of the original system and protein D will migrate downward to the bottom reservoir on the second extension unit as shown in Figure 29.

Unsymmetrical System:

The method of separation with this system is basically the same as the "Symmetrical System". This system is dependent on the way we decide to use the pH level as explained in the previous discourse. In this type of process, Case I and Case III, the separation method will become an unsymmetrical separation process system. Either Case I or Case III will have exactly the same process. So in the following paragraphs we will consider only Case I as an example.

We have two groups of proteins, one is protein A and the other are B, C and D, the isoelectric points of these proteins can be expressed as:

$$P_5 \prec I_D,\ I_C \text{ and } I_B \prec P_2 \prec I_A \prec P_1$$

As we proceed with the method, we select pH level P_2 for the reservoirs TR, R_2, ... R_{M-2} and BR, P_1 for the middle reservoirs ML_1, ML_3, ... ML_{M-1}, and P_5 for MR_1, MR_3, ... MR_{M-1} as in the original system. Then protein A will migrate upward to the top while the rest of proteins B, C and D migrate in the opposite direction to the bottom of the system. At steady state condition, the high concentration of protein A will be found in the top reservoir of the system. But the high concentration of all the rest of proteins B, C and D will be discovered in the bottom reservoir of the system. At this point we see that protein A is separated first, and the others are the mixture of proteins B, C and D which need more extension system to separate.

Isoelectric points of proteins B, C and D are

$$P_5 \prec I_D \prec P_4 \prec I_C \prec P_3 \prec I_B \prec P_2$$

We set up the second separation unit by selecting the pH level on either P_3 or P_4. If we choose P_3 then proteins can be grouped and their isoelectric points can be expressed as:

$$P_5 \prec I_D \text{ and } I_C \prec P_3 \prec I_B \prec P_2$$

while the other P_4, the isoelectric points of proteins will be:

$$P_5 \prec I_D \prec P_4 \prec I_C \text{ and } I_B \prec P_2$$

For this explanation, we use P_3 for reservoirs TR, R_2, ... R_{M-2} and BR on this first extension system, and allow P_2 for ML_1, ML_3, ... ML_{M-1} while MR_1, MR_3, ... MR_{M-1} will carry the pH level P_5. As explained in the previous cases, protein B will migrate toward to the top reservoir on this extension system, while proteins C and D will migrate downward in the opposite direction of B or toward the other end of the system where the last column carries a cation exchanger R^-. At steady state, a high concentration of B will be found at the top reservoir of the unit while the bottom reservoir contains a high concentration of the protein mixtures C and D which their isoelectric points stated as:

$$P_5 \prec I_D \prec P_4 \prec I_C \prec P_3$$

Once again, we need another extension unit to complete the separation. This extension will separate C from D. First of all, we choose P_4 for reservoirs TR, R_2, ... R_{M-2} and BR, while P_3 and P_5 will be carried by ML_1, ML_3, ... ML_{M-1}, and MR_1, MR_3, ... MR_{M-1}, respectively.

In the same manner, the protein C will migrate to the top reservoir of this second extension system, while protein D migrates in the opposite direction of C and reaches the bottom reservoir as shown in Figure 30.

For the separation of "K" components of protein mixtures where their isoelectric points are I_1, I_2, ... I_{K-1} and

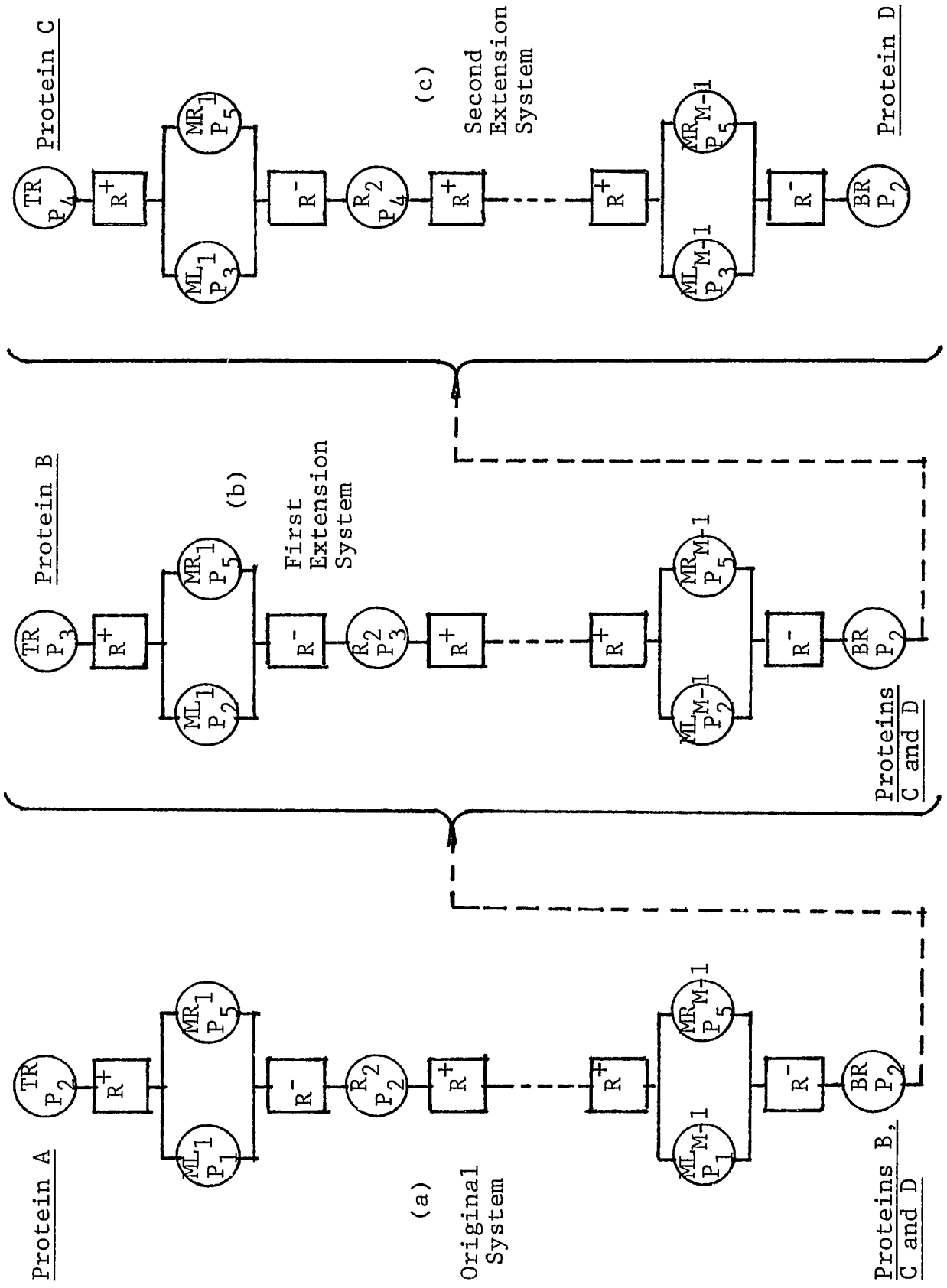


FIGURE 30 - SCHEMATIC OF PROCESS DESIGNING FOR AN UNSYMMETRICAL MULTI-SEPARATION SYSTEM.

I_K, the correlation between pH levels and isoelectric points can be expressed as:

$$P_{K+1} \prec I_K \prec P_K \ldots\ldots P_{11} \prec I_{10} \ldots\ldots I_2 \prec P_2 \prec I_1 \prec P_1$$

Assuming that, I_K, I_{10} and I_1 are selected as a major products. To begin with, the process will have to repeat the steps as mentioned above in the previous case. First, restate the isoelectric points and the coeeesponding pH level of these proteins in terms of:

$$P_{K+1} \prec I_K \prec P_K \prec\prec I_{10} \qquad \ldots\ldots I_1 \prec P_1$$

where I_{10} and I_1 are grouped together. P_K is selected for reservoirs TR, R_2, ... BR, choosing P_1 for the middle reservoirs ML_1, ML_3, ... ML_{M-1}, whereas P_{K+1} is selected fo MR_1, MR_3, ... MR_{M-1}. This is called the original system. Then protein I_K will move upward and be collected as one of the major products from the top reservoir of the system while the mixture of proteins I_{K-1}, I_{K-2}, ... I_{10} ... .. I_1 will move downward to the bottom reservoir.

The following step is decided upon to separate I_1 out of the mixture. The isoelectric points and pH levels must be written as:

$$P_K \prec I_{K-1}, I_{K-2}, \ldots\ldots I_{10}, I_9 \ldots\ldots I_2 \prec P_2 \prec I_1 \prec P_1$$

P_2, P_K and P_1 are chosen for reservoirs R_1, R_2, ...

R_{M+1}, middle reservoirs MR_1, MR_3, ... MR_{M-1} and ML_1, ML_3, ... ML_{M-1}, respectively. This new process will be called a first extension unit. Then I_1 is found at the bottom reservoir of this first extension system while the rest of proteins will move upward to the top of the column.

Again, the protein I_{10} can be separated from the mixture of I_{K-1}, I_{K-2}, ... I_{10}, I_9, ... and I_2 by grouping these proteins as:

$$P_K \prec I_{K-1}, \ldots I_{11} \prec P_{11} \prec I_{10}, \ldots I_2 \prec P_2$$

for the beginning step and selecting P_K, P_{11} and P_2 as a pH levels for use in the second extension unit. Next, proteins are grouped as:

$$P_{11} \prec I_{10} \prec P_{10} \prec I_9, I_8, \ldots \text{and } I_2 \prec P_2$$

where P_{11}, P_{10} and P_2 are chosen for the third extension unit. At the end of this step I_{10} is separated and the system is now completed. Altogether we required 4 units of these multi-column systems (one original unit plus three extension units) and these will be connected in series.

Chapter VII

RESULTS AND DISCUSSION

A. General

The experimental, analytical and computer solutions for pH-parametric pumps were used to generate concentration curves for both Batch and Semi-Continuous parametric pump at the various values of the operating parameters. These curves give the variation in solute separation, i.e., top and bottom product concentrations, with the change in number of cycles.

Once the experimental process had been established, the internal operation of the pH parametric pump was investigated using Equations 1.1 and 1.2 via graphical and mathematical base on elementary matrix algebra methods. Both fluid and solid phase concentrations were calculated by means of equilibrium theory with linear relationships (for dilute protein concentration) and simple material balances. Each movement of the two pistons push the liquid from top and bottom reservoirs alternately through the column(s), generating varoius concentration curves for any set of operating conditions.

The major variables affecting the shape of both Batch and Semi-Continuous pH-parametric pump concentration curves

are the number of cycles, dead volume, displacement volume and of course the parameter β, where β is defined as the ratio of the equilibrium constant for a low pH to the equilibrium constant for a high pH (See Equation 3.24).

B. One-Column System

First of all, we are concerned with the Batch operation system. Figure 31 illustrates the separation factor (S.F.) ($\langle y \rangle_n / \langle y_0 \rangle_n$) vs. n for a one column pH parametric pump. Initially, the feed solution containing a solute (haemoglobin or albumin) was present in the top reservoirs only. The column and the bottom reservoir dead volume were filled with the buffer solution pH = P_1(P_1 is varied from 8.0 to 8.5). The buffer solutions were made from monobasic and dibasic sodium phosphate. The top and bottom reservoir were respectively maintained at pH = 6 (P_2) and pH = 8 (P_1) so that the isoelectric point of haemoglobin would lie between the two pH levels. As a result of a change in the column pH, haemoglobin experiences a change in net charge, and migrates toward the high pH end on the bottom reservoir. Thus, the separation factor (S.F.) for haemoglobin increases with n and approaches a limiting value. For the case of albumin, $I_{albumin} = 4.7 < P_2$, and the net charge is always negative during upflow and downflow. As a result, the albumin concentration is unaffected by the parametric pumping operation and remains at zero.

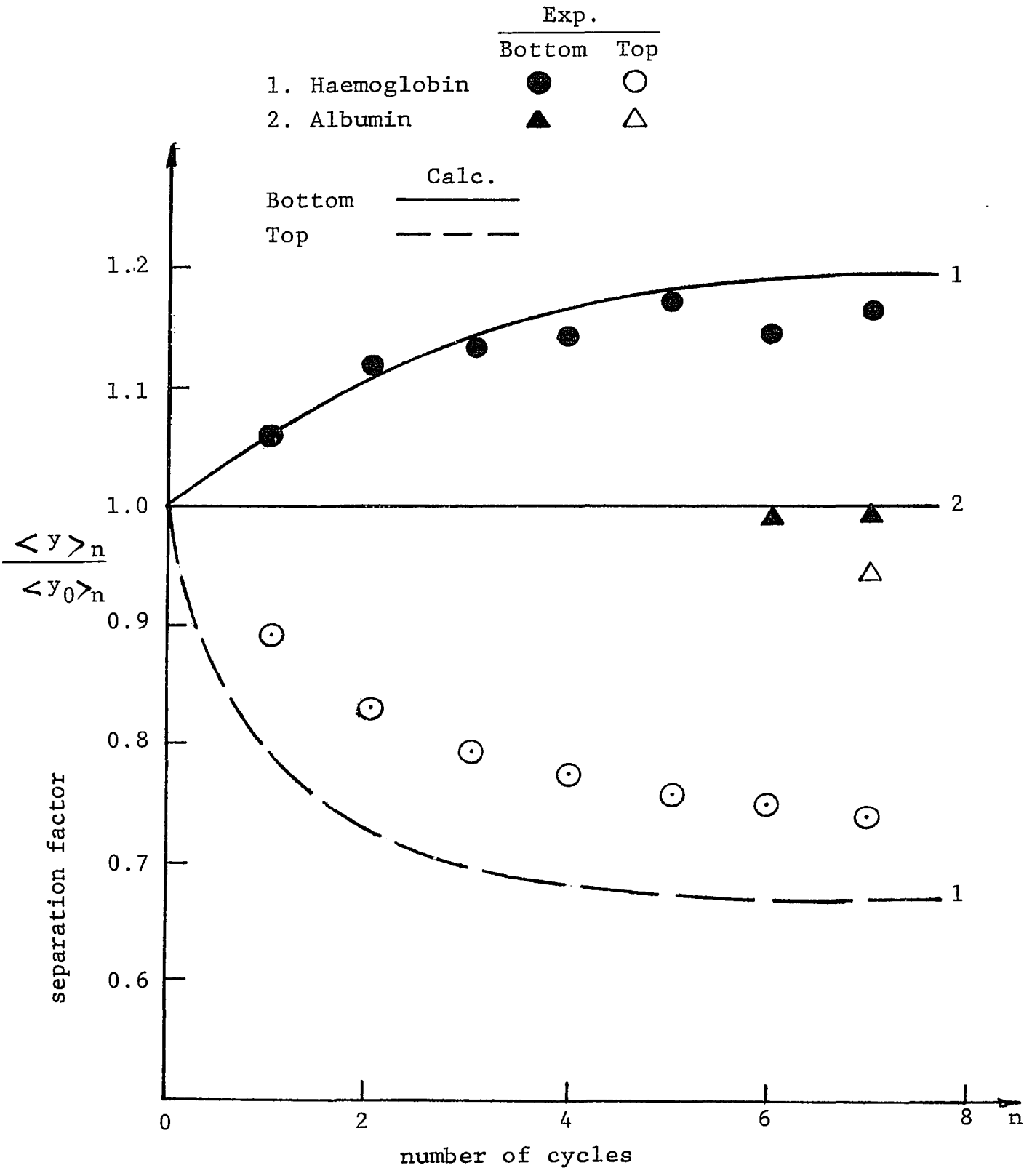


FIGURE 31 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN AND ALBUMIN vs. NUMBER OF CYCLES.

Figures 32 and 33 show the comparison of anion and cation exchanger column for haemoglobin and albumin respectively. As we know the isoelectric point of albumin is 4.7. The experimental results are shown in Figure 31. Instead of using pH = 8, pH = 4 was selected. The buffers used for this part of the experiments were mixtures of acetic acid, sodium acetate and sodium chloride. As the theory predicts, albumin is concentrated at the low pH end of R^+ column and at the high pH end of the R^- column which are opposite from the result for haemoglobin.

For the Semi-Continuous system the analysis of a few experiments using anion and cation exchangers and feed of single components only are involved. The operating parameters for these experimental runs can be found on Exhibit B-2 in Appendix B.

The purpose of these experiments was to investigate the flow migration of the individual components by varying the buffer conditions and also verify the equilibrium constants at the different pH level. The results, shown in Figure 21, not only explained how the equilibrium constant varies due to both the pH change and the ion exchanger, but give us more confidence by confirming the equilibrium theory at a low concentration of the solute (region of interest).

The first set of experiments investigates the anion column using a feed of pure haemoglobin. The ideal conditions

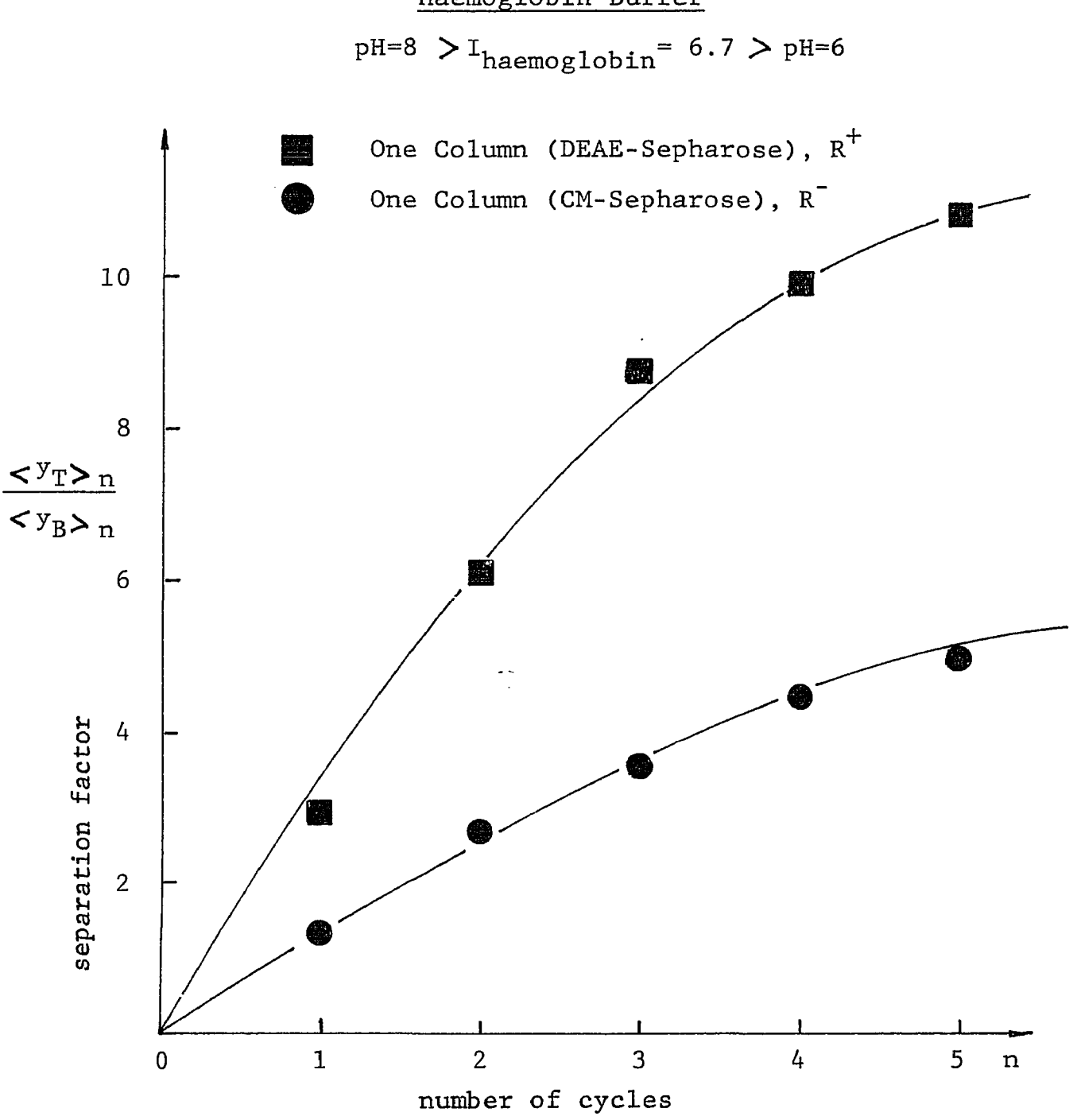


FIGURE 32 - COMPARISON OF ANION AND CATION COLUMN FOR HAEMOGLOBIN.

Albumin-Buffer

pH=6 > $I_{albumin}$ = 4.7 > pH=4

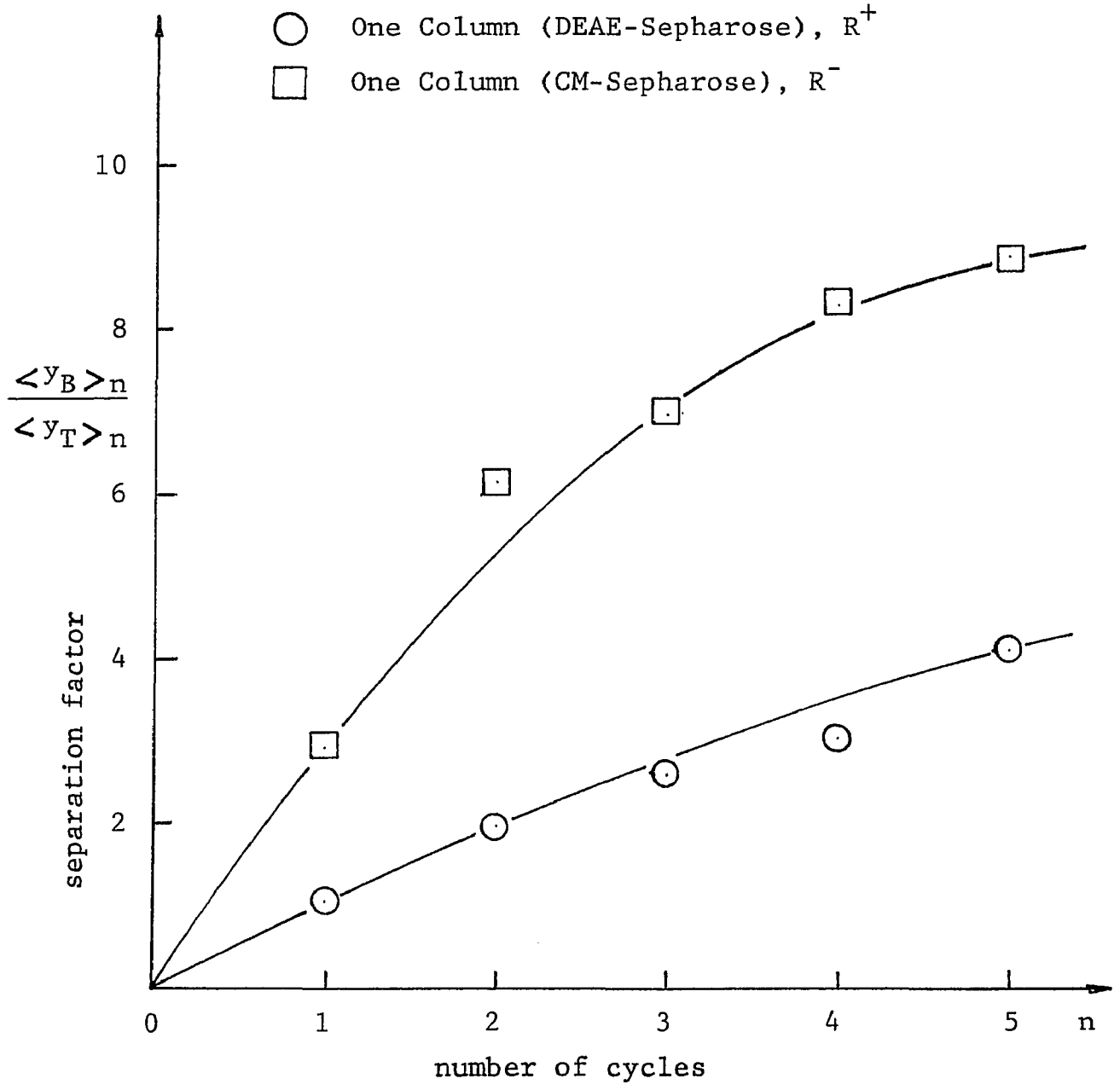


FIGURE 33 - COMPARISON OF ANION AND CATION COLUMN FOR ALBUMIN.

were to have a large concentration of haemoglobin in the 6.2 BR product stream. It was found that the experimental delivered the best results, which is shown in Figure 34.

The next group of experiments involved the use of an anion exchanger and a feed of pure albumin. The ideal condition were to have the concentration of albumin in the 6.2 top product stream low. The experimental results can be seen in Figure 35.

It should be noted that the buffer and sodium chloride concentrations were equal to 0.10 M and 0.05 M, respectively for all reservoirs and feeds.

The last group of experiments were conducted using the cation exchanger and a feed of pure haemoglobin. The ideal conditions were to have the concentration of haemoglobin in the 6.2 bottom product stream low. The results are shown in Figure 36.

C. Two-Column System

Mode 1: Three reservoirs Batch operation

This section is a combination of a two single column system connecting in series and they were packed alternately with an anion and a cation exchanger.

Before we discuss the results of this mode, we have to to understand the flow movement of the individual components

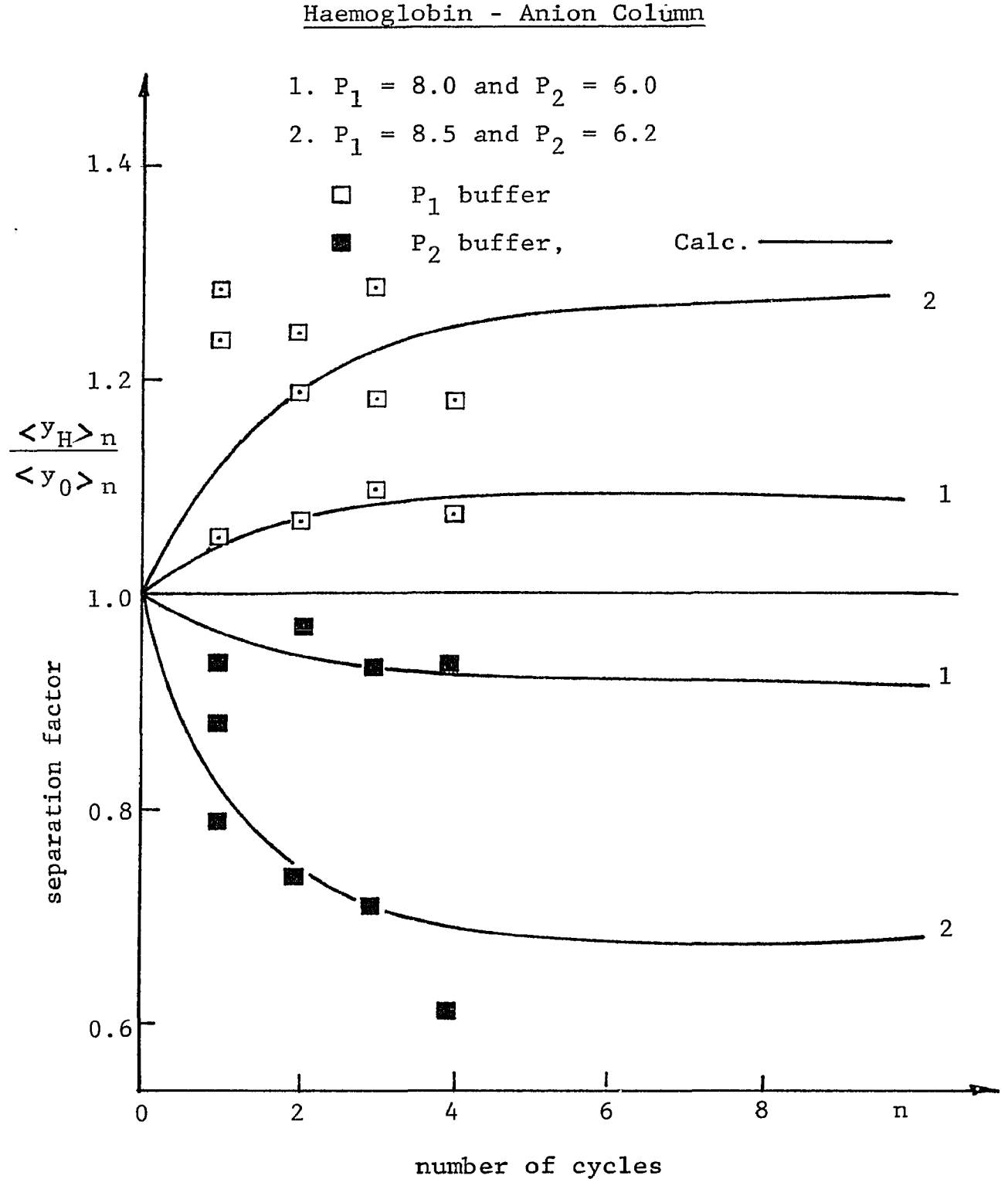


FIGURE 34 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN IN A SINGLE-ANION-COLUMN SYSTEM.

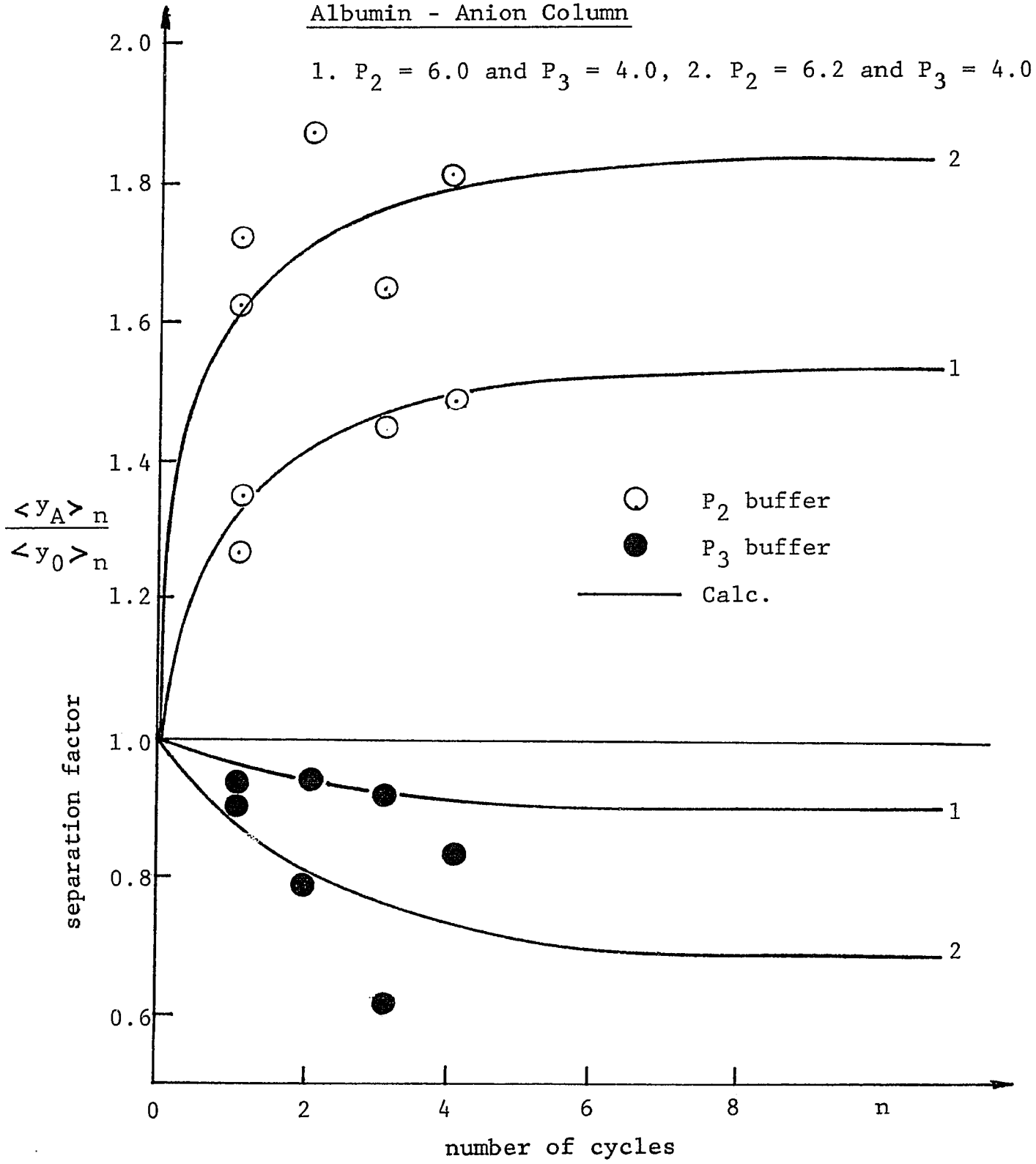


FIGURE 35 - THE CONCENTRATION TRANSIENTS OF ALBUMIN IN A SINGLE-ANION-COLUMN SYSTEM.

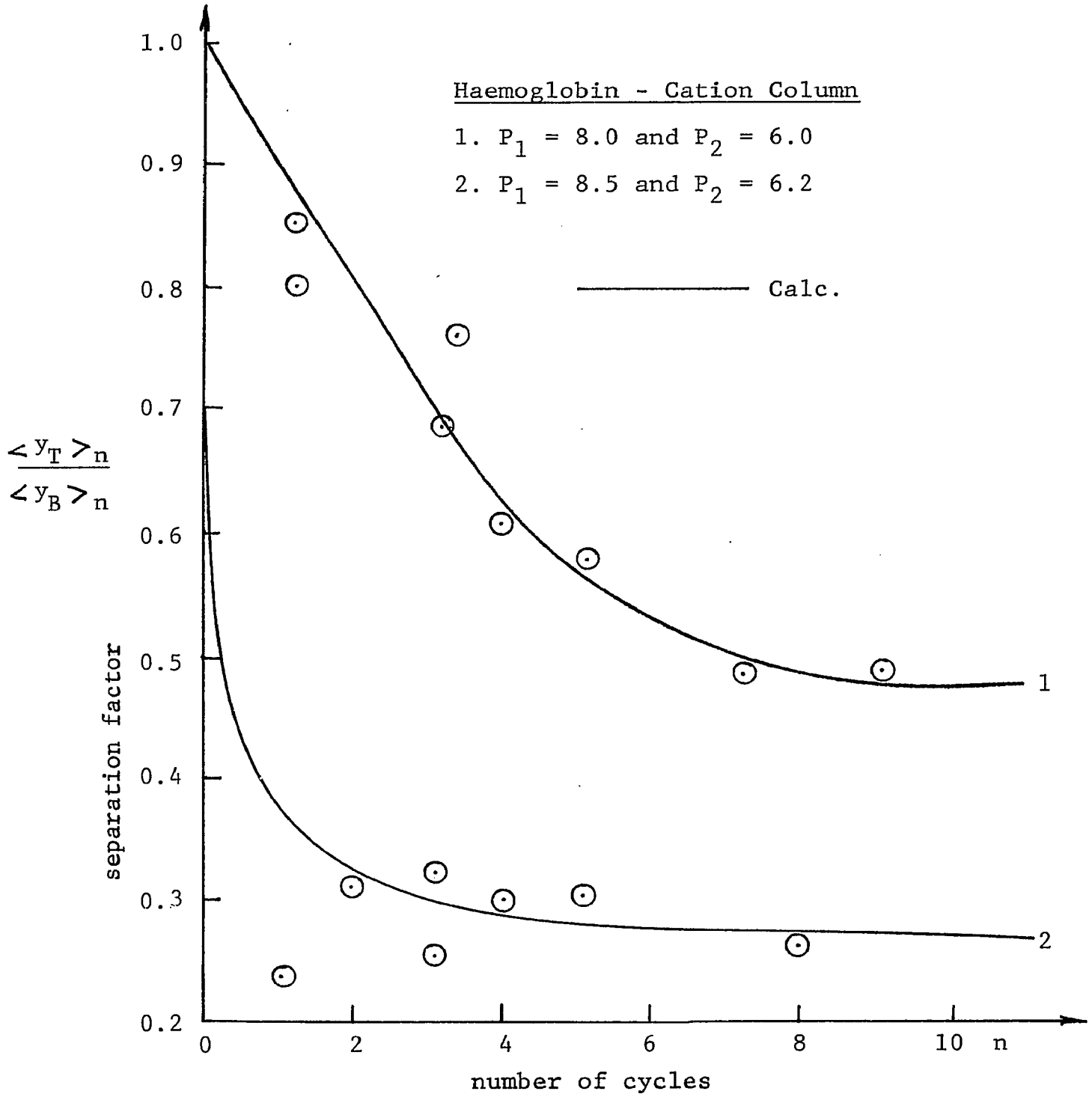


FIGURE 36 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN IN A SINGLE-CATION-COLUMN SYSTEM.

in the system. The haemoglobin having the higher isoelectric point (= 6.7), will migrate the 8.5 (P_1) reservoir through the anion (R^+) column to the 6.2 (P_2) reservoir. Albumin having a lower isoelectric point (= 4.7), compare with P_1 and P_2, then the system will have no mass transfer on albumin.

In the same manner, if the column was packed with cation (R^-) the haemoglobin will migrate from 6.2 reservoir through R^- column to 8.5 reservoir, while no change for the concentration of albumin occur in any reservoirs.

Figures 37 and 38 show the results of running pure haemoglobin and pure albumin. We also found that, for the two column system, a better result on haemoglobin is occured, where there is no change in albumin concentration

Mode 2: Four Reservoirs Batch Operation

A general understanding of the flow movement of the individual component of the four reservoirs system must be explained before we can discuss the results. The haemoglobin having the higher isoelectric point (I.P.), will migrate from the 6.2 bottom reservoir, through the cation column to the 8.5 middle reservoir; and from the 8.5 middle reservoir, through the anion column to the 6.2 top reservoir. The albumin, having the lower isoelectric point (I.P.), will migrate from the 6.2 top reservoir, through the anion column to the 4.0 middle reservoir; and from the 4.0 middle reser-

Albumin

pH = 6.2 > $I_{albumin}$ = 4.7 > pH = 4.0

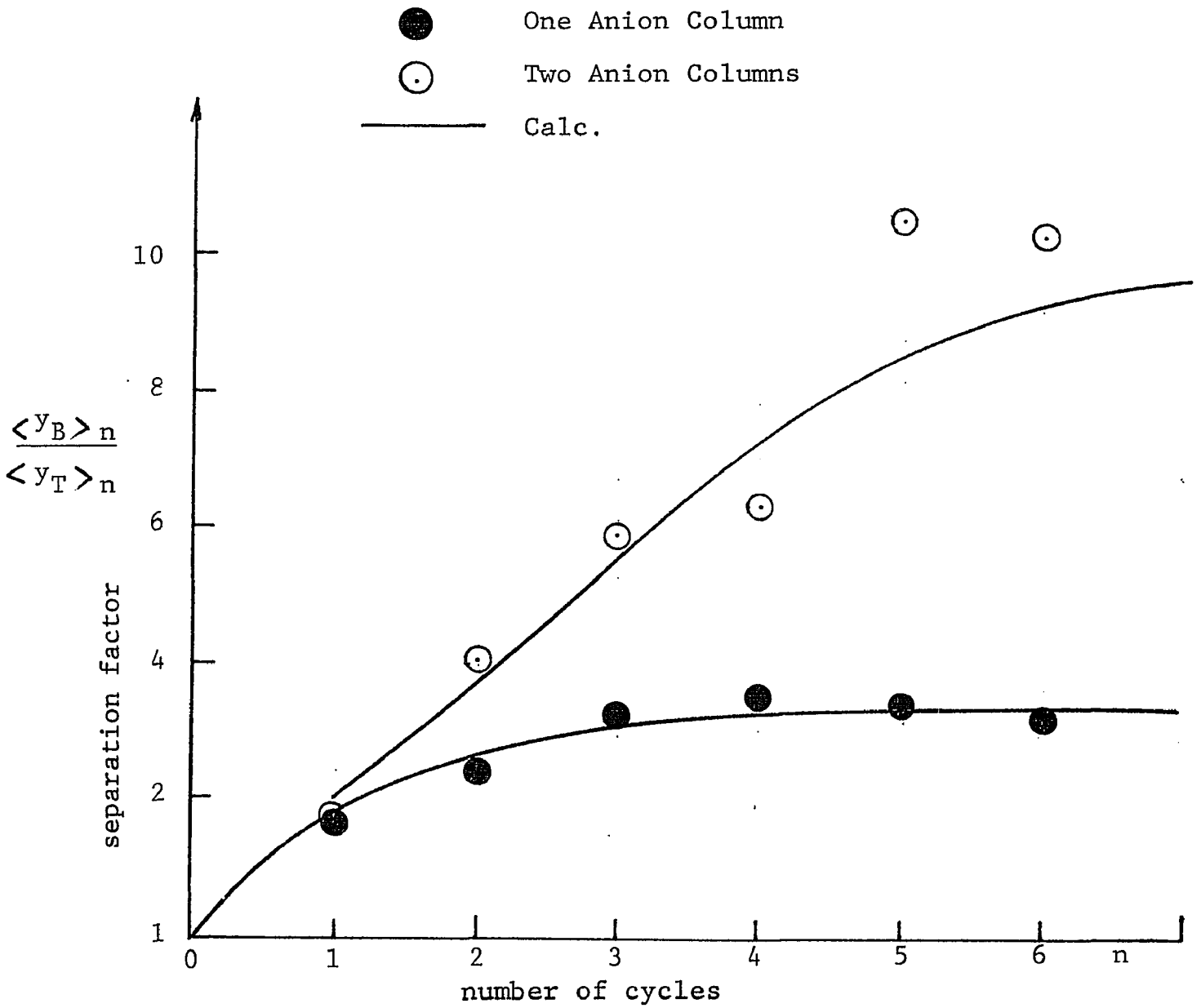


FIGURE 37 - COMPARISON OF ALBUMIN CONCENTRATION BETWEEN ONE- AND TWO-COLUMN SYSTEM.

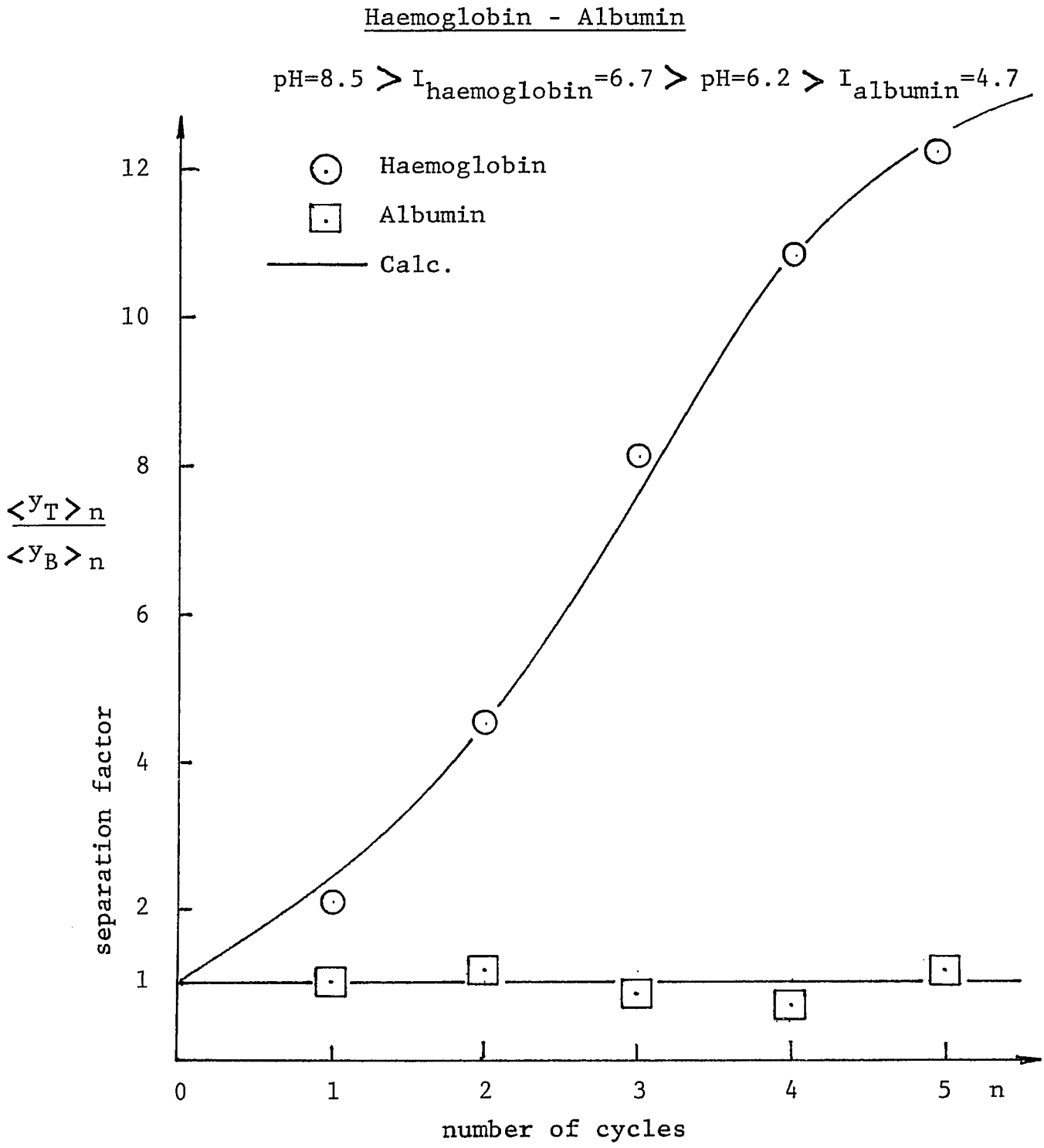


FIGURE 38 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN AND ALBUMIN vs. NUMBER OF CYCLES: MODE 1.

voir through the cation column to the 6.2 bottom reservoir. Thus, the 6.2 top and the 6.2 bottom reservoir are examined.

Experimental parameters are tabulated in Exhibit B-4 which were conducted by initially having a feed mixture of haemoglobin and albumin in the 4.0 and 8.5 middle reservoirs, and having a pure buffer solution in the 6.2 top and 6.2 bottom reservoirs. This was done to examine the flow migration of haemoglobin and albumin in the system. The 6.2 top and bottom reservoirs buffer and sodium chloride concentration in the 4.0 and 8.5 middle reservoirs varied to aid in the protein migration.

The main objective of these experiments was to have the concentration of haemoglobin greater to that of albumin in the 6.2 top reservoir and to have the concentration of albumin greater to that of haemoglobin in the 6.2 bottom reservoir. By examining Figures 39, 40 and 41, we can see that the concentration of albumin is greater than the concentration of haemoglobin in the 6.2 bottom reservoir. We can also see from the figures that the concentration of haemoglobin is less than the concentration of albumin in the 6.2 top reservoir. This result proved to be negative for the 6.2 top reservoir.

The next set of experiments were designed to investigate the migration of the individual components in the Four Reservoir Batch System.

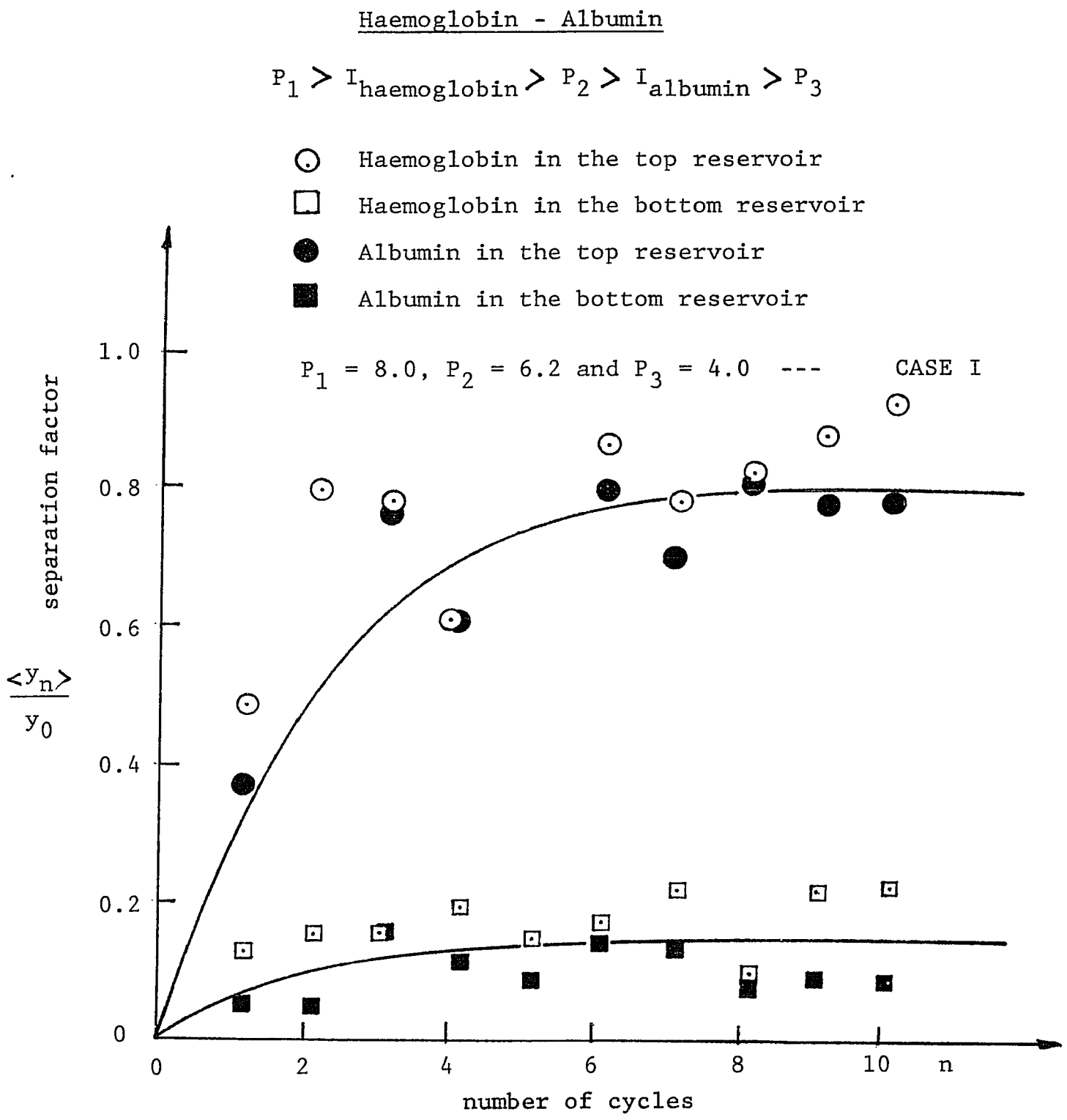


FIGURE 39 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN AND ALBUMIN vs. NUMBER OF CYCLES: MODE 2. (CASE I)

Haemoglobin - Albumin

$$P_1 > I_{haemoglobin} > P_2 > I_{albumin} > P_3$$

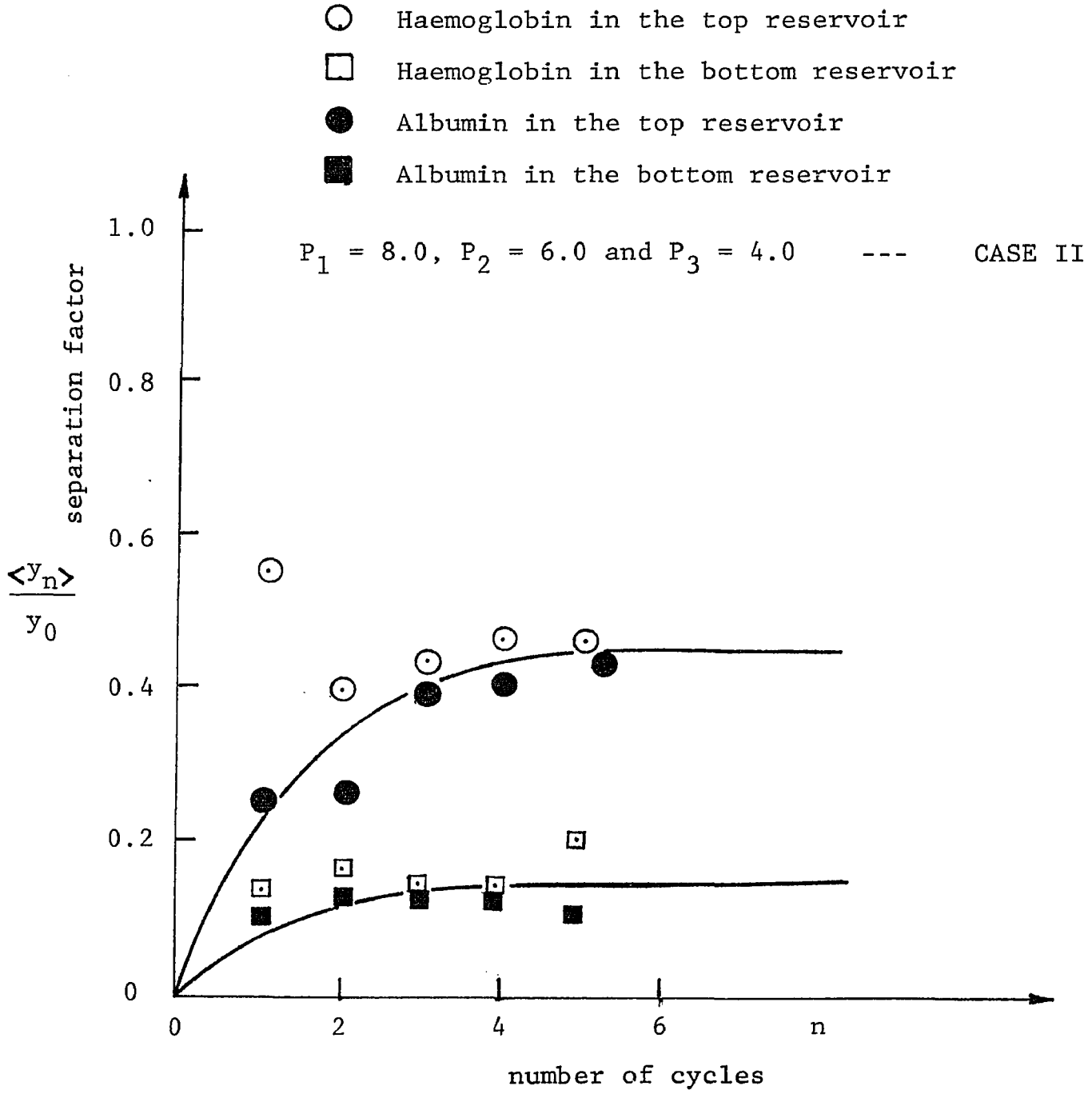


FIGURE 40 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN AND ALBUMIN vs. NUMBER OF CYCLES: MODE 2. (CASE II)

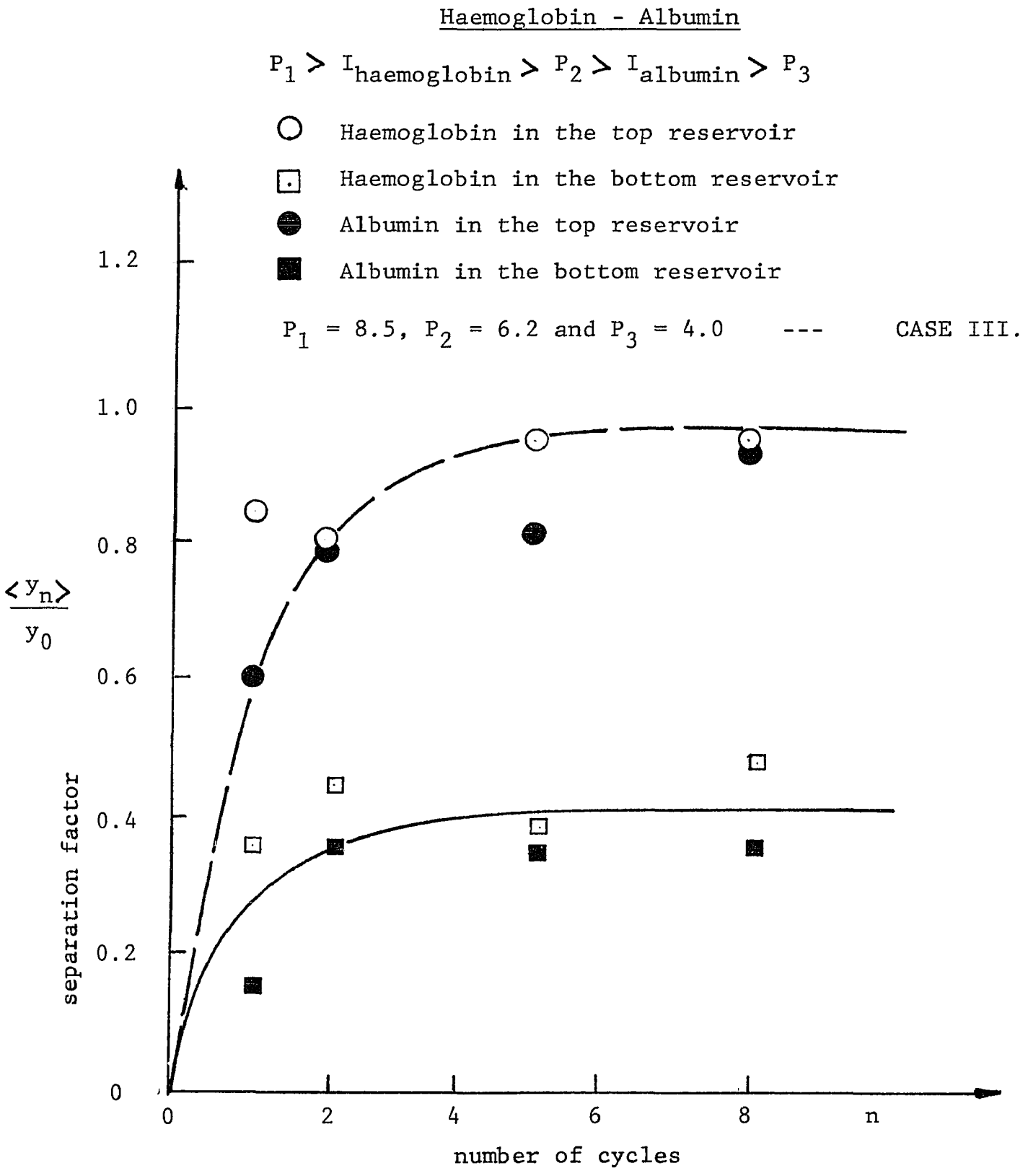


FIGURE 41 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN AND ALBUMIN vs. NUMBER OF CYCLES: MODE 2. (CASE III)

This experiment had pure albumin feed in the 4.0 and the 8.5 middle reservoirs and pure buffer in the 6.2 bottom and top reservoirs. The results of using this experiment proved to be positive (i.e. y_A 6.2 bottom $>$ y_A 6.2 top). See Figure 42. The next logical step was to use the same buffer conditions and run the same experiment with pure haemoglobin.

Mode 2: Four Reservoirs Semi-Continuous Operation.

A general understanding of the flow movement of the individual components of the Four Reservoirs Semi-Continuous System. The haemoglobin having the higher isoelectric point (I.P.), will emerge from the 6.2 top product where albumin having the lower isoelectric point (I.P.) will emerge from 6.2 bottom product. The other intermediat steps were analyzed for protein concentration.

Experimental results, see Figures 43 and 44, were identical with respect to the operating parameters. These experiments were conducted with a feed of pure haemoglobin. The results that were obtained were positive. The concentration of haemoglobin was greater in the 6.2 top product stream than in the 6.2 bottom product stream. The next step was to run another experiment with pure albumin to observe how the albumin migrates.

Figure 45 shows the results which were operated under

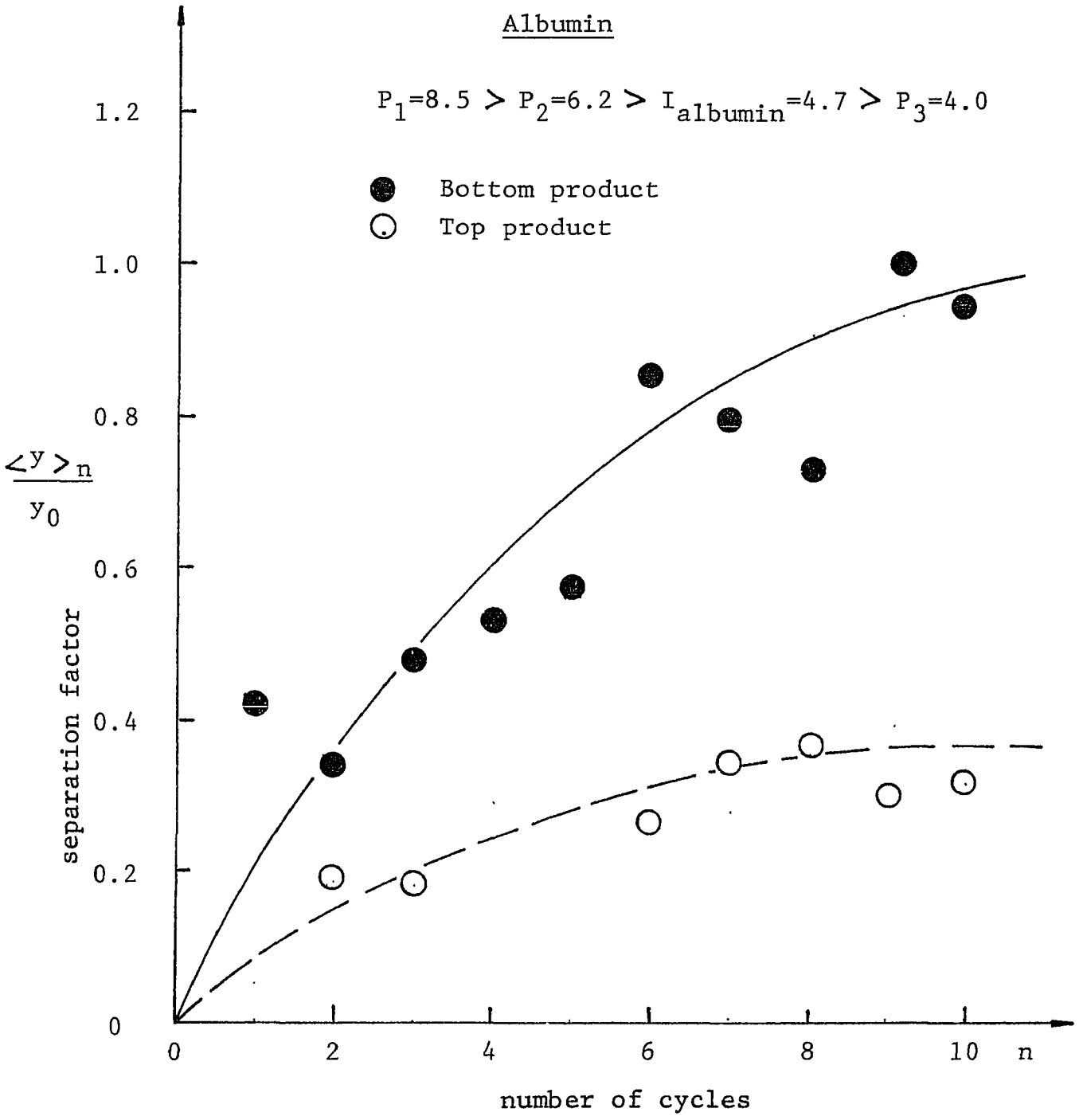


FIGURE 42 - THE CONCENTRATION TRANSIENTS OF ALBUMIN vs. NUMBER OF CYCLES: MODE 2.

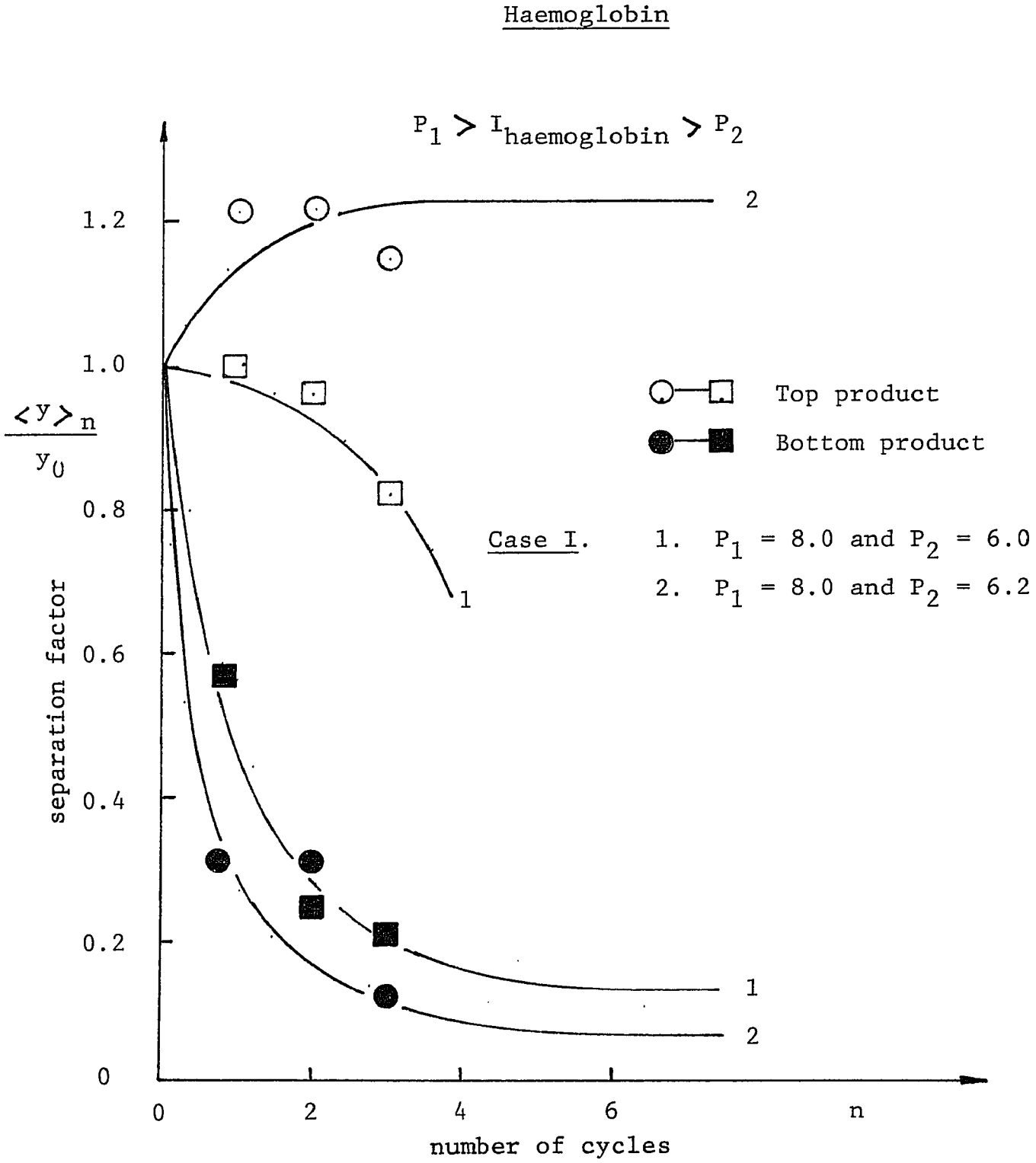


FIGURE 43 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN vs. NUMBER OF CYCLES: MODE 2 (CASE I).

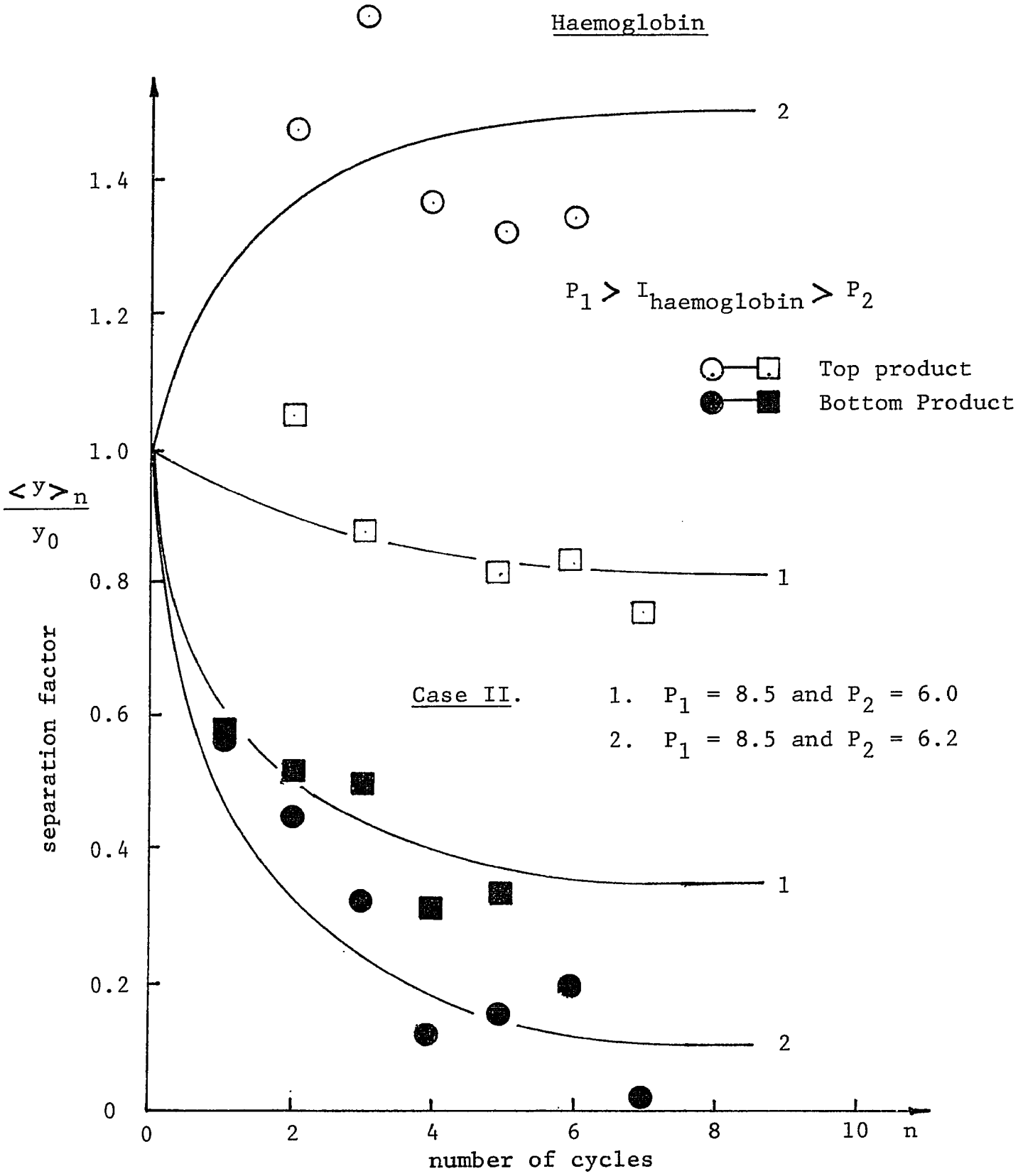


FIGURE 44 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN vs. NUMBER OF CYCLES: MODE 2 (CASE II).

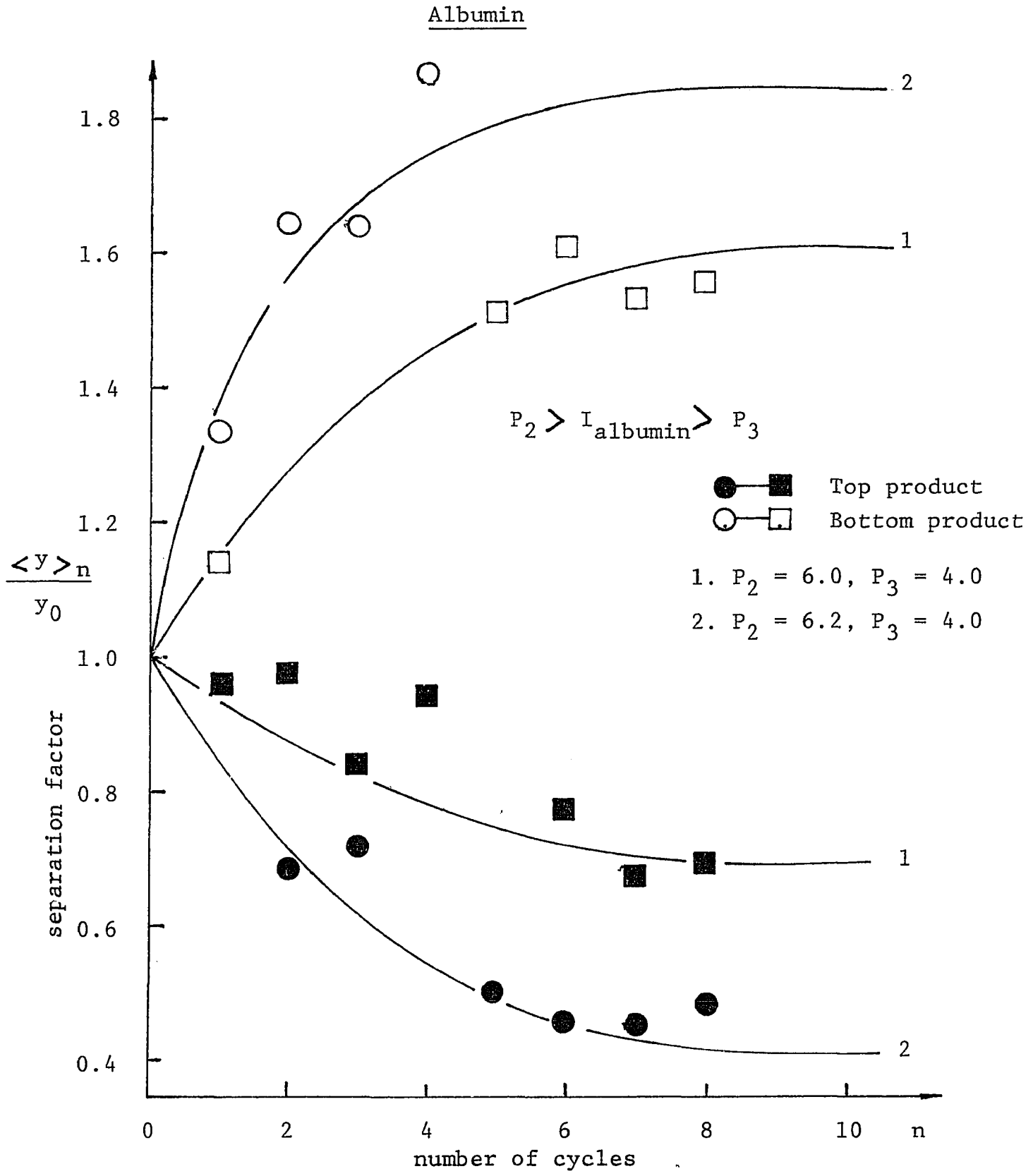


FIGURE 45 - THE CONCENTRATION TRANSIENTS OF ALBUMIN vs. NUMBER OF CYCLES: MODE 2.

the same buffer and sodium chloride conditions as the in the previous paragraph. The feed was a pure albumin. The results that were obtained were positive. The concentration of albumin was greater in the 6.2 bottom product stream than in the 6.2 top product stream. The next step was to run to run the mixture of haemoglobin and albumin by using the same buffer conditions that were used for the individual run.

The experiment (Figure 46) was conducted with a mixture of haemoglobin and albumin. The results that were obtained were positive. The concentration of haemoglobin in the 6.2 top product stream was greater than the concentration of albumin in the 6.2 top product stream. The y_H, (y_H/y_{HO}), values for the 6.2 top product streams were 1.42 and 1.61 respectively. The y_A, (y_A/y_{AO}), values were in the order of 0.19 and 0.09 for the 6.2 top product streams. The concentration of albumin in 6.2 bottom product stream was greater than the concentration of haemoglobin in the bottom product stream. The y_A, (y_A/y_{AO}), y_H and (y_H/y_{HO}) values were 1.58, 1.39, 0.15 and 0.31 respectively in the 6.2 bottom product stream. The buffer concentration for this experiment was 0.10 M and the sodium chloride concentration was 0.05 M for all reservoirs and feed streams. Thus, the experimental parameters that were used in this experiment and calculation were considered acceptable.

Mode 3: Five Reservoirs Batch Operation.

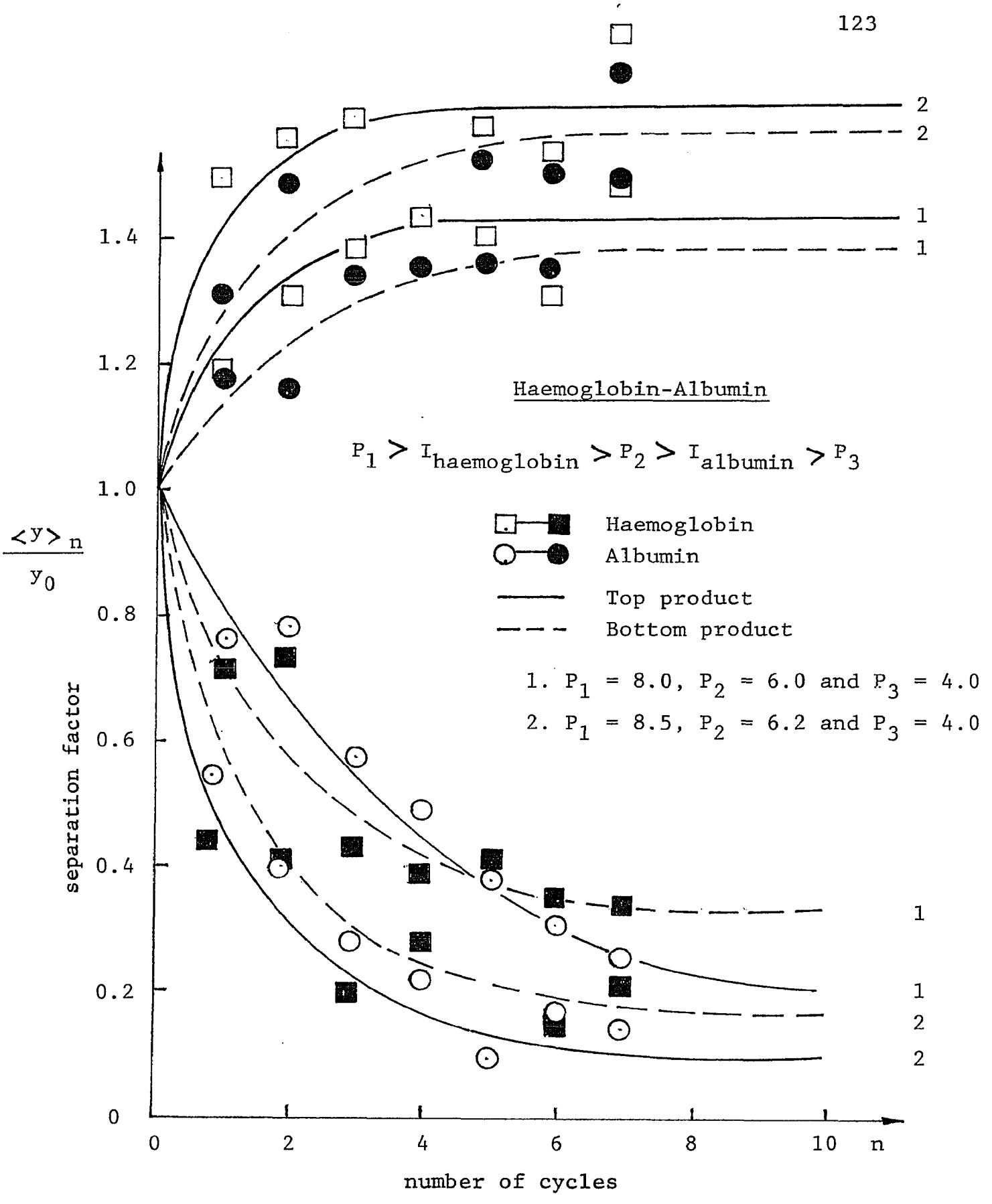


FIGURE 46 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN-ALBUMIN vs. NUMBER OF CYCLES: MODE 2.

Before we can discuss the various results of this system, we have to understand the flow movement of the individual components in the system. The haemoglobin having the higher isoelectric point (I.P.), will migrate from the 8.5 top reservoir through the anion column to the 6.2 middle reservoir; it will then move from the 6.2 middle reservoir through the cation column and into the 8.5 bottom reservoir. The albumin having the lower isoelectric point, will migrate from the 4.0 bottom reservoir through the cation column and to the 6.2 middle reservoir; then from the 6.2 middle reservoir, it will migrate through the anion column and into the 4.0 top reservoir.

Figure 47 can be explained that the haemoglobin did migrate to the 8.5 bottom reservoir, and the albumin also moved to this reservoir. This phenomenon implies that the buffer strength of the 8.5 bottom reservoir will remove not only the haemoglobin but also the albumin from the cation exchanger. By examining the 4.0 top reservoir, we found that the albumin migrated to the 4.0 top reservoir; yet, the buffer strength of the 4.0 top reservoir did not allow the removal of the haemoglobin from the solid phase of the anion exchanger. This type of result was obtained when the the buffer strength was 0.15 M and 0.20 M, and for a sodium chloride of 0.05 M and 0.05 M for experimental results shown in Figures 47 and 48 respectively. The y_H value (equivalent to $< y_H/y_{HO} >$ in the previous pages) refers to the feed

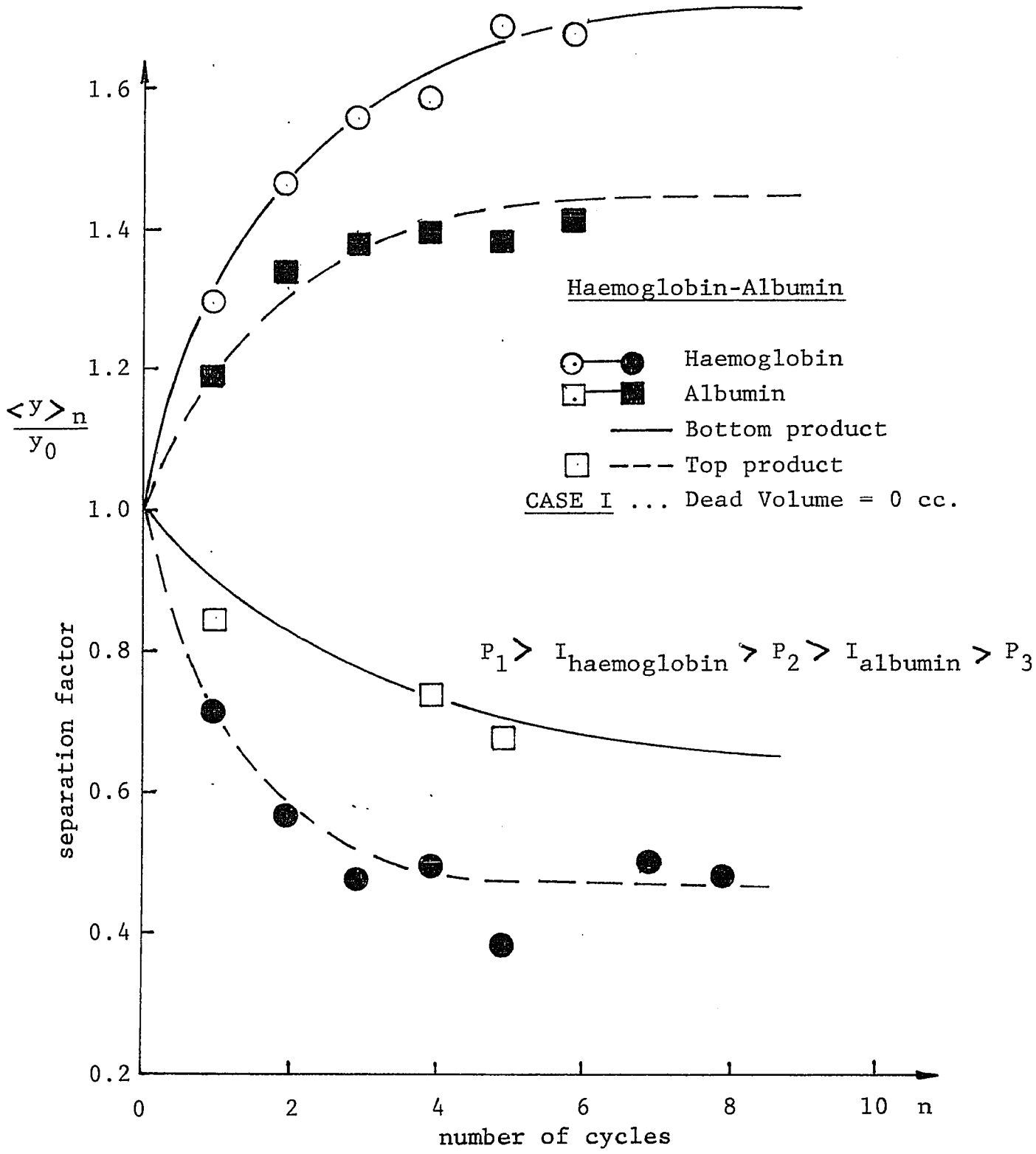


FIGURE 47 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN-ALBUMIN vs. NUMBER OF CYCLES: MODE 3 (CASE I).

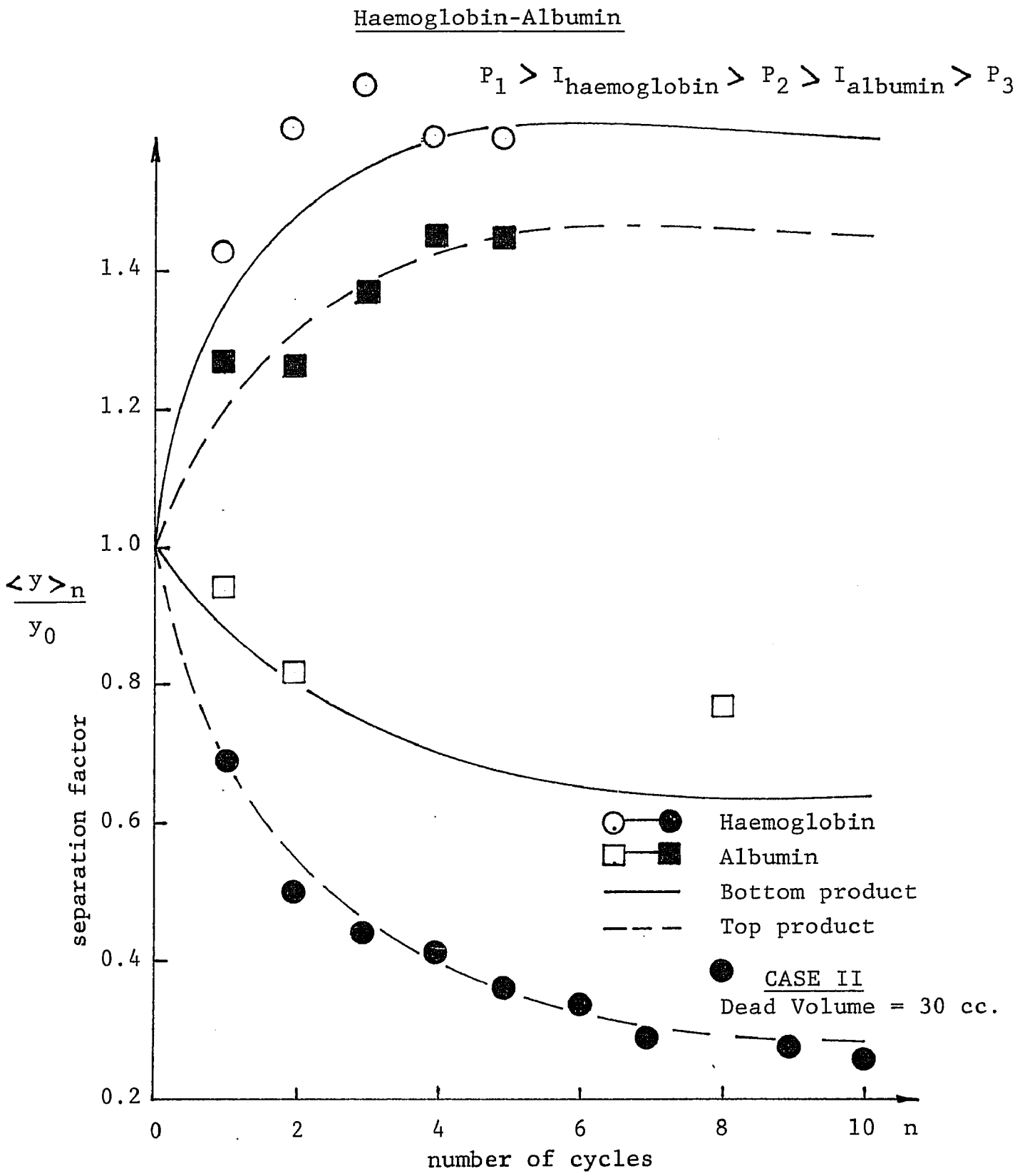


FIGURE 48 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN-ALBUMIN vs. NUMBER OF CYCLES: MODE 3 (CASE II)

concentration. If this value is equal to 1.0, this indicates that the haemoglobin is at the initial condition (assuming the buffer solution has haemoglobin present). The same explanation is true for albumin (y_A).

The results, shown in Figure 49, were conducted using the same buffer and sodium chloride conditions as mentioned before in the previous page. The major difference was in the 4.0 top reservoir and 8.5 bottom reservoir. The 4.0 top reservoir had only albumin while the 8.5 bottom reservoir had only haemoglobin. This was done to see to what extent the haemoglobin would enter the 4.0 top reservoir and albumin would enter the 8.5 bottom reservoir. The results of this experiment is also confirmed the previous results as discussed in the earlier pages.

Mode 4: Two Reservoirs Batch Operation.

This section is included a few experiments to confirm the mathematical model. The experiments were investigated by running individual components. First of all, with pure haemoglobin where pH = 6.2 (P_2) and pH = 8.5 (P_1) were used. Later on, we performed the experiment on pure albumin where pH = 6.2 (P_2) and pH = 4.0 (P_3) were designed.

The haemoglobin having a higher isoelectric point (I.P.), will migrate from the 6.2 bootom reservoir through the cation column, and through the pH-Converter, then it migrate from

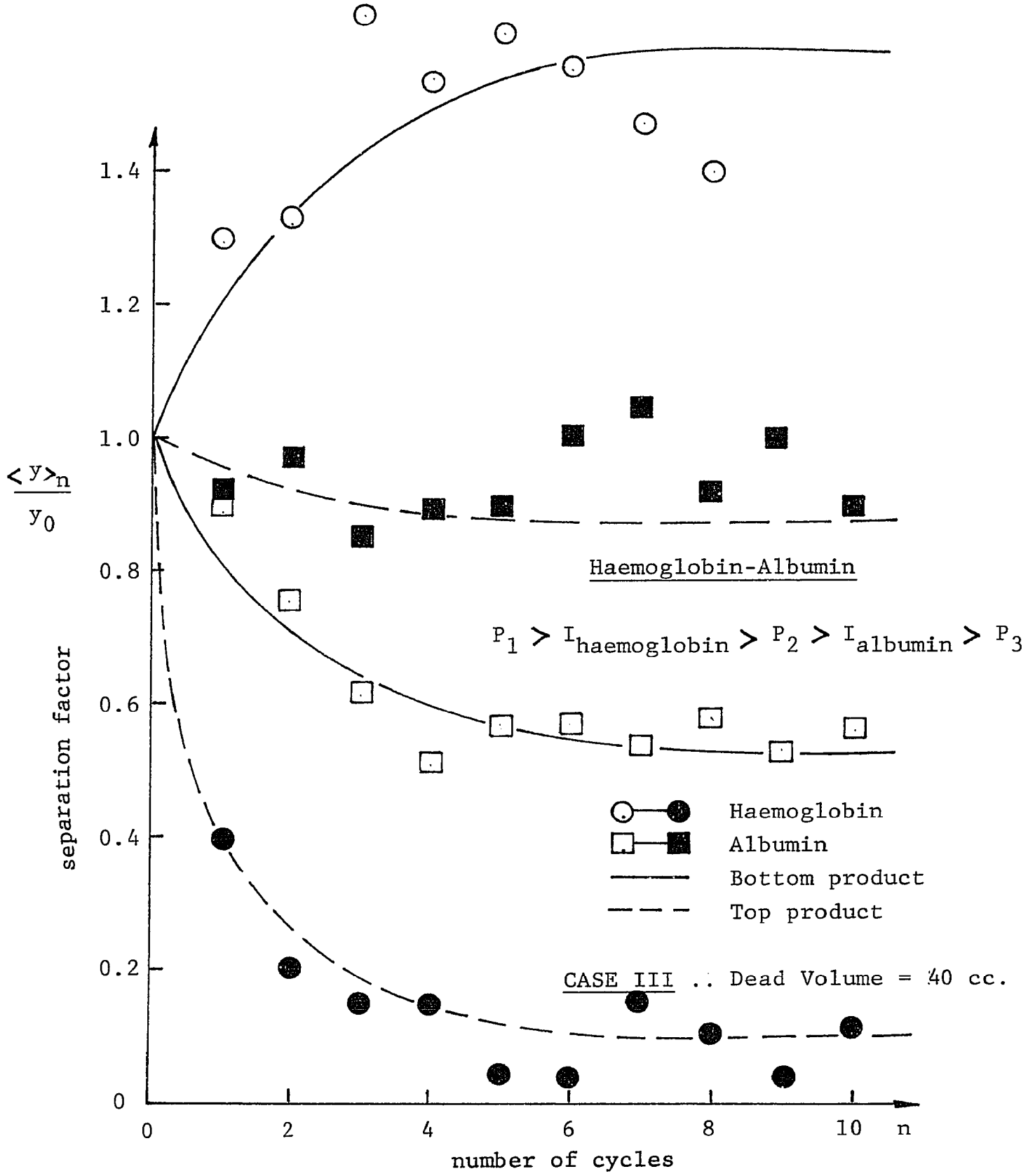


FIGURE 49 - THE CONCENTRATION TRANSIENTS OF HAEMOGLOBIN-ALBUMIN vs. NUMBER OF CYCLES: MODE 3 (CASE III).

the pH-Converter through another cation column into the 8.5 top reservoir. In the same manner, albumin will migrate from the 4.0 bottom reservoir through the cation column into the pH-Converter and go through another cation column into the 6.2 top reservoir.

Figures 50 and 51 show how the separation factor of haemoglobin and albumin respectively, were developed as the number of cycles were increased. The experimental results are agree well with the calculation value for both haemoglobin and albumin.

Mode 4: Two Reservoirs Semi-Continuous Operation.

The experiment were performed by running a mixture of haemoglobin and albumin by using pH = 8.5 (P_1) and pH = 6.2 (P_2). The isoelectric point of haemoglobin are in the range of 6.2 to 8.5, then the separation of haemoglobin was occured. The flow movement of haemoglobin is the same as we mentioned in the previous paragraph. The isoelectric point of albumin is 4.7 and lower than either P_1 or P_2, then there is no separation on albumin. Figure 52 shows the separation factor vs. n (number of cycles) for both haemoglobin and albumin.

D. Separation Of Proteins Via Multi-Column

This section is atheoretical study which extend our study from two column system into a multi-column system. We established that, Mode 2: Four reservoirs Batch Operation,

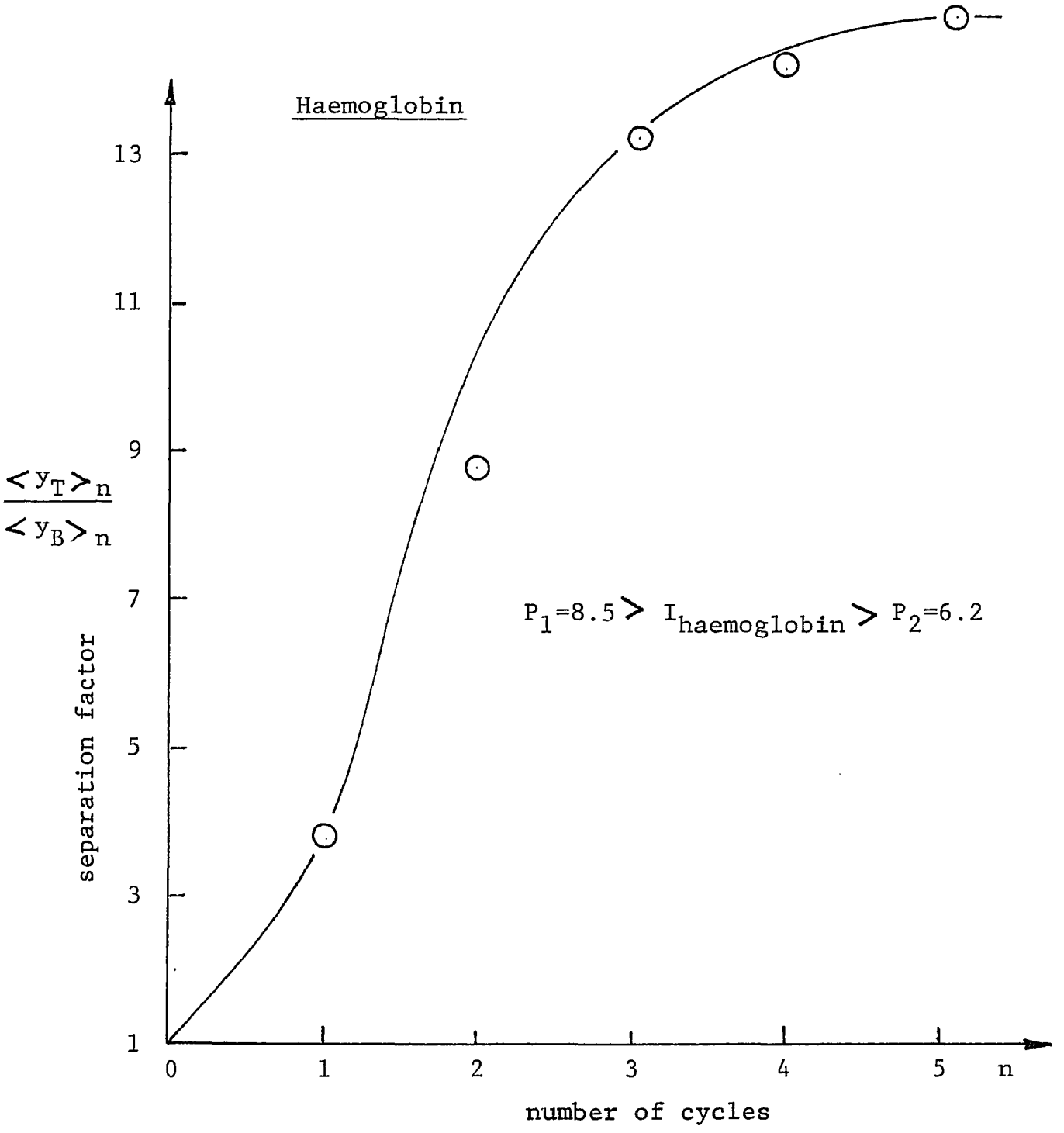


FIGURE 50 - SEPARATION FACTOR OF HAEMOGLOBIN vs. NUMBER OF CYCLES: MODE 4.

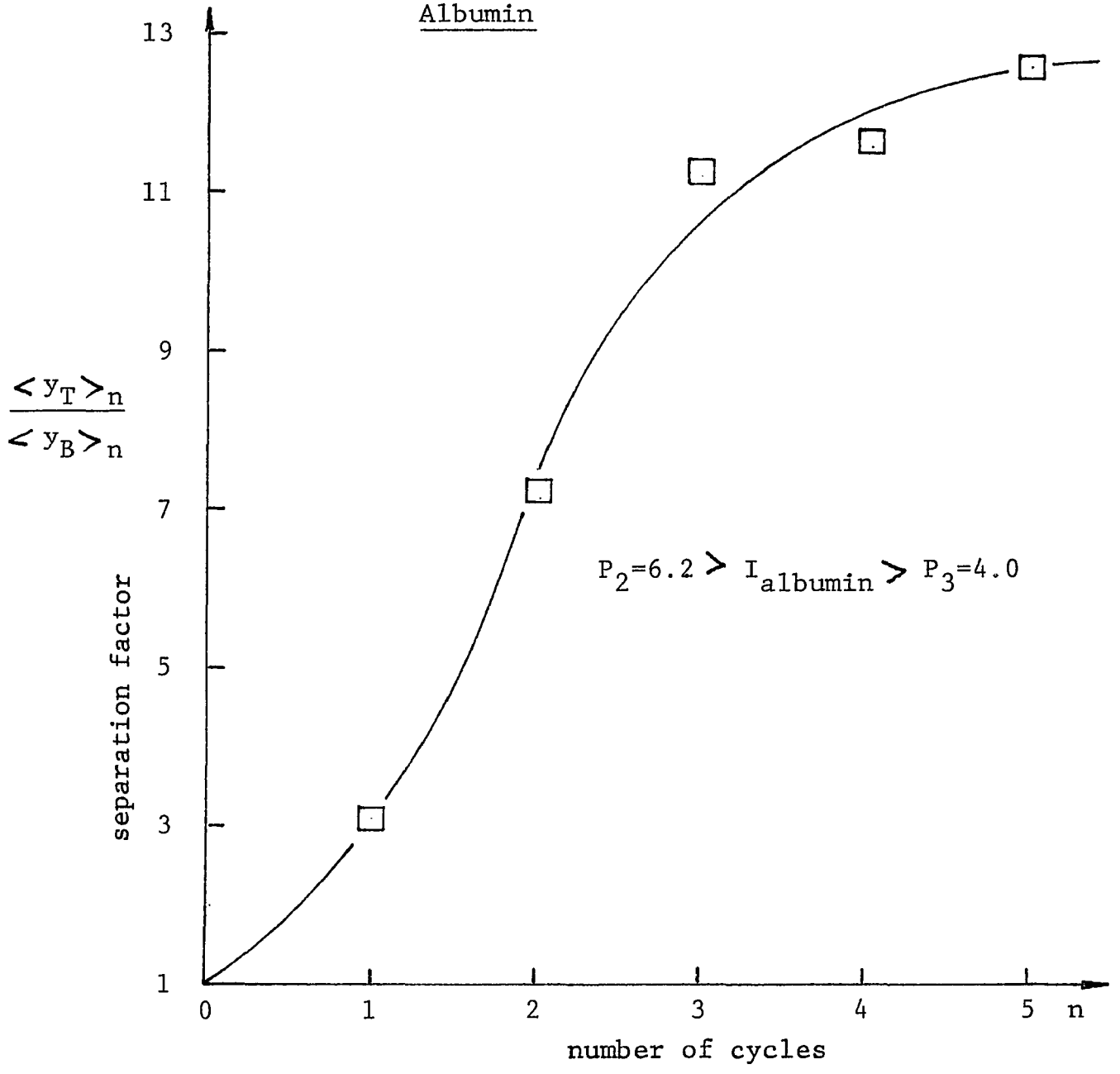


FIGURE 51 - SEPARATION FACTOR OF ALBUMIN vs. NUMBER OF CYCLES: MODE 4.

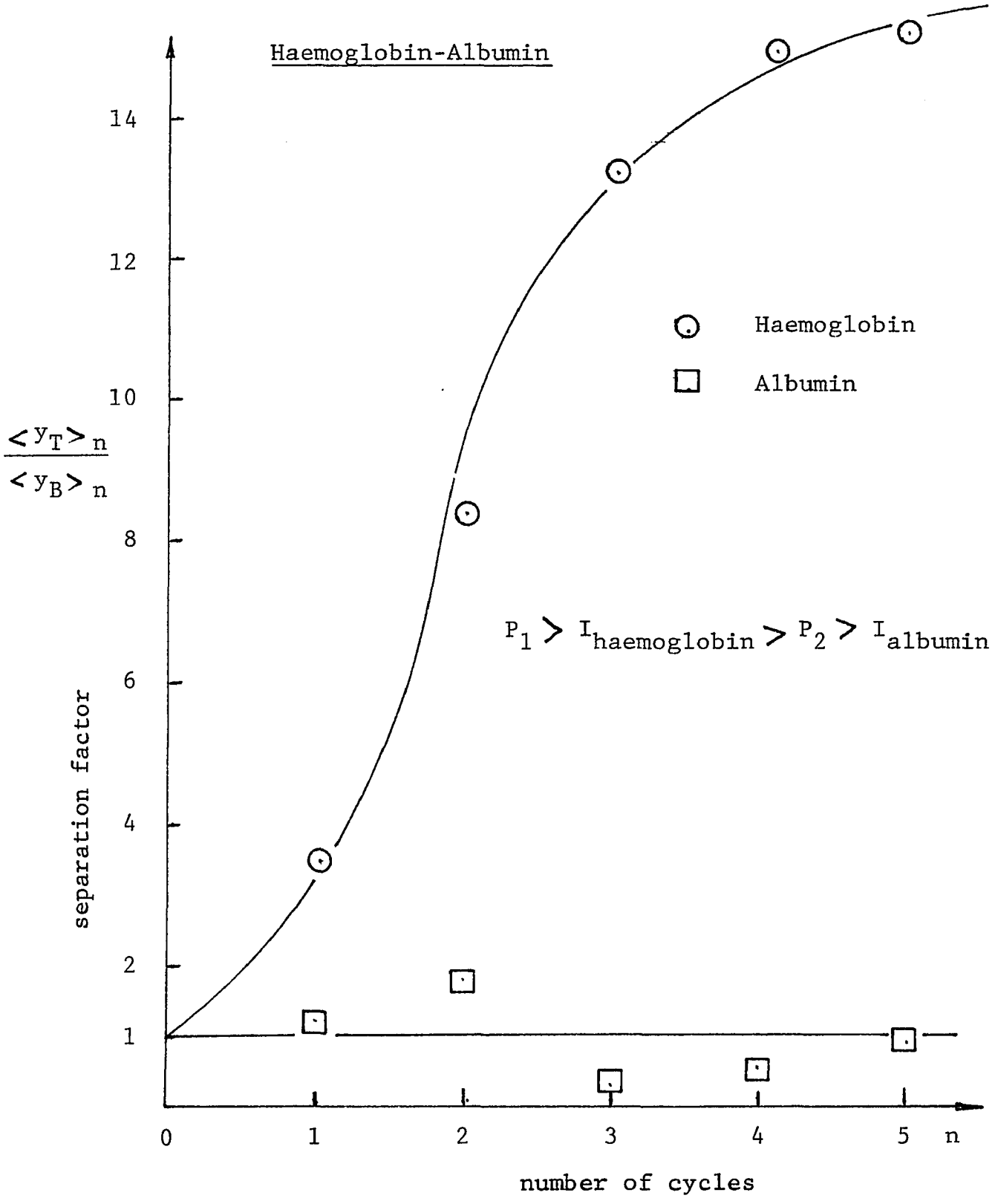


FIGURE 52 - SEPARATION FACTOR OF HAEMOGLOBIN AND ALBUMIN vs. NUMBER OF CYCLES: MODE 4.

can extend well to a multi-column system and be able to explained by using a graphical method. Our discussion will begin from a simple case and introduce a new symbols of proteins as A, B, C etc., just for our convenience for this explanation as well as the pH levels i.e., P_1, P_2, P_3 and so on.

Two Proteins - Two Columns:

Since the graphical method can predict the steady state concentrations of both top and bottom products for a single column system well, we are able to extend this concept of the graphical method to the two column system where the process description has been described in Chapter II.

To understand the flow movement of individual protein component, we consider the expression of isoelectric point and the pH level. Protein A having the higher isoelectric point (I.P.), will migrate from the P_2 bottom reservoir, through the cation column to the P_1 middle reservoir; from the P_1 middle reservoir, through the anion column to the P_2 top reservoir. The protein B having the lower isoelectric point(I.P.) will migrate from the P_2 top reservoir, through the anion column to the P_3 middle reservoir; and from the P_3 middle reservoir through the cation column to the P_2 bottom reservoir. Thus the enriched concentration of protein A and protein B will be found from the P_2 top reservoir and the P_2 bottom reservoir respectively.

Once the system operates for anumber of cycles, the steady state values of protein A and B reached, the results shown in Figures 53 and 54 as protein A and B are moved in the opposite direction indicate, that where protein A migrates upward to the top reservoir (pH = P_2), protein B moves downward to the bottom reservoir (pH = P_2).

The separation factor of protein is defined as $< y_T >_\infty / < y_B >_\infty$. For any protein mixture, to which this process can be applied, the S.F. (separation factor) will be high or low depending upon the value of the equilibrium constants for anion and cation exchanger which correspond to the pH in the top and bottom reservoirs, $k^+_{P_2}$ and $k^-_{P_2}$ respectively.

Figures 55 and 56 show the plot of S.F. versus the number of cycles at a different value of β for the protein A and B respectively. As β increases the S.F. is also increases. Figures 53 and 54 also show the results where both top product, $< y_T >_\infty$ and bottom product, $< y_B >_\infty$ of the proteins A and B are located.

Two Proteins - Multi Column:

This section investigates how the S.F. is effected if the number of columns is increased. Figures 57 and 58 show that as we increase the number of columns, the top and the bottom products of protein A are increasing and decreasing

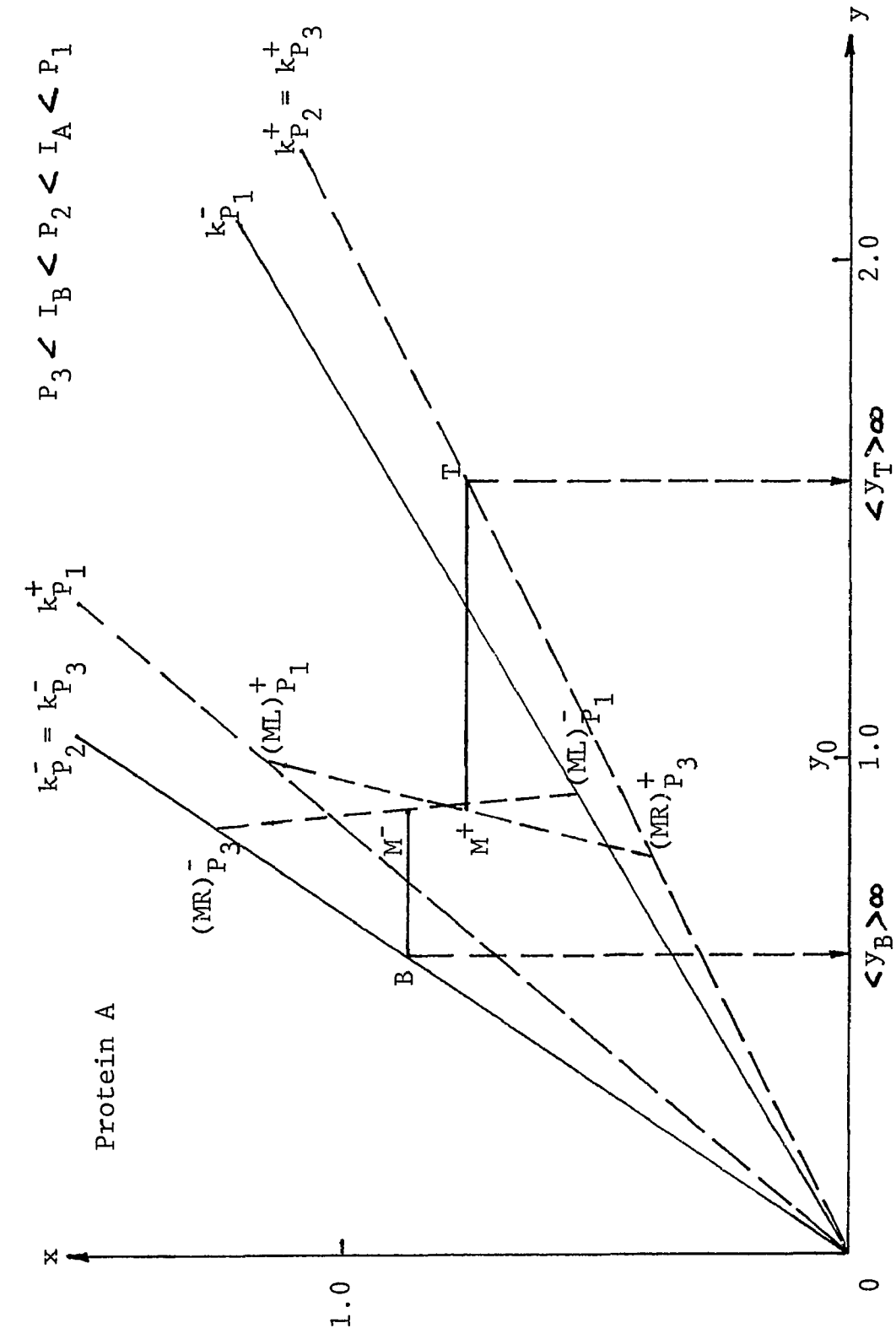
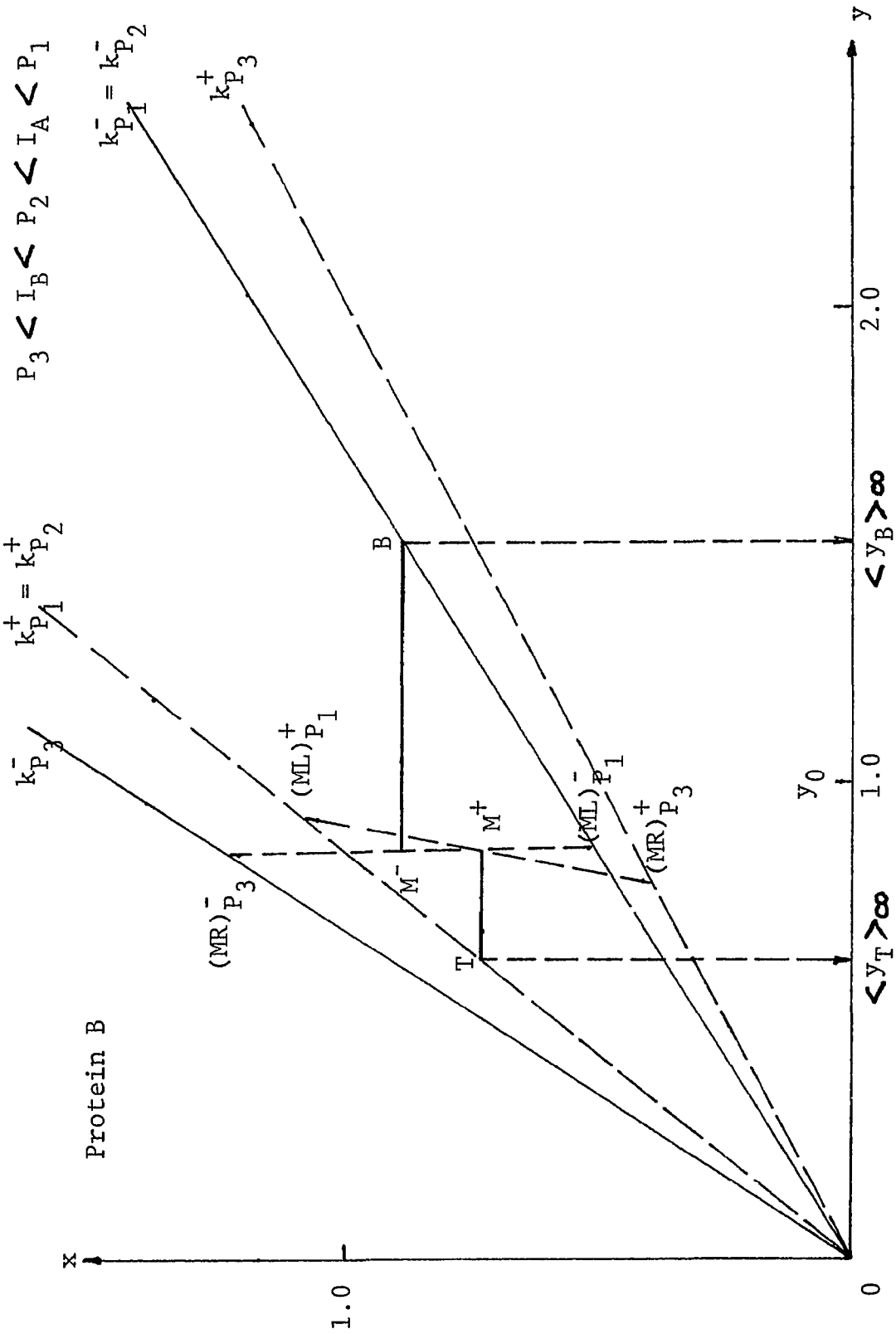


FIGURE 53 - GRAPHICAL SOLUTION OF PROTEIN A VIA TWO-COLUMN SYSTEM.



x, protein concentration in the solid phase

y, protein concentration in the fluid phase

FIGURE 54 - GRAPHICAL SOLUTION OF PROTEIN B VIA TWO-COLUMN SYSTEM.

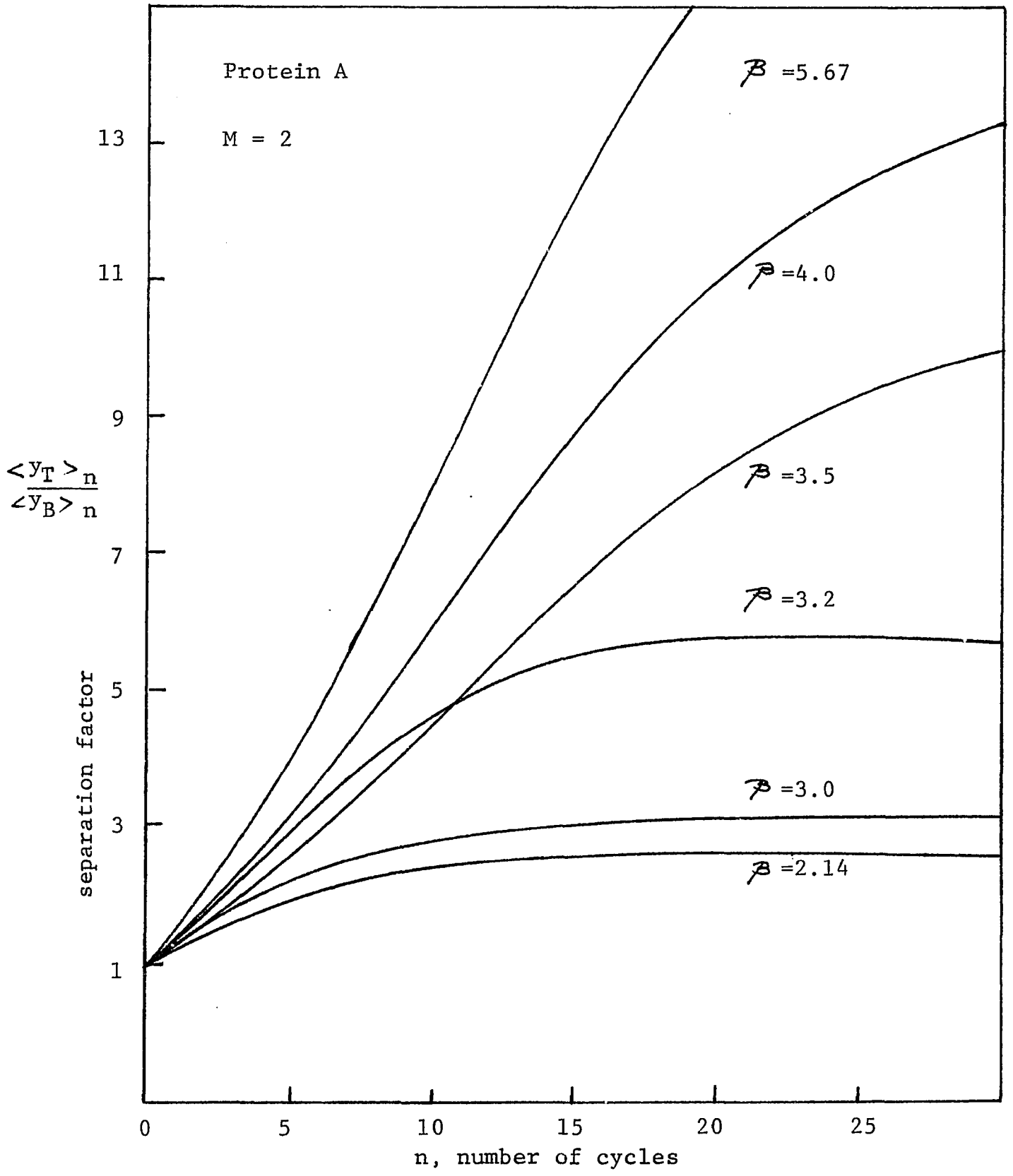


FIGURE 55 - EFFECT OF $\mathcal{B}$ ON CONCENTRATION TRANSIENTS FOR PROTEIN A

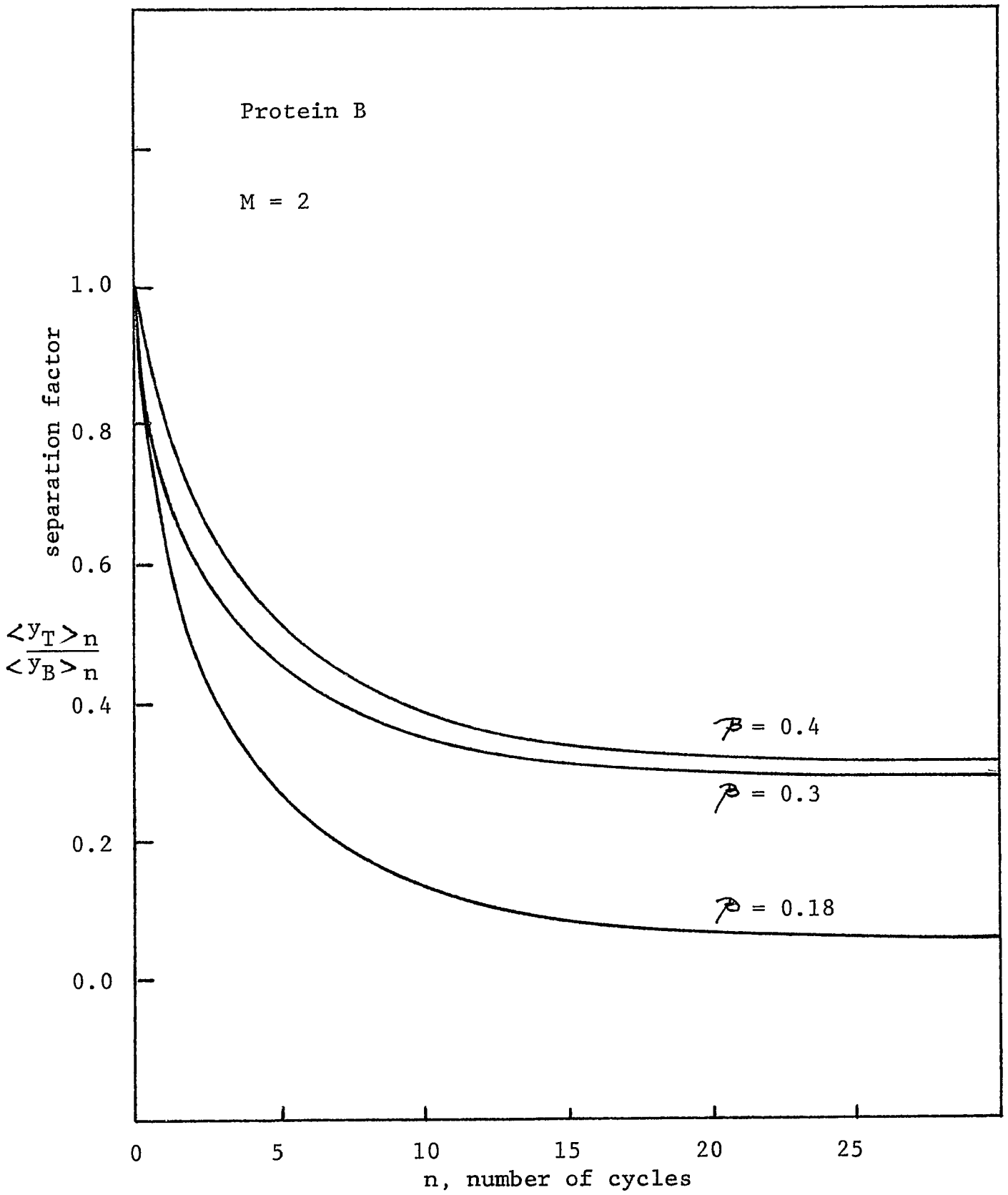


FIGURE 56 - EFFECT OF $\mathcal{P}$ ON CONCENTRATION TRANSIENTS FOR PROTEIN B

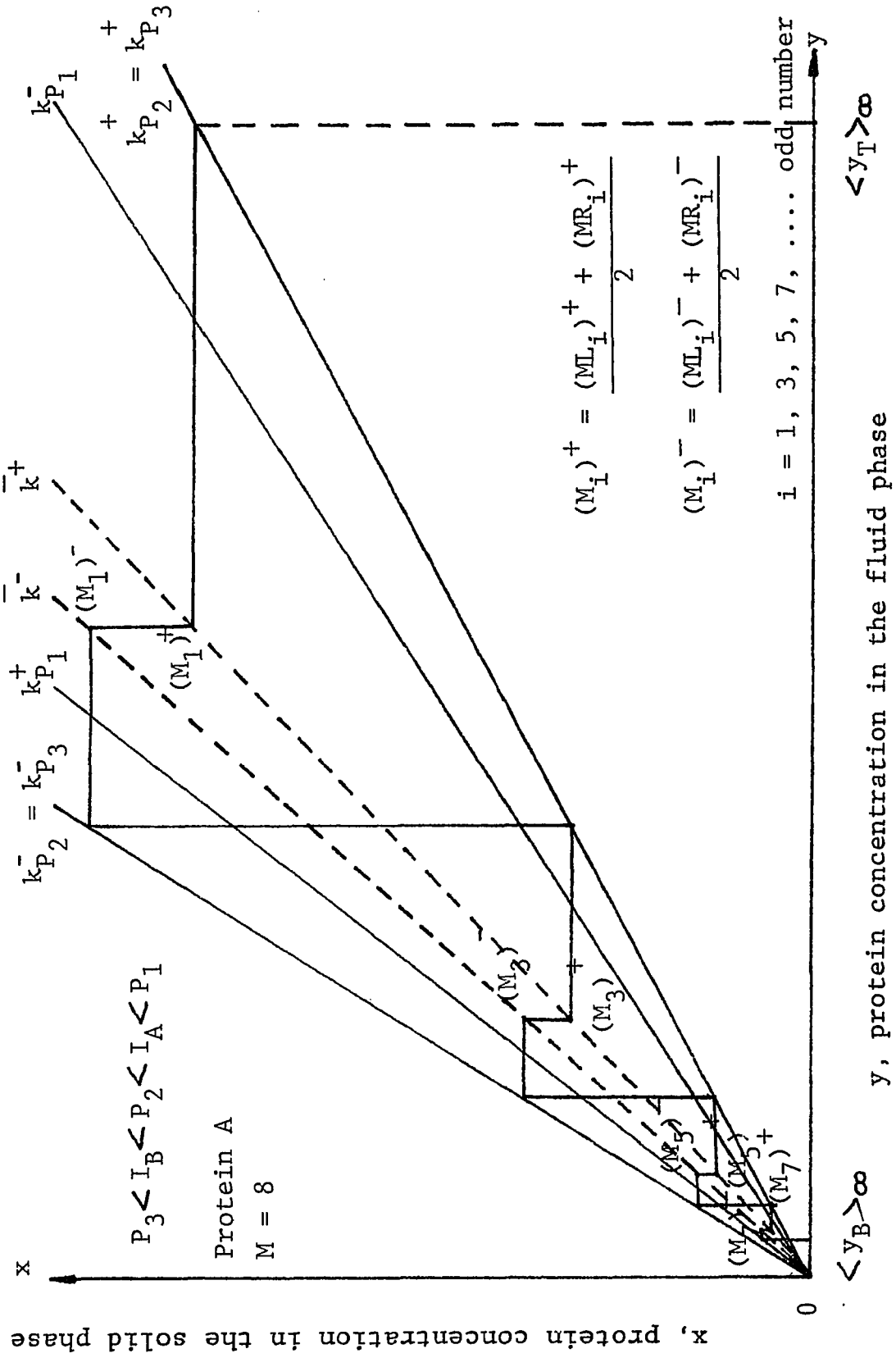


FIGURE 57 - GRAPHICAL SOLUTION OF PROTEIN A VIA MULTI-COLUMN SYSTEM, SEPARATION FROM TWO COMPONENTS OF PROTEIN.

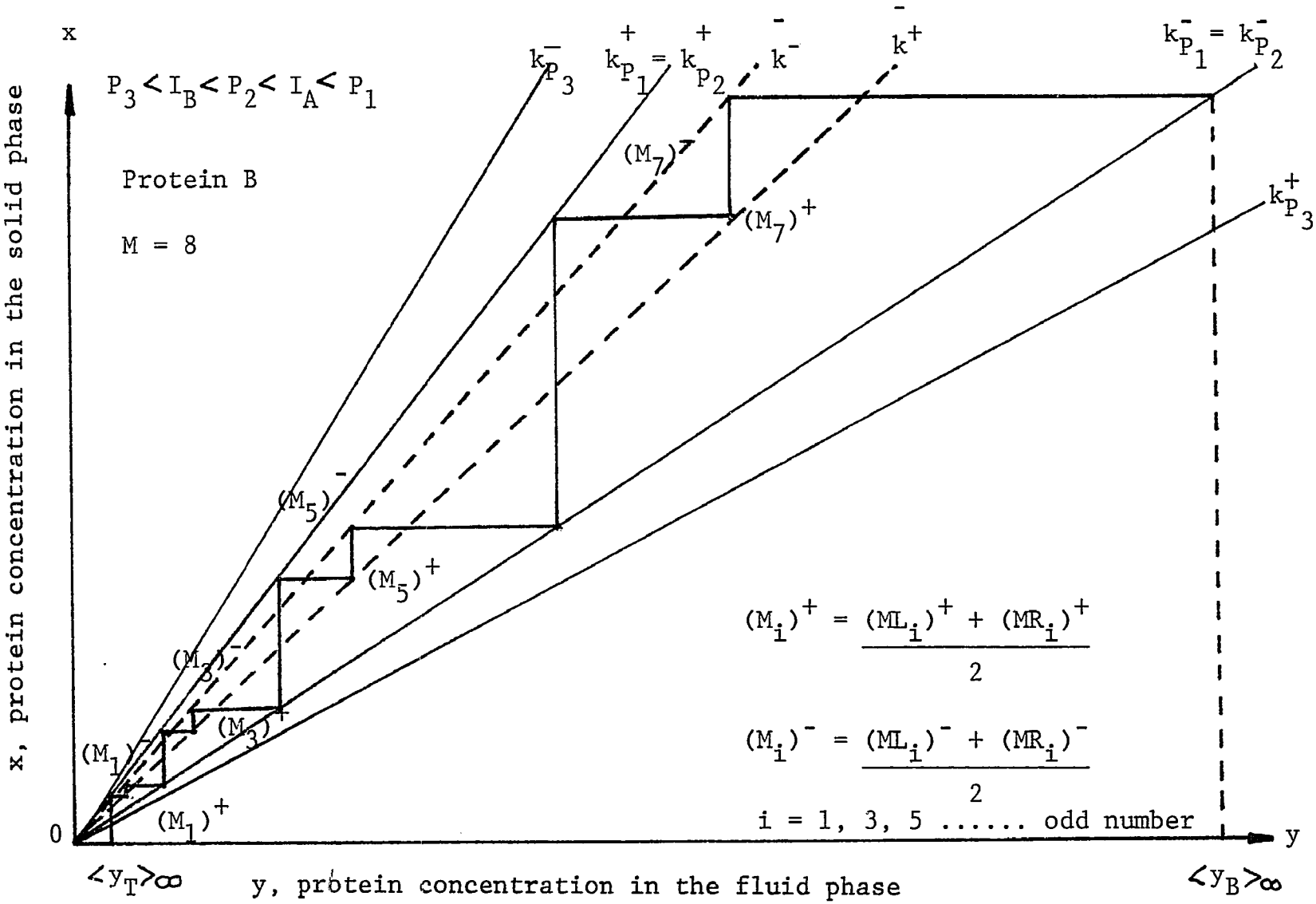


FIGURE 58 - GRAPHICAL SOLUTION OF PROTEIN B VIA MULTI-COLUMN SYSTEM, SEPARATION FROM TWO COMPONENTS OF PROTEIN

respectively compare with the two-column system as shown in Figures 53 and 54. Bottom product of protein B is also increasing while the top product is decreasing as we increase the number of columns. Figures 57 and 58 show that where the top and bottom products of both proteins A and B are located, and also locates the concentrations of protein A and protein B for each correspondind column.

Figures 53 and 54 show the straight lines which are connected from a point T to a point M^+, from M^+ to M^- and from a point M^- to a point B. Then, the line which connects the point T and M^+, represents the anion exchanger column while the other line which connects the point M^- and B, represents the cation exchanger column. From this step, the two "Assymtotic lines" can be drawn by conecting the origin to the point M^- and the origin to the point M^+ where their slopes are $\bar{k}^-$ and $\bar{k}^+$ respectively.

If we fix all those parameters except the number of columns, then the two assymtotic lines are drawn at a slopes of $\bar{k}^-$ and $\bar{k}^+$ (see Figures 57 and 58). Once either top or bottom product is known, then we are able to graph and predict the other product. The example of this graphical method, to predict the bottom product where top product is known, is shown in Figure 59 for a six-column system. The known top product is located at point T on the equilibrium line, slope is equal to $k^+_{P_2}$ (= $k^+_{P_3}$). The horizontal line

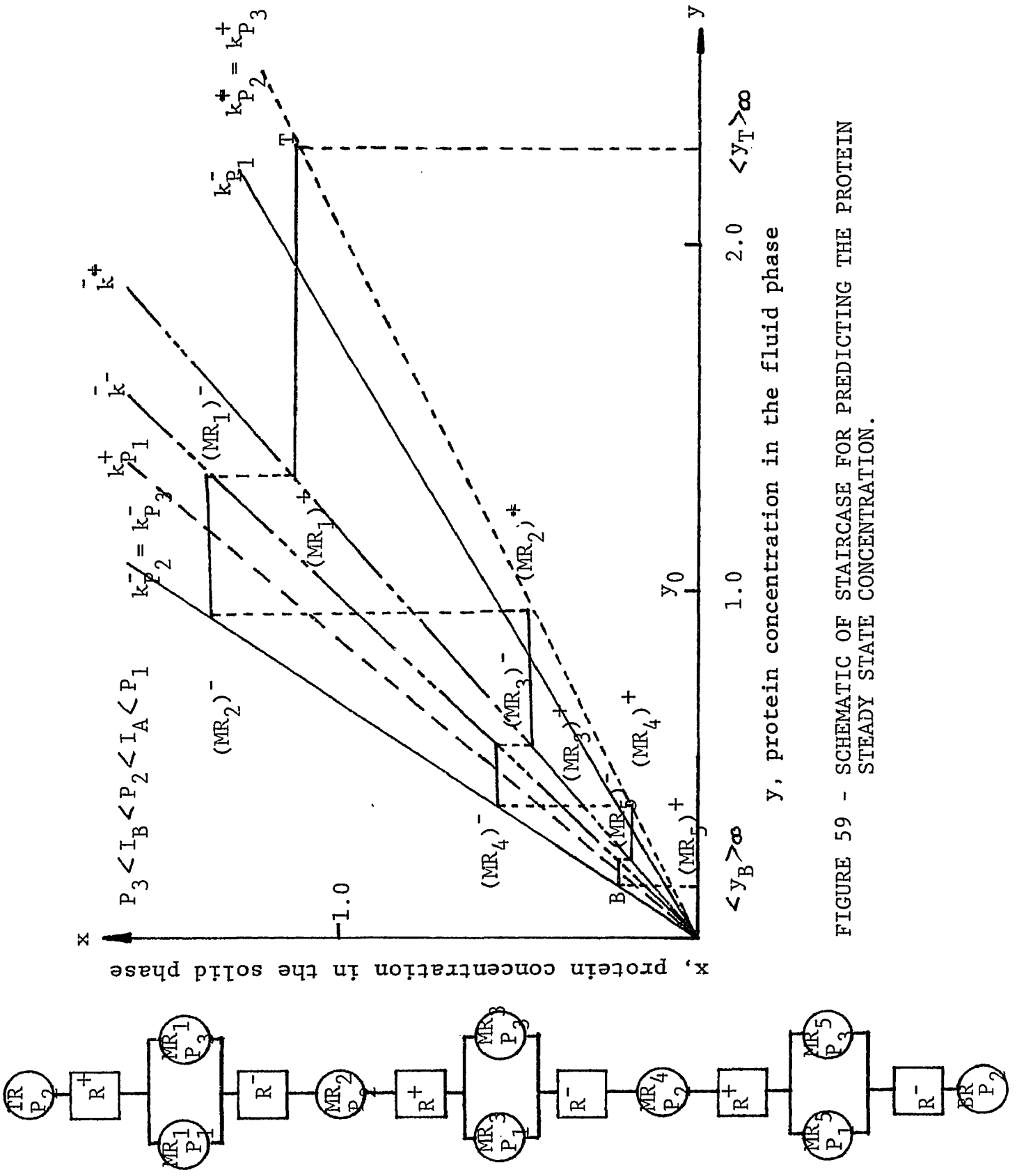


FIGURE 59 - SCHEMATIC OF STAIRCASE FOR PREDICTING THE PROTEIN STEADY STATE CONCENTRATION.

$T(MR_1^+)$ represents the column 1 (R^+) where the point X_1^+ indicates the solute concentration in the solid phase. The vertical line $(MR_1)^+(MR_1)^-$ is drawn where MR_1^+ and MR_1^- are the average concentrations of the middle reservoirs as mentioned previously. Next, we draw the horizontal line $(MR_1)^-(MR_2)^-$ which represents column 2 (R^-) while X_2^- is the concentration of protein in the solid phase. The concentration of protein in MR_2 reservoir be located at Y_2 by drawing a vertical line from the point $(MR_2)^-$ to the y-axis, the intersection of this vertical line and the line $k_{P_2}^+ = k_{P_3}^+$ is $(MR_2)^+$. In the same we can draw the lines $(MR_2)^+(MR_3)^+$, $(MR_3)^+(MR_3)^-$, $(MR_3)^-(MR_4)^-$ and so on until the last line $(MR_5)^-B$ is drawn. Once B is known then the bottom product $\langle y_B \rangle_\infty$ can be located. Clearly shown in Figure 60, the plot of S.F. versus M (number of columns) on semi-log scale. As the number of columns is increasing, the S.F. intends to increase a great deal.

Four Proteins - Multi Column:

This process operates similar to the separation of two proteins as described in the previous section, the different is the number of proteins, which is increased to four components: A, B, C and D. If the system considers the separation of these four proteins individually as the major products, then the separation process can operate based on the selection of pH levels as explained in Chapter V.

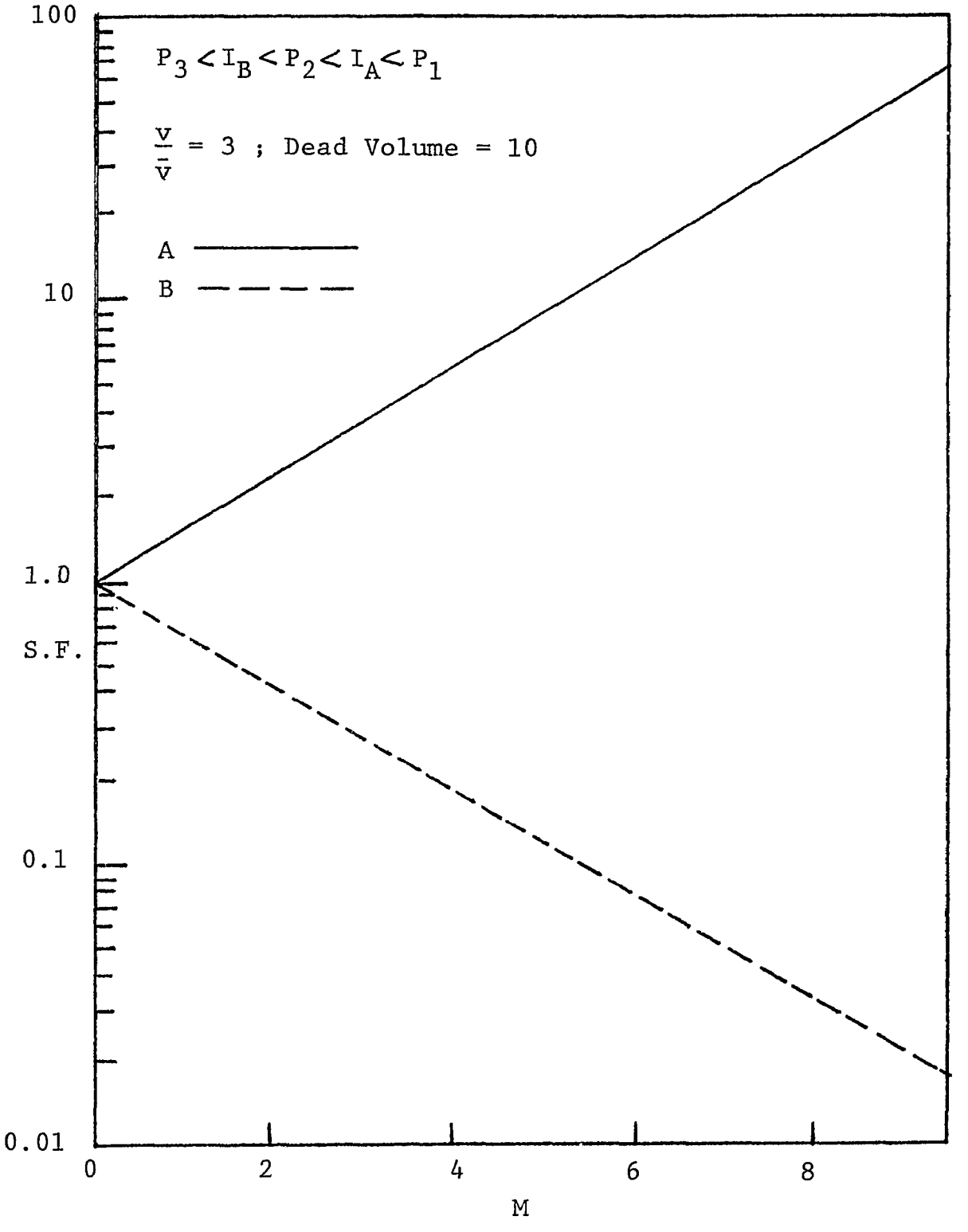


FIGURE 60 - STEADY STATE SEPARATION FACTORS OF TWO PROTEINS vs. NUMBER OF COLUMNS (M).

Case I The isoelectric points of this protein mixture can stated as:

$$P_5 < I_D,\ I_C \text{ and } I_B < P_2 < I_A < P_1$$

where we let B, C and D be the group of proteins with their isoelectric points (I_B, I_C and I_D) in between P_5 and P_2. The pH levels P_1, P_2 and P_5 are chosen for this first separation process. As the protein A having the higher isoelectric compared with the rest, A will migrate from the P_2 bottom reservoir (BR), through the cation column (M) to the P_1 middle reservoir (MR_{M-1}). Again from reservoir, MR_{M-1}, protein A will migrate through the anion column (M-1) to the P_2 reservoir (R_{M-2}) and keep repeating the flow step by migrating up through cation column (M-2) to the P_1 middle reservoir (MR_{M-3}) and so on until protein A reaches the top reservoir. As A is moving upward, proteins B, C and D having the lower isoelectric point s, compared with protein A, will migrate down from the P_2 top reservoir through anion column (M = 1) to the P_5 middle reservoir (ML_1). Then from the reservoir ML_1, protein mixture B, C and D migrate through cation column (M = 2) to the P_2 reservoir (R_2). In the same manner as protein A, the mixture of B, C and D will migrate down through anion column (M = 3) to P_5 reservoir (ML_3) and through cation column (M = 4) to P_2 reservoir (R_4) and so on until these protein mixtures reach the P_2 bottom reservoir. Graphical solution of both protein A and the mixture of B, C

and D are shown in the Figures 61 and 62 respectively. At the steady state condition, the enriched concentration of protein A will be found in the top reservoir while the bottom reservoir will obtain the enriched concentration of proteins B, C and D. The separation factor of protein A and the mixtures of B, C and D are plotted and shown in Figure 63. Up to this point, protein A is separated while protein B, C and D still mixed together and are ready to be separated on the first extension unit.

The isoelectric points of protein mixtures can be expressed and related to the pH level in terms of:

$$P_5 < I_D \text{ and } I_C < P_3 < I_B < P_2$$

For this first extension unit, the P_2, P_3 and P_5 to be used, are selected as explained earlier in this chapter. Protein B having a higher isoelectric point, it will migrate from the P_3 bottom reservoir through cation column (M) to the P_2 middle reservoir (MR_{M-1}) and through anion column (M-1) to the P_3 reservoir (R_{M-2}) and continue flowing until the protein B reaches the P_3 top reservoir. Protein mixtures C and D having a lower isoelectric points, will migrate down from the P_3 top reservoir through anion column (M = 1) to the P_5 middle reservoir (ML_1) and through cation column (M = 2) to the P_3 reservoir which locates toward to the bottom of this extension unit, until the movement reaches the P_3 bottom reservoir. Figures 64 and 65 show clearly the

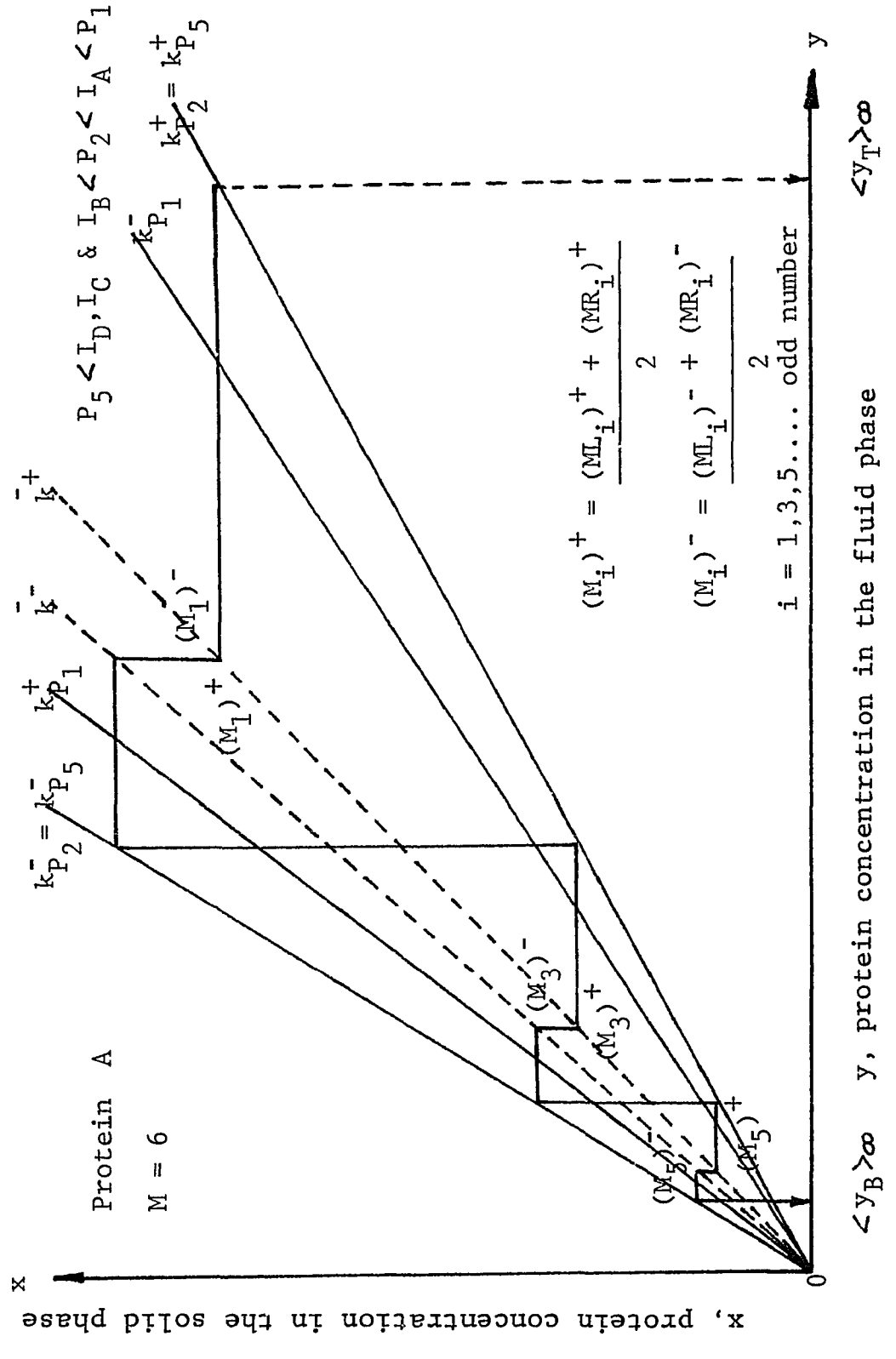


FIGURE 61 - GRAPHICAL SOLUTION OF PROTEIN A VIA MULTI-COLUMN SYSTEM, SEPARATION FROM MULTI-COMPONENT OF PROTEIN.

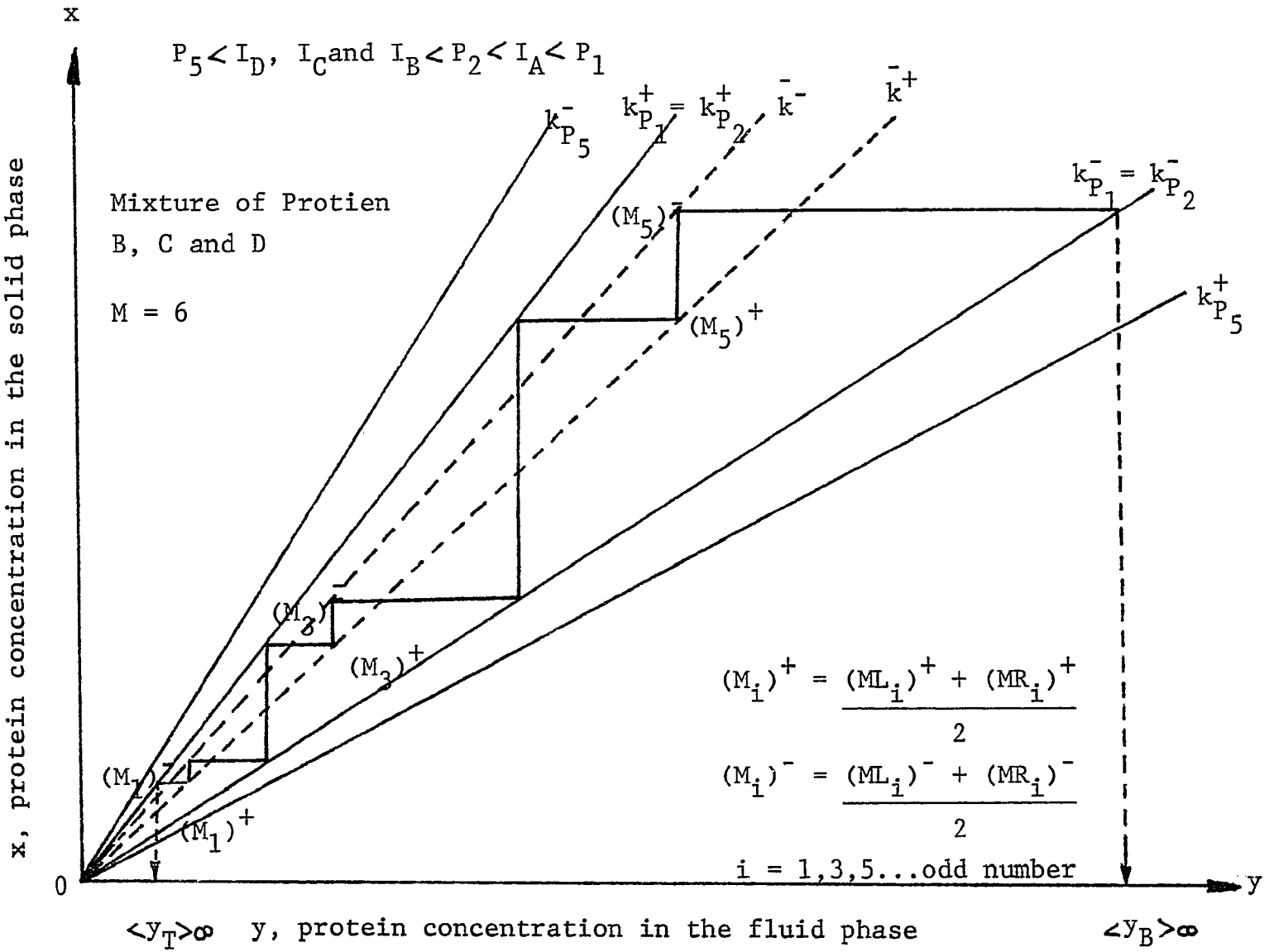


FIGURE 62 - GRAPHICAL SOLUTION FOR PROTEIN MIXTURES B, C, AND D VIA MULTI-COLUMN SYSTEM.

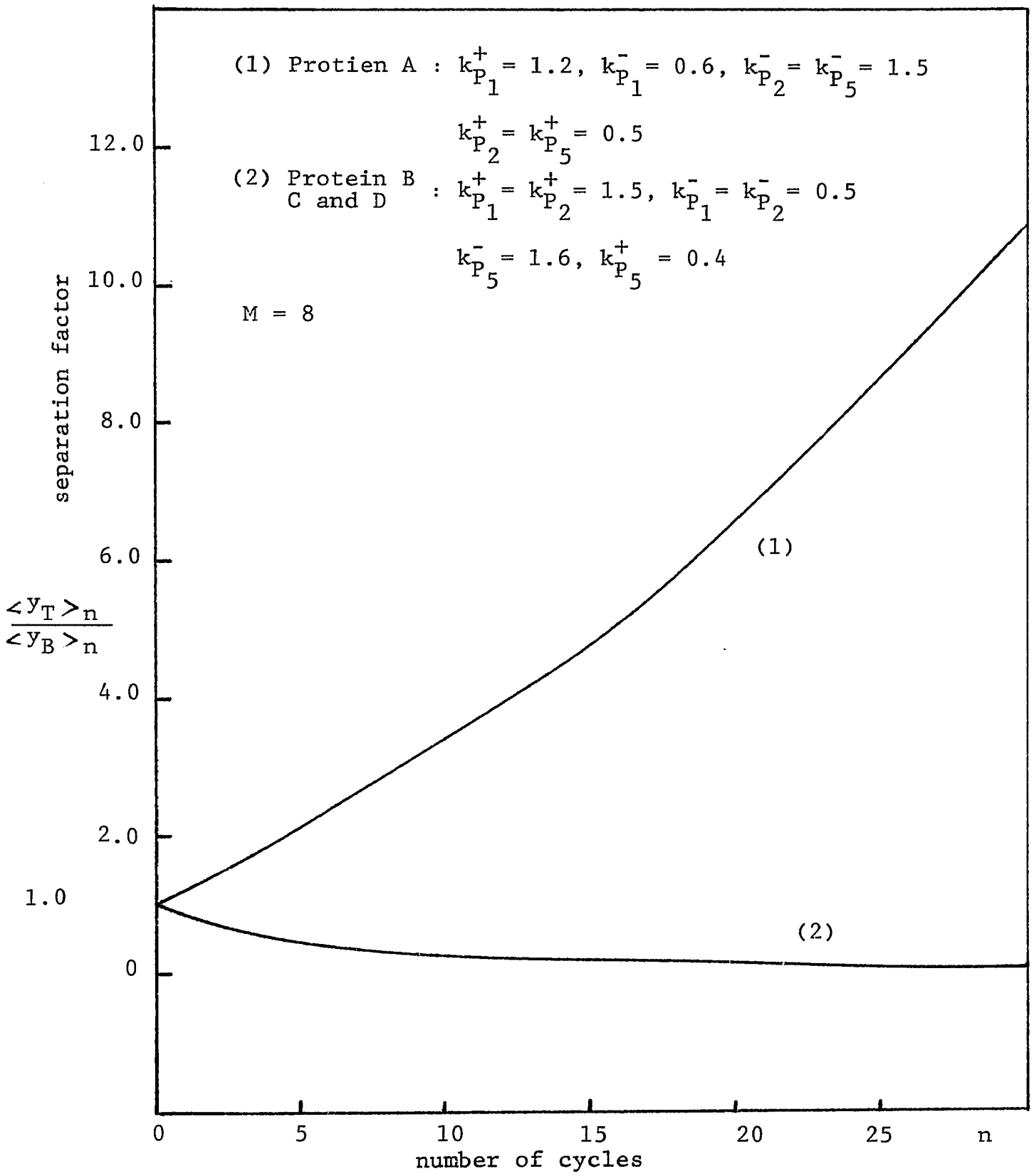


FIGURE 63- THE CONCENTRATION TRANSIENTS OF PROTEIN A AND PROTEIN MIXTURES B, C AND D vs. NUMBER OF CYCLES.

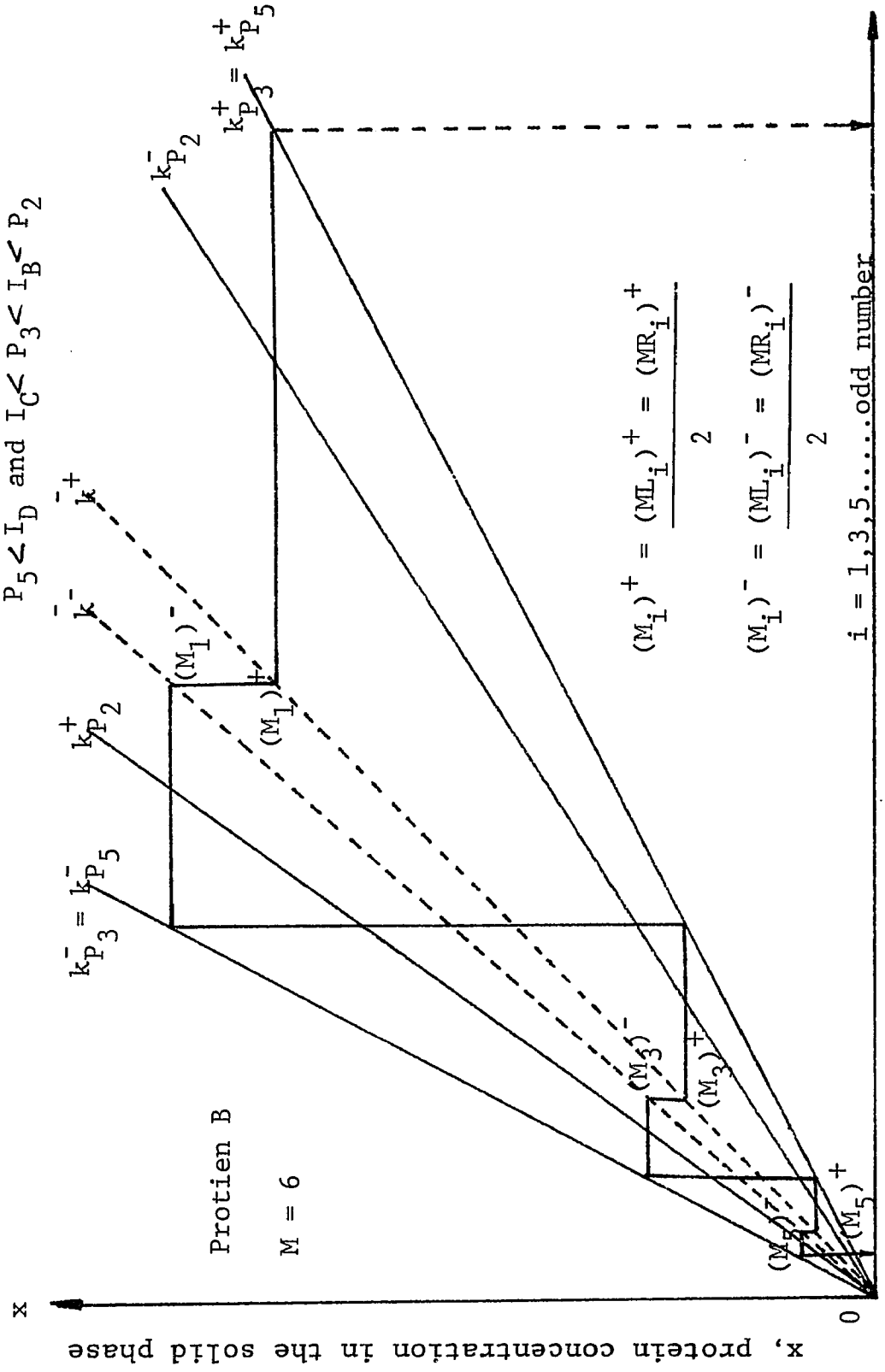


FIGURE 64 - GRAPHICAL SOLUTION FOR PROTEIN B VIA MULTI-COLUMN SYSTEM, SEPARATION FROM MULTI-COMPONENT OF PROTEIN.

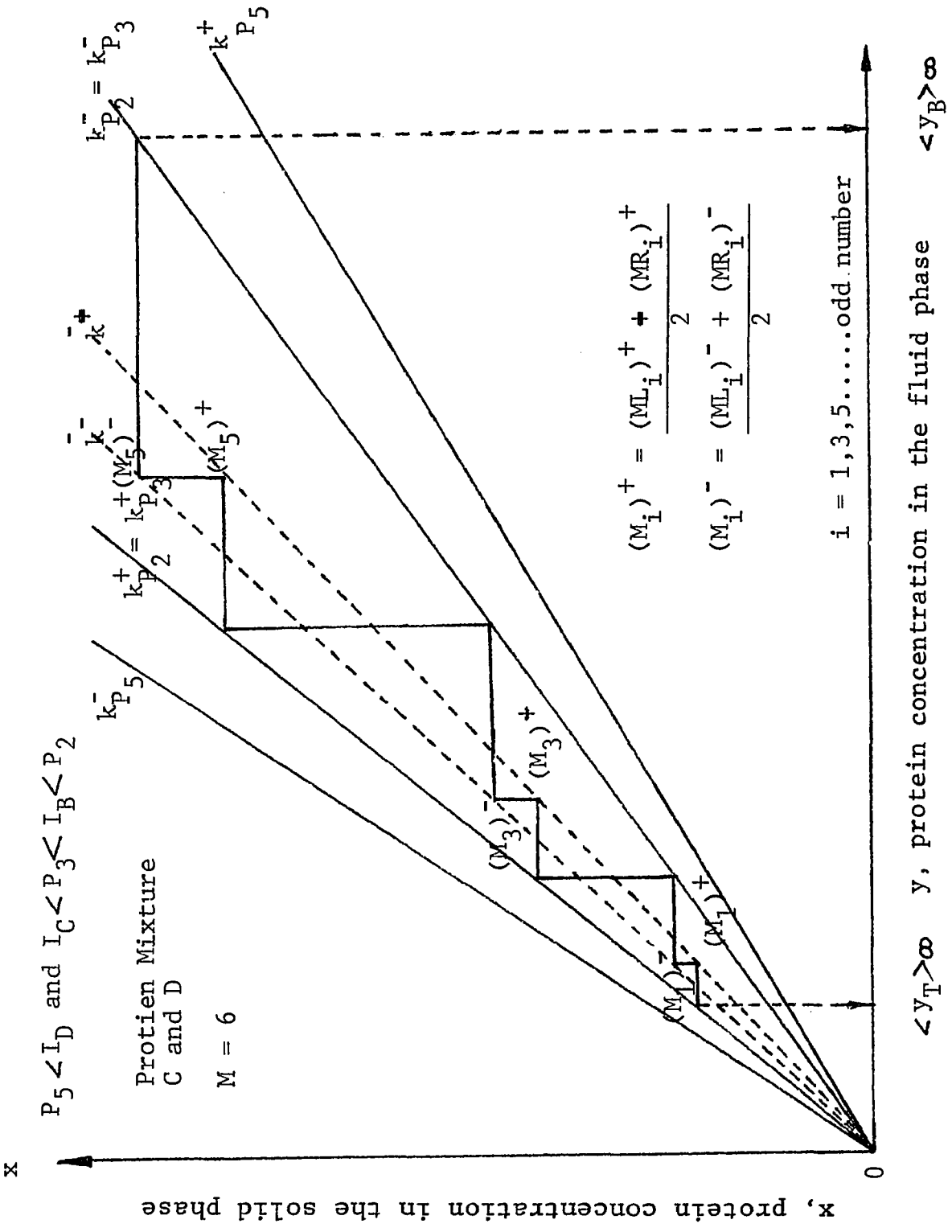


FIGURE 65 - GRAPHICAL SOLUTION FOR PROTEIN MIXTURES C AND D VIA MULTI-COLUMN SYSTEM.

graphical analysis and predict the top and the bottom products for both proteins B and mixtures of C and D. Once again, when the steady atate is reached the enriched concentration of protein B will be obtained from the top reservoir while the enriched concentration of protein mixtures C and D is found from the bottom reservoir and another extension unit is need to separate C and D. The transient concentrations of protein B and a mixture of C and D are shown in Figure 66.

For this second extension unit, the unit will be the last which is added into the system due to the number of solutes which remain (protein C and D). The isoelectric points of C and D can correlate with the pH level and be stated as:

$$P_5 < I_D < P_4 < I_C < P_3$$

The pH levels P_3, P_4 and P_5 are chosen for this second extension unit. As we explained in the earlier part of the chapter, protein having a higher isoelectric point will migrate from the P_4 bottom reservoir through cation column (M) to the P_3 middle reservoir (MR_{M-1}) and through anion column (M-1) to the P_4 reservoir (R_{M-2}) until this flow movement reaches the P_4 top reservoir. However, while protein C is moving, protein D which has a lower isoelectric point will migrate from the P_4 top reservoir through anion column (M = 1) to the P_5 middle reservoir (ML_1) and migrate

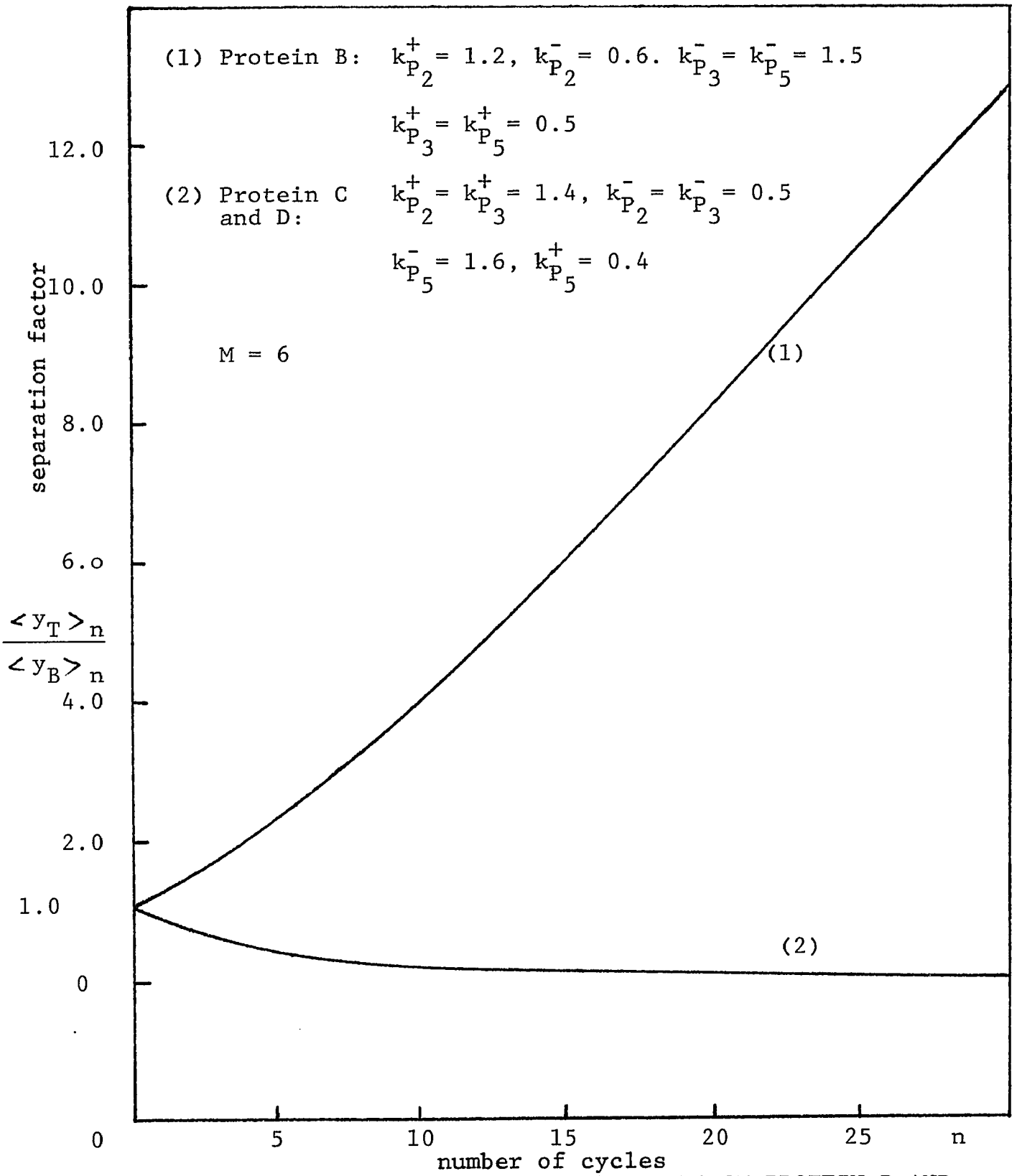


FIGURE 66 - THE CONCENTRATION TRANSIENTS OF PROTEIN B AND PROTEIN MIXTURES C AND D vs. NUMBER OF CYCLES.

through cation column (M = 2) to the P_4 reservoir toward to the bottom of the unit until the movement of flow reaches the P_4 bottom reservoir. Figures 67 and 68 show the graphical solution of both proteins C and D respectively. Also the transient concentration of proteins C and D are plotted versus the number of cycles and shown in Figure 69

Case II In this case, P_3 is selected for reservoirs TR, R_2, R_4 ..... R_{M-2} and BR. Then the P_3 will split the proteins into two groups; A, B and C, D. The isoelectric point can express as:

$$P_5 < I_D \text{ and } I_C < P_3 < I_B \text{ and } I_A < P_1$$

The pH level P_1 is chosen for the middle reservoirs ML_1, ML_3 ..... ML_{M-1} and the P_5 is chosen for MR_1, MR_3 ..... MR_{M-1}. The protein mixtures A and B having the higher isoelectric points than the P_3 and protein C and D, will migrate from the P_3 bottom reservoir (BR), through the cation column (M) to the P_1 middle reservoir (ML_{M-1}). From this middle reservoir, protein mixtures A and B will continue migrating through the anion column (M-1) to the P_3 reservoir (R_{M-2}) and migrating further in the same manner until the mixture of A and B reach the P_3, top reservoir (TR). As proteins A and B are moving upward, the mixture of proteins C and D will migrate in the opposite direction of A and B due to the fact that, their isoelectric points are lower than the P_3 and

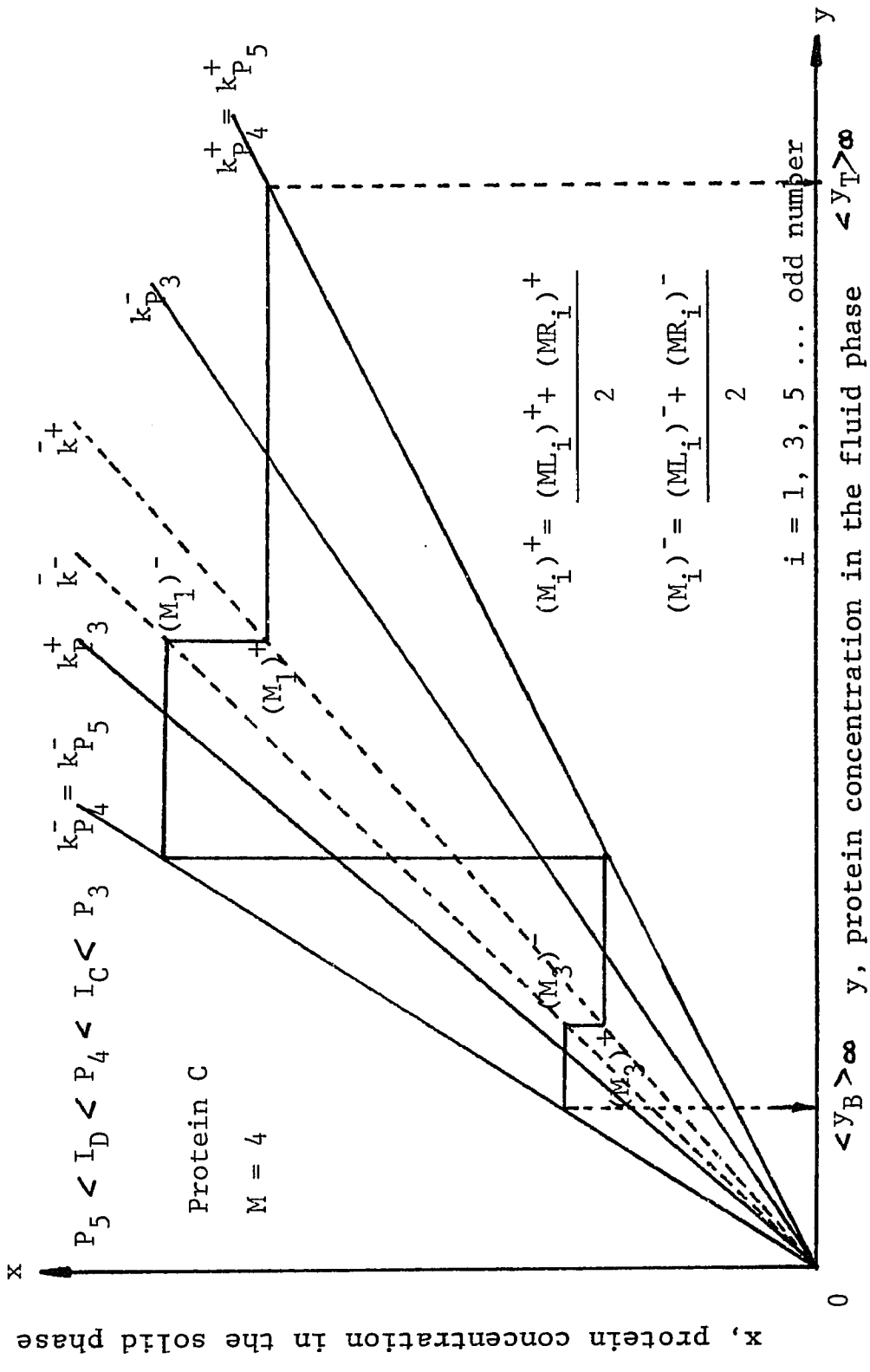


FIGURE 67 - GRAPHICAL SOLUTION OF PROTEIN C VIA MULTI-COLUMN SYSTEM.

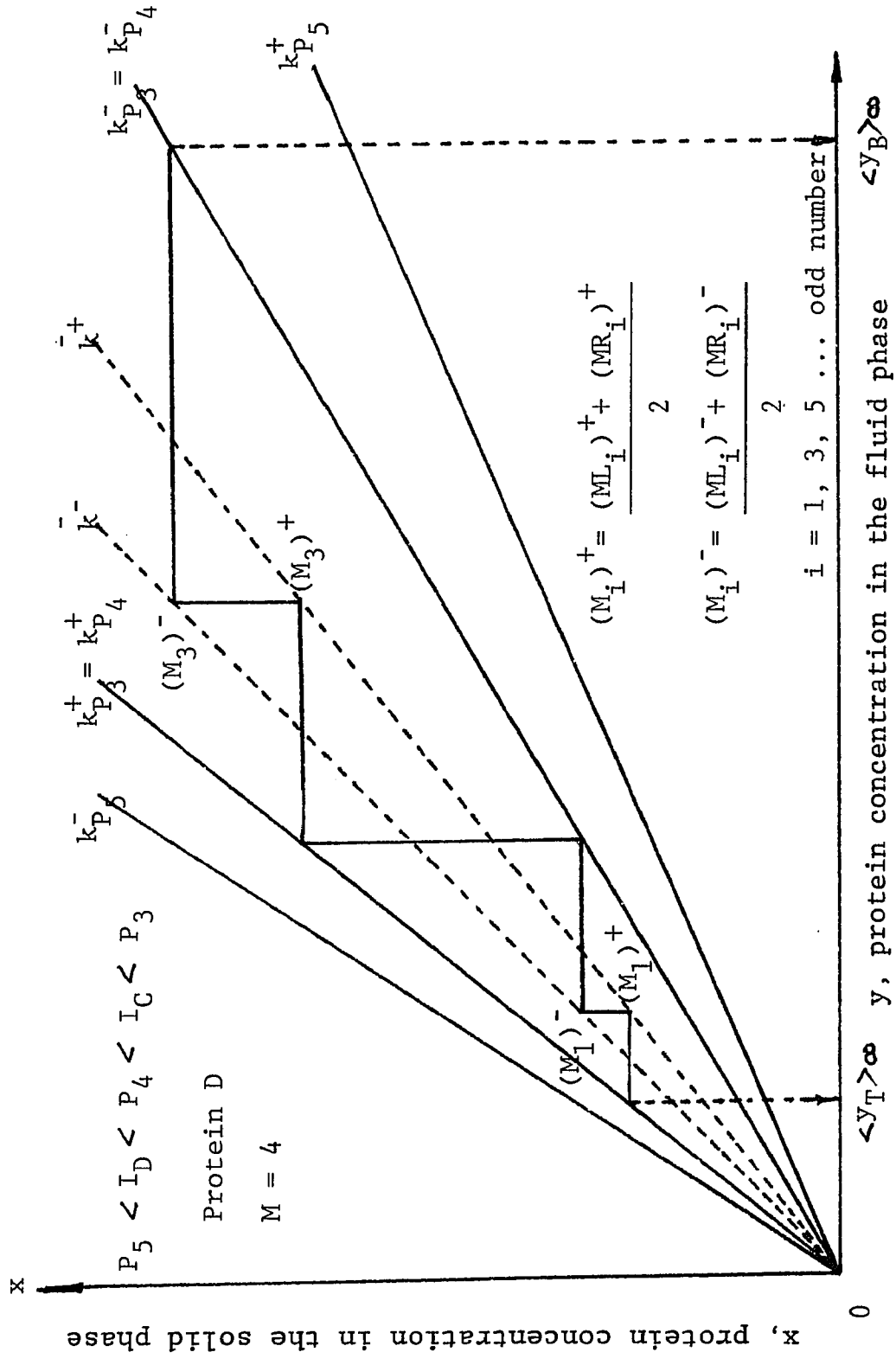


FIGURE 68 - GRAPHICAL SOLUTION OF PROTEIN D VIA MULTI-COLUMN SYSTEM.

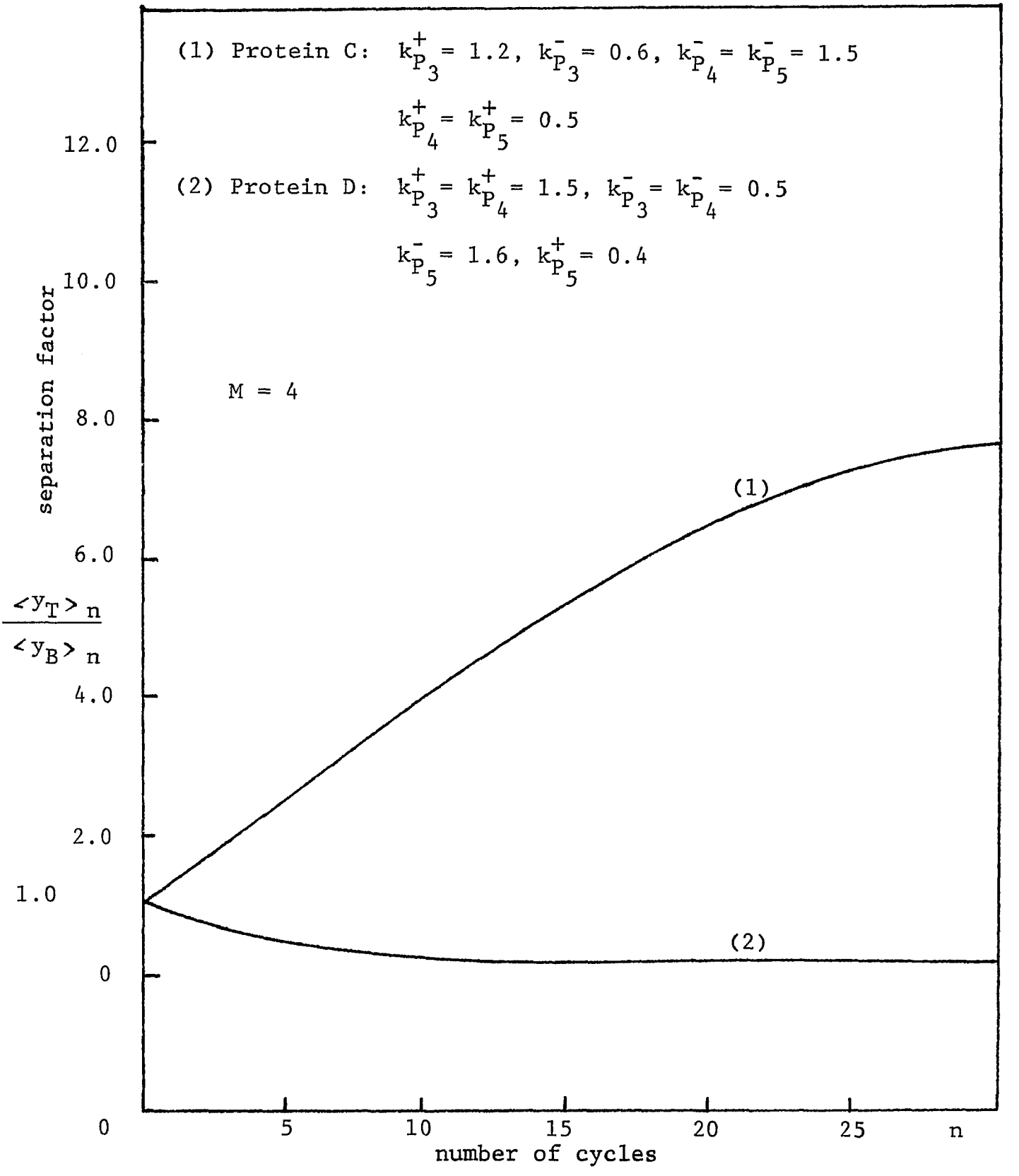


FIGURE 69 - THE CONCENTRATION TRANSIENTS OF PROTEIN C AND PROTEIN D vs. NUMBER OF CYCLES.

proteins A and B. The proteins C and D will migrate down from the P_3 top reservoir (TR) through anion column (M = 1) to the P_5 middle reservoir (MR_1). Then from reservoir MR_1, proteins C and D migrate through cation column (M = 2) to the P_3 reservoir (R_2) and so on until this flow movement of the protein mixture reaches the P_3 bottom reservoir. Figure 70 shows the graphical solution for protein mixtures A and B, where the graphical solution for proteins C and D is clearly shown in Figure 71. As the steady state condition is reached, the enriched concentration of proteins A and B will be obtained from the top reservoir while the bottom reservoir collects the enriched concentration of the mixture of proteins C and D. The concentration transients of the mixture A and B, and the mixture C and D are plotted versus the number of cycles and show in the Figure 72.

The next step is the separation of the mixture A and B or C and D. At the beginning we had a mixture of four proteins for separation ; up to this point the mixture of proteins is reduced down to two components, either A and B or C and D. The separation of two proteins via multi column has been explained, also the graphical solution of proteins A and B, and proteins C and D are shown and discussed earlier in this chapter.

For mixtures of proteins of more than four components, the separation process begins in the same manner as for the

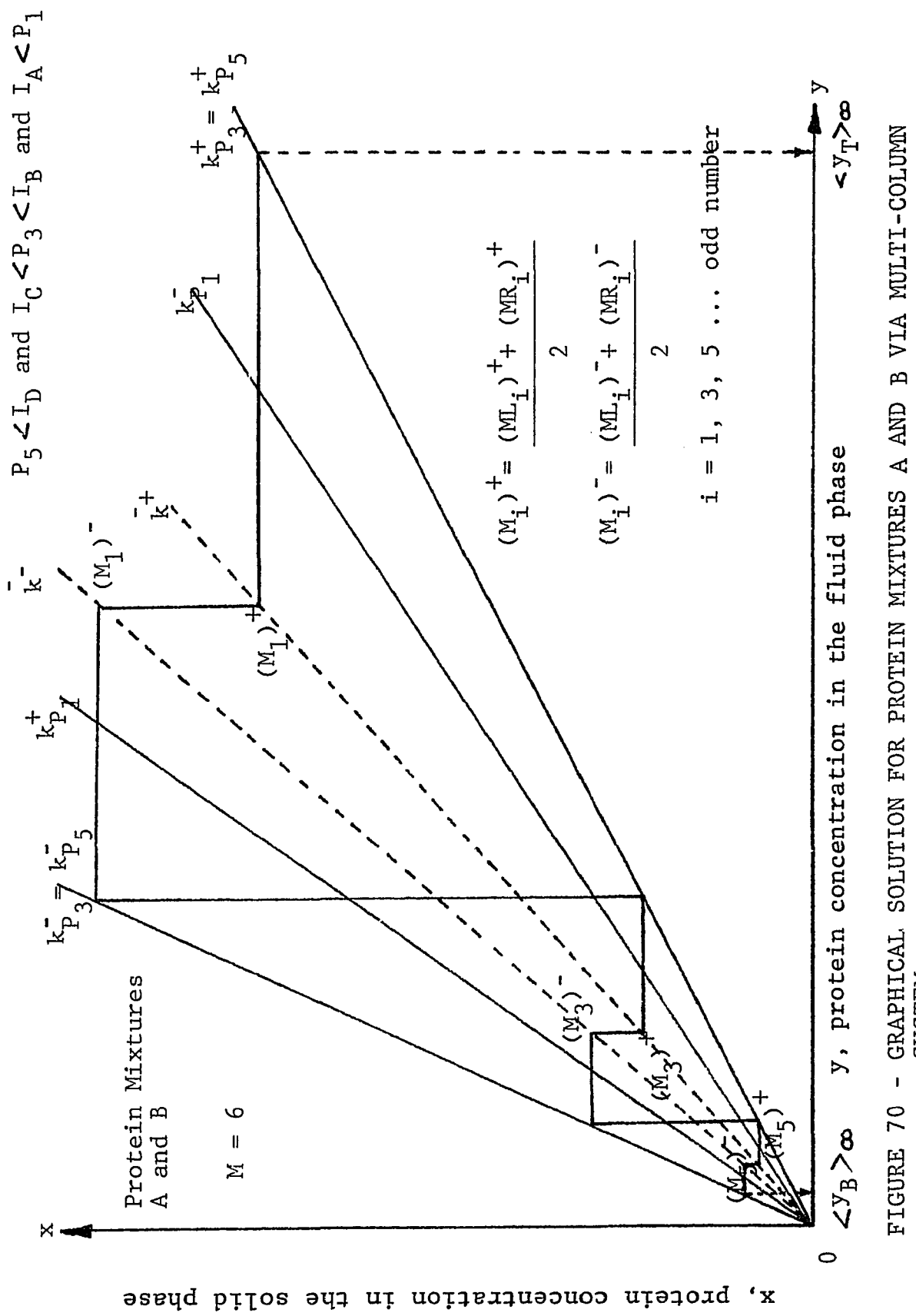


FIGURE 70 - GRAPHICAL SOLUTION FOR PROTEIN MIXTURES A AND B VIA MULTI-COLUMN SYSTEM.

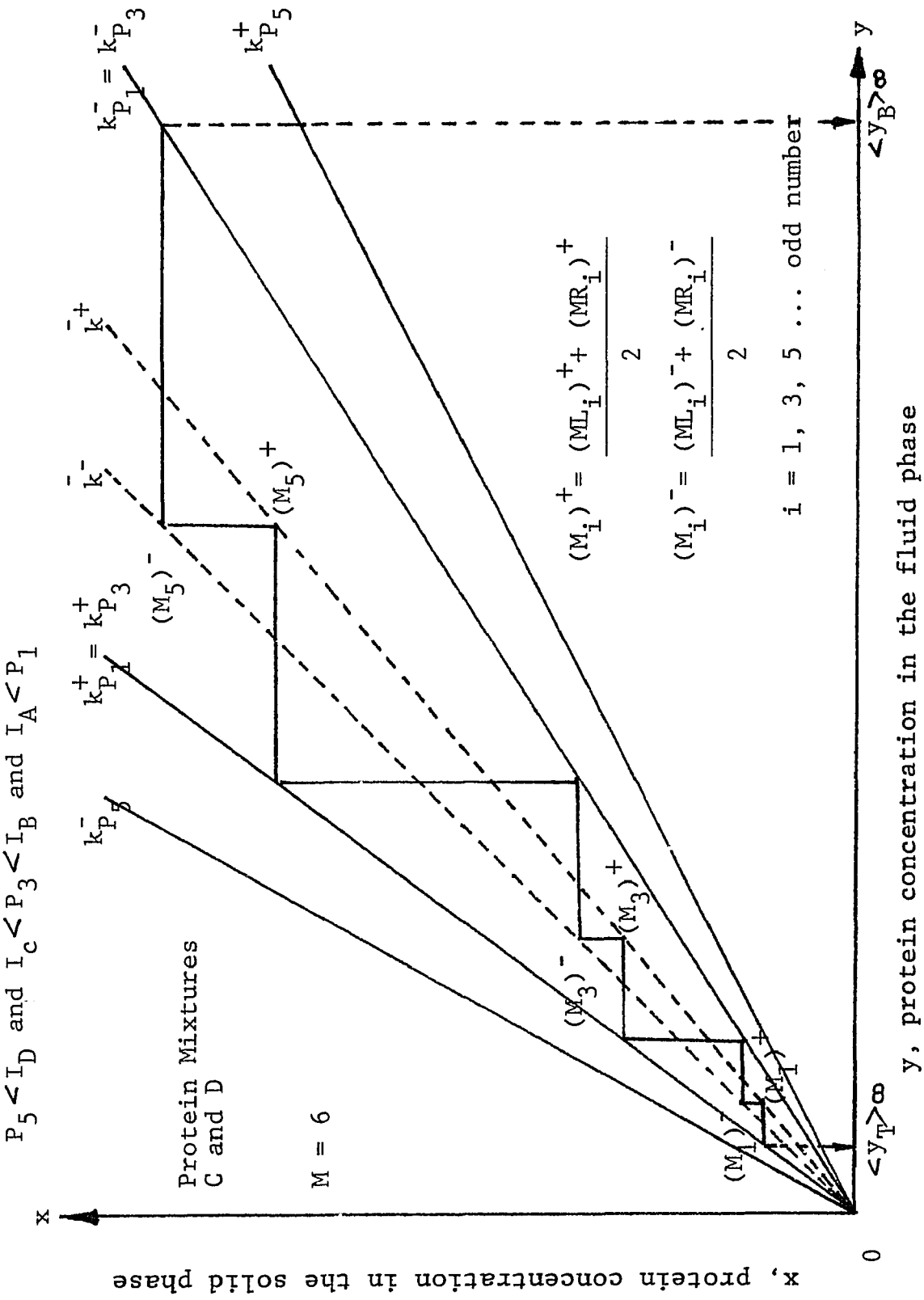


FIGURE 71 - GRAPHICAL SOLUTION FOR PROTEIN MIXTURES C AND D VIA MULTI-COLUMN SYSTEM.

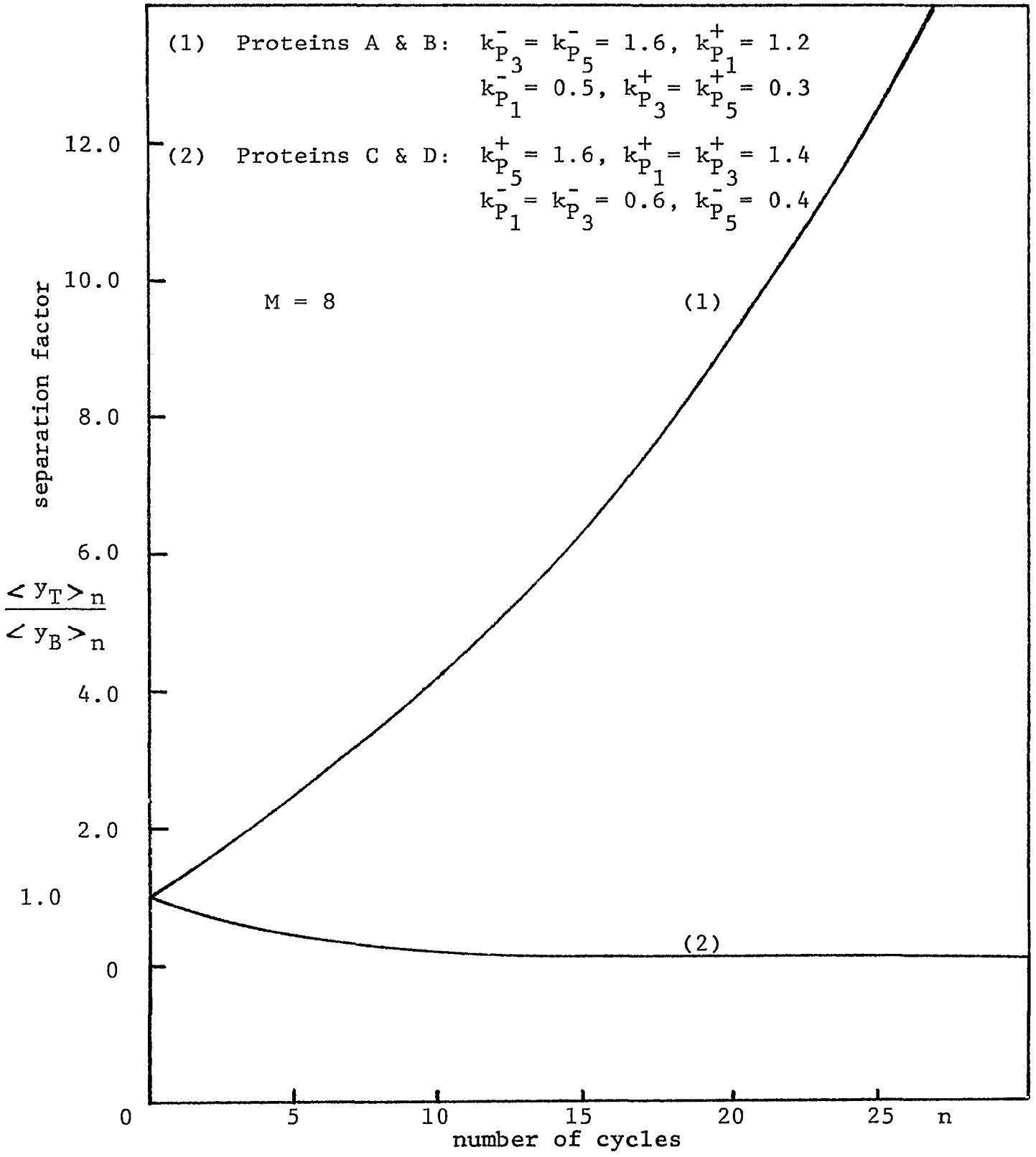


FIGURE 72 - THE CONCENTRATION TRANSIENTS OF PROTEINS A & B AND PROTEINS C & D vs. NUMBER OF CYCLES.

four components of proteins mixture. First of all, we are grouping the mixture into two groups of proteins by selecting the pH level, one is the highest (higher than the highest isoelectric point of protein component), one is the lowest (lower than the lowest isoelectric point of the protein component) and the last one is the middle one (the one which separates proteins into two groups). Once the process is completed, both top and bottom products are obtained and then we repeat the same step again, by taking either top or bottom product to separate until the separation of the desired product(s) is reached.

E. A Theoretical Study Via Mathematical Farmalism Based On Elementary Matrix Algebra

This work can not complete without discussion on this title. After we have been investigated on the subject for a length of time. The experiment was performed and dicussed in the earlier part of this chapter for Mode 4. Figures 73 and 74 show the calculation curves of protein concentrations in the six sections of liquid phase (five columns and one top reservoir), and five sections (columns) of solid phase respectively. The calculations were made at the beginning of the cycles, after equilibration at the low pH. The parameters used, were given in the Appendix A and also shown on the figures. Similarly, Figures 75 and 76 show the results of a different parameters used. As expected from the smaller

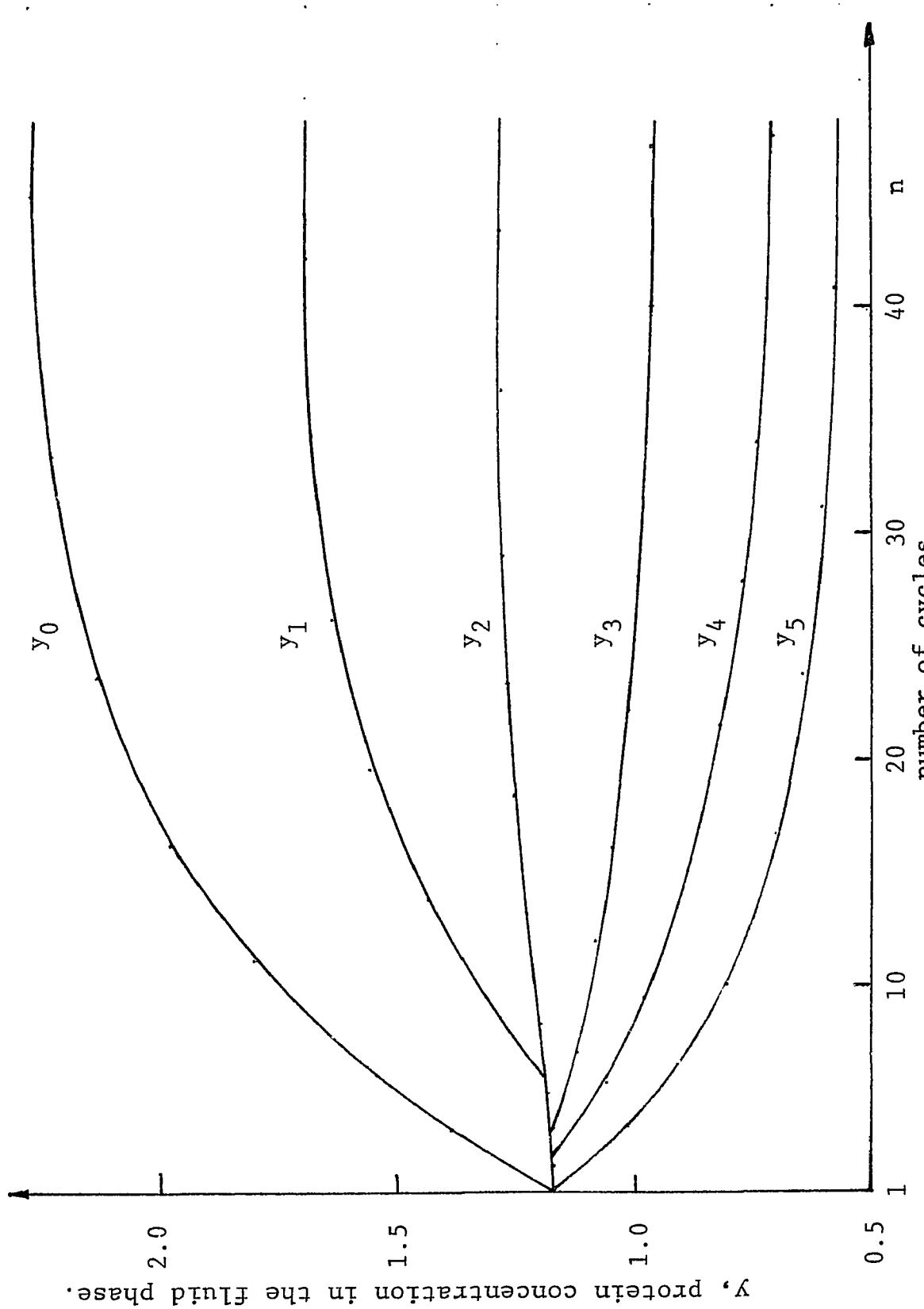


FIGURE 73 - THE CONCENTRATION TRANSIENTS OF PROTEIN IN THE FLUID PHASE vs. NUMBER OF CYCLES (CASE I).

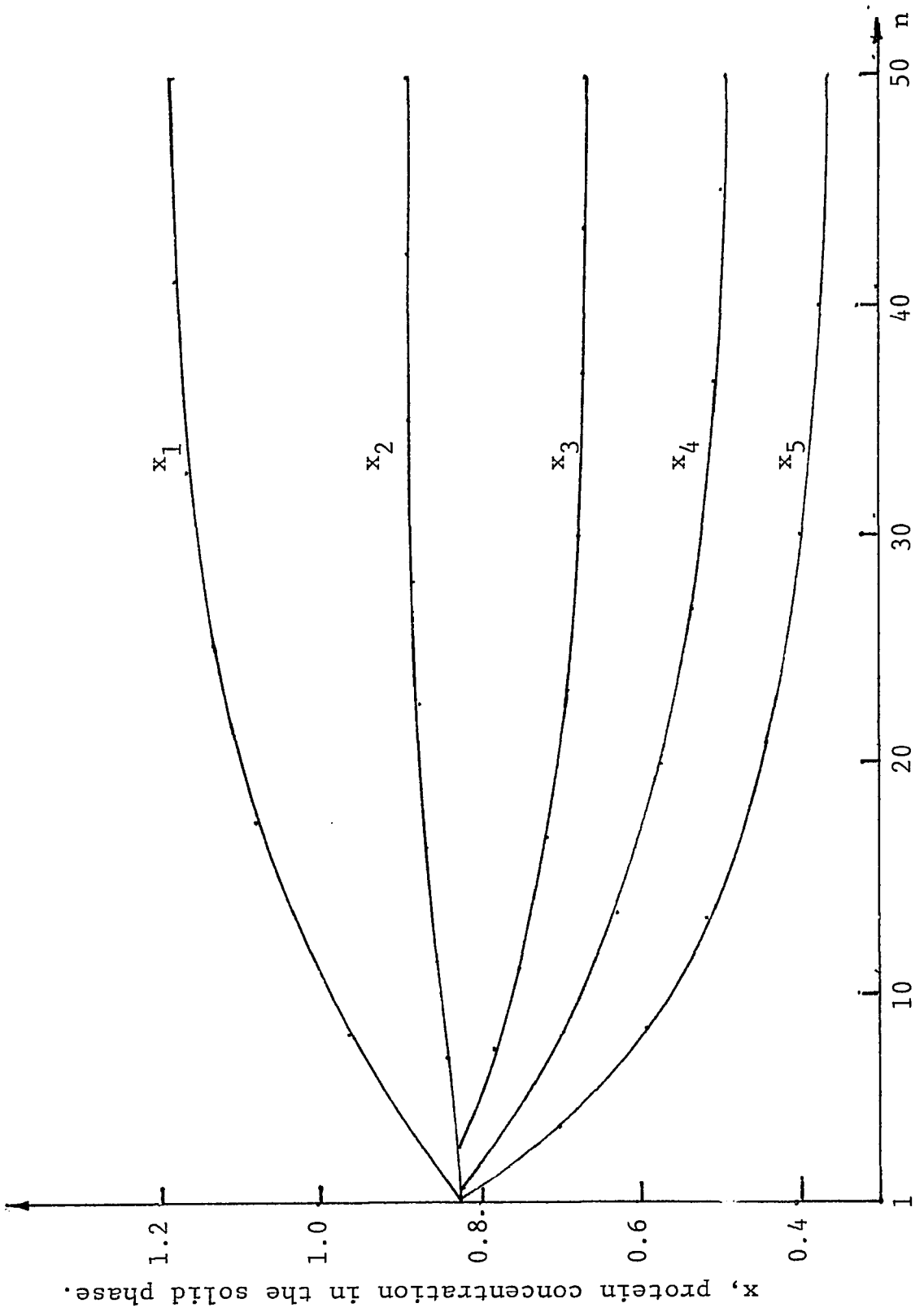


FIGURE 74 - THE CONCENTRATION TRANSIENTS OF PROTEIN IN THE SOLID PHASE vs. NUMBER OF CYCLES (CASE I).

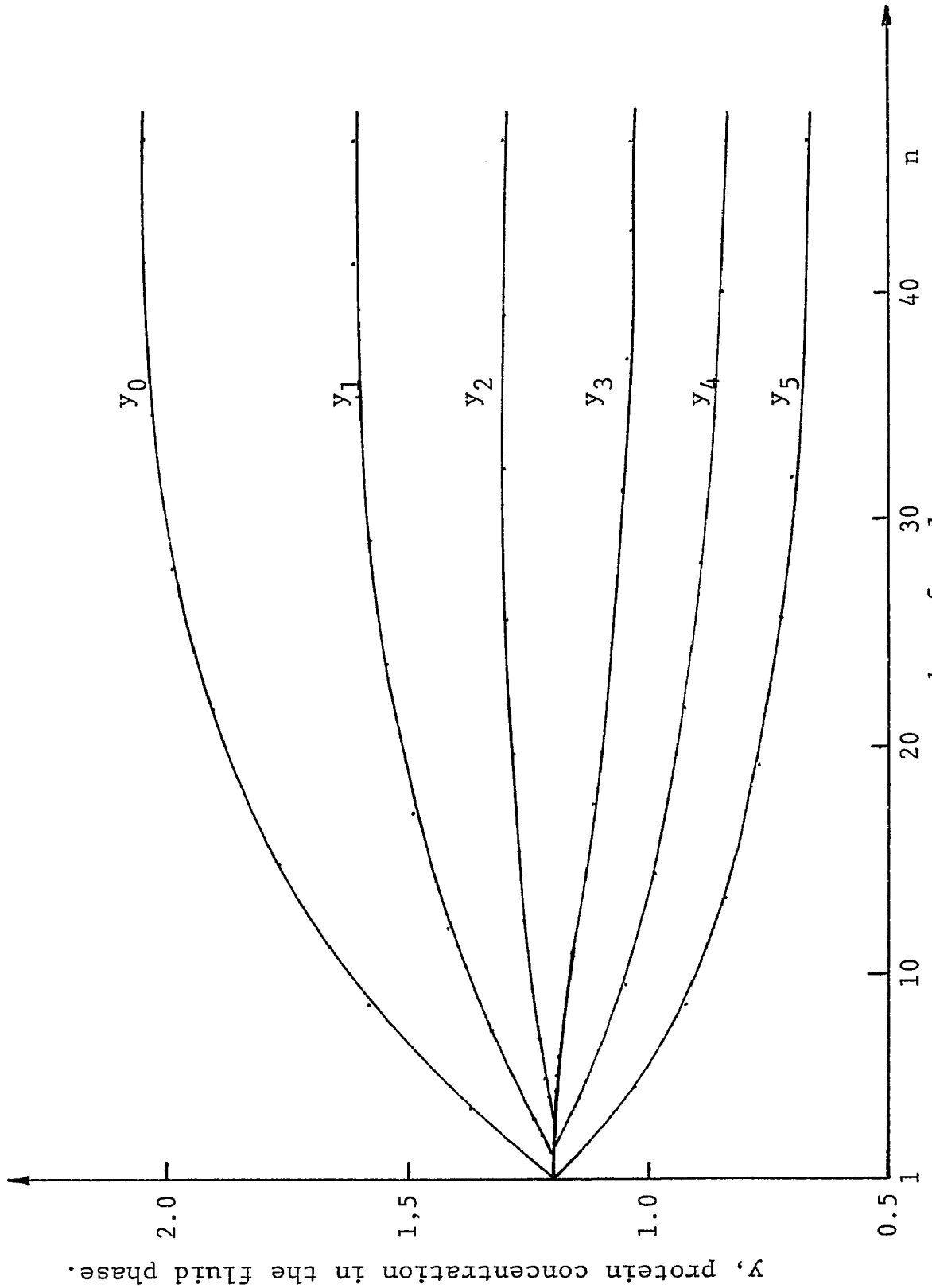


FIGURE 75 - THE CONCENTRATION TRANSIENTS OF PROTEIN IN THE FLUID PHASE vs. NUMBER OF CYCLES (CASE II).

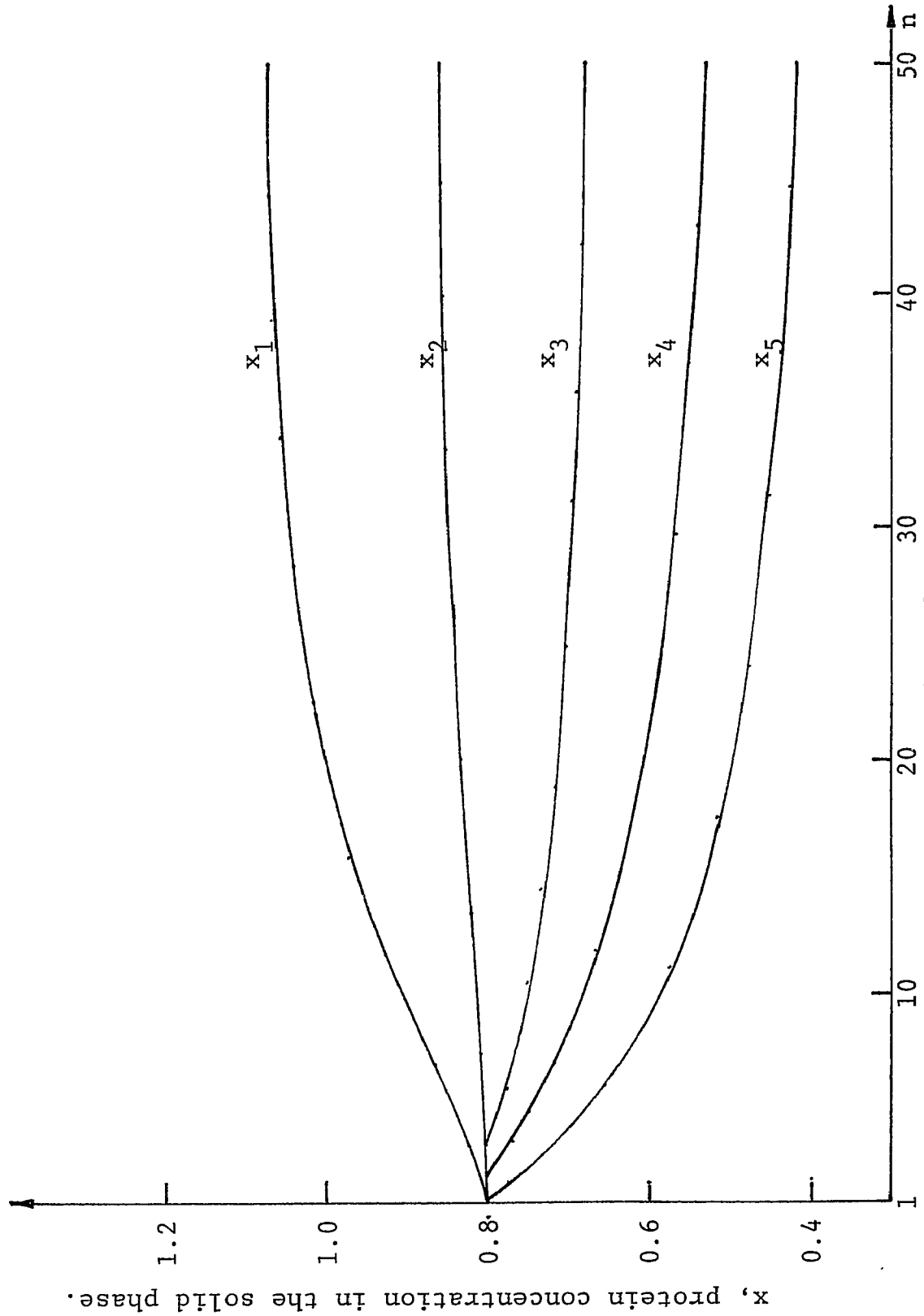


FIGURE 76 - THE CONCENTRATION TRANSIENTS OF PROTEIN IN THE SOLID PHASE vs. NUMBER OF CYCLES (CASE II).

value of β, the maximal separation (see Figures 75 and 76) are smaller than the results show on Figures 73 and 74. More detail of extending the study of this subject is explained and discussed in Appendix A.

The technique of separation of protein via a pH-parametric pumps seem to work well as discussed in this chapter from Mode 1 through Mode 4. We also established that, Mode 2 and Mode 4 are able to extend the model into a multi-column system. The advantage of Mode 2 is, unlimit on the number of protein components for the separation process. This work is only study on the Batch operation basis and also found the difficulty of extending this work into either Semi-Continuous or Continuous process. Mode 4 is able to run either Batch or Semi-Continuous for a multi-column system. The limitation of work is the pH level. As we observed, the pH level used for this mode is two (either P_1 and P_2 or P_2 and P_3), the protein can purify, must has its isoelectric point fall in the range of P_1 and P_2. But some how, this process is work well for recovery any protein component.

The extendind of this work still carry on both experimental study and theoretical study by Chen and his students.

Chapter VIII

SUMMARY OF CONCLUSIONS

This work establishes the reliability of the model equations for predicting the behavior of batch and semi-continuous equilibrium via a pH-parametric pumps. We have examined one-, two- and multi-column system by both experimental and theoretical study. The model is based on the linear equations of change for the liquid -solid system with the diffusion term of negligible importance, a liquid-film controlling mass transfer rate expression, and a linear equilibrium relation between the liquid and solid phases.

This work further establishes the reliability of the method of graphical and the method of using the elementary matrix algebra to predict the concentration curve on protein separation.

There is little to choose between this two computational schemes. The graphical method is more fundamental and more efficient on predicting the steady state value while the matrix algebra method can give us more detail in a transient state.

The agreement between the experimental results and the behavior predicted by computational methods is good. The

qualitative differences between separations achieved under varying operating conditions is correctly predicted by both experiments and calculations.

For the process designning on both batch and semi-continuous pH-parametric pumps, the following were noted:

1. Any separation process in this paper, the process is worked on either one component or two components of solute.
2. The separation system is required to use either two or three pH levels at a time.
3. Literally, scaling up column parameters results in no improved separation.
4. Decreasing the reservoir dead volume, decreases the time of operating to reach the steady state condition

NOMENCLATURE

a, b, c, d, e, p	coefficients in material balance equations, defined by Eqn. 3.13 (dimensionless)
A, B, ...	protein component
I	stage number
I_i	isoelectric point of i
IS_1	ionic strength in the bottom reservoir
IS_2	ionic strength in the top reservoir
J	transfer step
k	x/y, equilibrium constant
k_{P_i}	equilibrium constant at pH = P_i
M	number of columns
n	number of cycles of pump operation
N	number of stages or cells
P_i	pH level i and higher than pH level i+1 (i = 1, is the highest)
Q	reservoir displacement rate, cm^3/sec
t_i	duration i, sec
V	volume of fluid phase per stage, cm^3
$\bar{V}$	volume of solid phase per stages, cm^3

V_B	bottom reservoir dead volume, cm^3
V_{MR}	middle reservoir dead volume, cm^3
V_T	top reservoir dead volume, cm^3
W	total mass of protein present in the system, gm
x	concentration of solute in the solid phase, gm mole/cm^3
y	concentration of solute in the fluid phase, gm mole/cm^3
y_0	concentration of solute in the feed, gm mole/cm^3
$\langle y_B \rangle_n$	average concentration of solute in the bottom reservoir at n^{th} cycle, gm mole/cm^3
$\langle y_T \rangle_n$	average concentration of solute in the top reservoir at n^{th} cycle, gm mole/cm^3
$\langle y_B \rangle_\infty$	steady state concentration of solute in the bottom reservoir, gm mole/cm^3
$\langle y_T \rangle_\infty$	steady state concentration of solute in the top reservoir, gm mole/cm^3

Greek Letters

α	(reservoir displacement)/(column void volume)
β	$k_{P_i}^- / k_{P_i}^+$ (dimensionless)
π/ω	duration of upflow or down flow, sec
$\lambda_0, \lambda_1, \ldots \lambda_n$	eigenvalues of matrix $[\underset{\sim}{M}]$ (dimensionless)
ϱ	ratio of volumes of liquid phase to solid phase (dimensionless)
γ	dead volume ratio, defined by Eqn. A-4.1 (dimensionless)

Vector and matrix quantities

$[\underset{\sim}{A}_j]$	matrice defined by Eqn. 3.20
$\underset{\sim}{C}$	column vector defined by Eqn. A-3.1
$\underset{\sim}{F}_1, \underset{\sim}{F}_h$	feed vectors in open parapump, defined by Eqns. A-4.3 and A-4.4
$[\underset{\sim}{I}]$	unity matrix
$[\underset{\sim}{M}], [\underset{\sim}{\Theta}]$	matrices of coefficients in material balance equations, defined by Eqns. 3.8, 3.10 and 3.12
$[\underset{\sim}{S}], [\underset{\sim}{S}^{-1}]$	matrix of column eigenvectors of $[\underset{\sim}{M}]$, and inverse

$[\underset{\sim}{D}]$	diagonal matrix of eigenvalues

Indices, Subscripts and Superscripts

+	anion exchanger
-	cation exchanger
R^+	anion exchanger
R^-	cation exchanger
*, (∞)	define the cyclic steady state
l,h	designate variables defined at the low pH and at the high pH respectively

APPENDIX A

ANALYTICAL DETAILING

The following material is the information and detailing for a mathematical formalism based on elementary matrix algebra which explained in Chapter III. There follow, in order,

Exhibit A-1, Calculation of Eigenvalues of $[\underset{\sim}{M}]$.

Exhibit A-2, The structure of $[\underset{\sim}{M}]^{\infty}$.

Exhibit A-3, Example of analysis of transient region.

Exhibit A-4, Extension of the theoretical approach.

Exhibit A-5, Tables of the computational results for Five-Column system

Exhibit A-1

CALCULATION OF EIGENVALUES OF $[\underset{\sim}{M}]$

The eigenvalues of M are calculated from the equation

$$P_N(\lambda) = \det [\underset{\sim}{M} - \lambda \underset{\sim}{I}] =$$

$$\begin{vmatrix} d-p\lambda & e & 0 & 0 & 0 & 0 \\ a & b-p\lambda & c & 0 & 0 & 0 \\ 0 & a & b-p\lambda & c & 0 & 0 \\ 0 & 0 & a & b-p\lambda & c & 0 \\ 0 & 0 & 0 & a & b-p\lambda & c \\ 0 & 0 & 0 & 0 & 0 & a+b-p\lambda \end{vmatrix} = 0 \qquad \text{(A-1.1)}$$

Since the determinant is tridiagonal, it may be expanded easily, by elements of the last column for example. Let $P_{N-1}(\lambda)$ be the determinant obtained by deleting the last row and the last column, and let $P_{N-j}(\lambda)$ be the determinant obtained by deleting the j last rows and columns. We then have (with N = 6):

$$\begin{aligned} P_6(\lambda) &= (a+b-p\lambda)\, P_5(\lambda) - ac\, P_4(\lambda) \\ P_5(\lambda) &= (b-p\lambda)\, P_4(\lambda) - ac\, P_3(\lambda) \\ P_4(\lambda) &= (b-p\lambda)\, P_3(\lambda) - ac\, P_2(\lambda) \\ P_3(\lambda) &= (b-p\lambda)\, P_2(\lambda) - ac\, P_1(\lambda) \end{aligned} \qquad \text{(A-1.2)}$$

$$P_2(\lambda) = (b-p\lambda)\, P_1(\lambda) - ae\, P_0(\lambda)$$

$$P_1(\lambda) = (d-p\lambda)\, P_0(\lambda)$$

$$P_0(\lambda) = 1$$

A trial value of λ is assumed and the successive expressions P_i(i = 1 to N) are calculated. An eigenvalue is found when $P_N(\lambda) = 0$.

The localsation of the eigenvalues, and thus the trial values, is facilitated by the fact that the expressions P_i form a series, which permits the following test:

Assume a value λ' and calculate the sequence P_0 .... P_N. Let $V(\lambda')$ be the number of sign changes in this series. Assume an other trial value λ'' and determine in the same way the number of sign changes in the sequence $V(\lambda'')$. The difference $V(\lambda') - V(\lambda'')$ gives the number of real eigenvalues in the interval (λ', λ''). This property may be used to show that all eigenvalues are real. We assume λ' very large and positive and λ'' very large and negative. It is then easy to see that

$\lambda' >> 0$	$\lambda'' << 0$
$P_0 = 1 > 0$	$P_0 = 1 > 0$
$P_1 = d-p\lambda < 0$	$P_1 = d-p\lambda > 0$
$P_2 = (b-p\lambda)\, P_1 - ac > 0$	$P_2 = (b-p\lambda)\, P_1 - ac > 0$
$P_3 < 0$	$P_3 > 0$

$P_4 > 0$ $\qquad$ $P_4 > 0$

$P_5 < 0$ $\qquad$ $P_5 > 0$

$P_6 > 0$ $\qquad$ $P_6 > 0$

The numbers of sign changes are $V(\lambda) = N$ and $V(\lambda') = 0$ and the number of real eigenvalues, positive or negative, is N. Thus all eigenvalues are real.

The sturm sequence A-1.2 may also conveniently be used to show that $\lambda = 1$ is an eigenvalue. For $\lambda = 1$, we have

$$P_0 = 1 > 0$$

$$P_1 = d - p = -k^h(\beta + k^l) < 0$$

$$P_2 = (b-p)\, P_1 - ac = -aP_1$$

$$P_3 = (b-p)\, P_2 - acP_1 = -aP_2 - cP_2 - acP_1 = -aP_2$$

$$P_j = (b-p)\, P_{j-1} - acP_{j-2} = -aP_{j-1} - cP_{j-1} - acP_{j-2}$$

and since $P_{j-1} = -aP_{j-2}$

we have

$$P_j = -aP_{j-1}$$

For the last polynomial P_N, which represents the characteristic equation, we have:

$$P_N = (a+b-p)\, P_{N-1} - ac\, P_{N-2}$$

with $a + b - p = - c$

and $P_{N-1} = - aP_{N-2}$

thus $P_N = 0$

and $\lambda = 1$ satisfies the characteristic equation and is thus eigenvalue, independently of the values of the parameters. Incidentally, this calculation shows that $V(\lambda' = 1) = N-1$. Since $V(\lambda' \gg 0) = N$, there is a single real root in the interval $(+1, +\infty)$ which is precisely $\lambda = 1$. No eigenvalues can thus have a value larger than one. A somewhat more tedious calculation can be made for $\lambda' = 0$, showing that $V(\lambda' = 0) = 0$. This ensures that all eigenvalues are positive, and finally, we must have

$$0 \leq \lambda_0 \leq \lambda_1 \leq \ldots\ldots \leq \lambda_N = 1$$

Figure A-1.1 shows the plot of $\overset{n}{i}$ as a function of the number of cycles (n) on the Semi-Logarithmic ordinate.

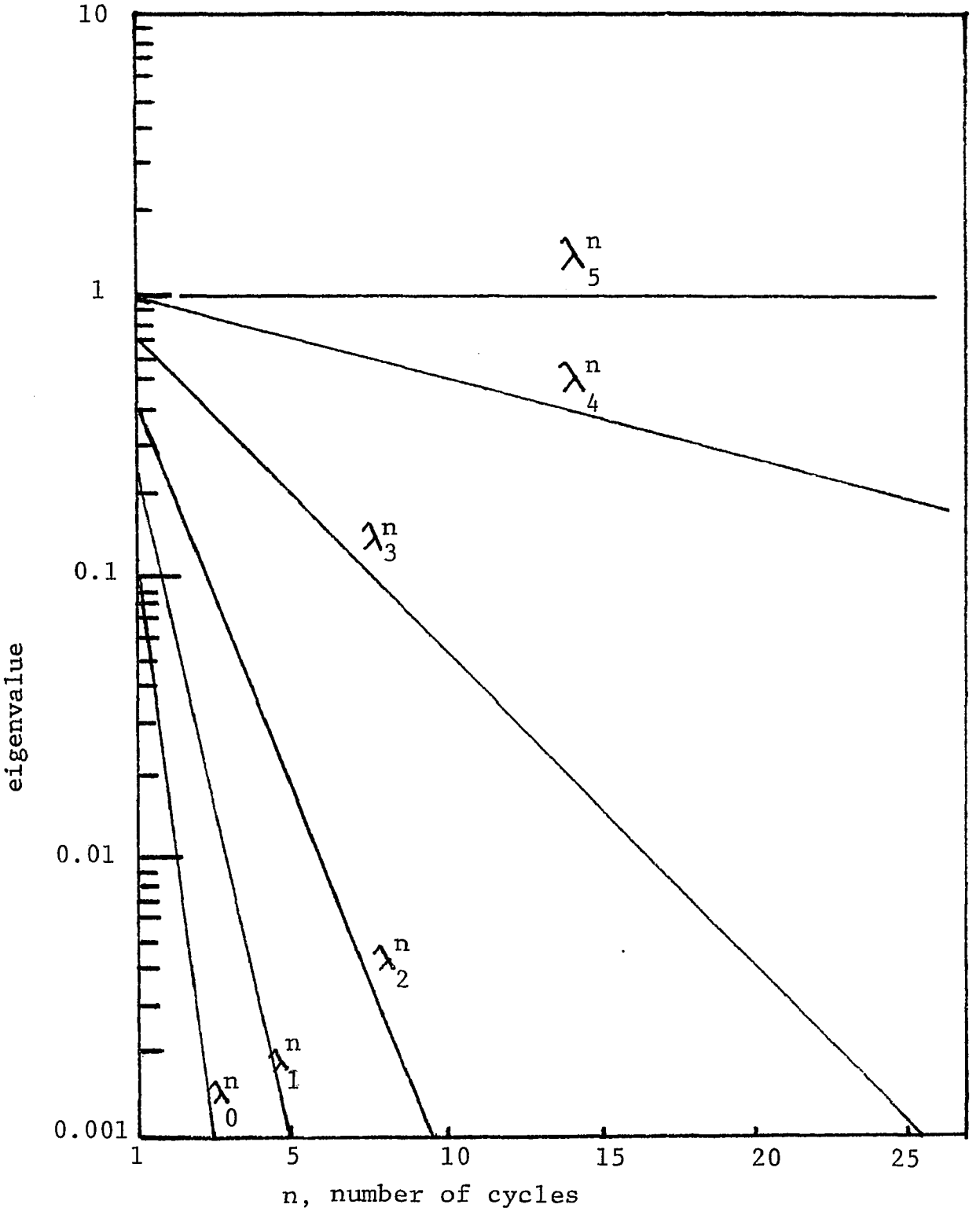


FIGURE A-1.1 - THE PLOT OF λ_i^n vs. NUMBER OF CYCLES.

Exhibit A-2

THE STRUCTURE OF $[\underset{\sim}{M}]^{\infty}$

For large values of n, that is at cyclic steady state, M^n must satisfy for N+1 equations

$$\lim_{n \to \infty} [\underset{\sim}{M}]^n \underset{\sim}{Y}(0) = \underset{\sim}{Y}(\infty) \qquad \text{(A-2.1)}$$

independently of the initial distribution $\underset{\sim}{Y}(0)$. The concentrations $(w_0, w_1, \ldots\ldots w_N)$ of $\underset{\sim}{Y}(0)$, and the concentration $(y_0^*, y_1^*, \ldots\ldots y_N^*)$ of $Y(\infty)$ are related only by the condition of conservation of mass of solute in the whole system. This condition is expressed by:

$$W = \bar{V} \left(\varsigma w_0 + \sum_{i=1}^{N} (\varsigma + k^1) w_i \right)$$

$$= \bar{V} \left(\varsigma y_0^* + \sum_{i=1}^{N} (\varsigma + k^1) y_i^* \right) \qquad \text{(A-2.2)}$$

Replacing y_i^* by $y_0^* \beta^{-i}$, from Equation 3.24, factoring out y_0^* in the right hand side and re-managing, we obtain

$$y_0^* = A w_0 + B \sum_{j=1}^{N} w_j \qquad \text{(A-2.3)}$$

where:

$$A = \frac{\varsigma}{\varsigma + (\varsigma + k^1) \sum_{q=1}^{N} \beta^{-q}} \; ; \quad B = \frac{\varsigma + k^1}{\varsigma} A \qquad \text{(A-2.4)}$$

Cleary the N+2 equations A-2.1 and A-2.3 may hold for any set of w_j only if they are redundant: we may thus identify, for example Equation A-2.3 with the i^{th} equation of A-2.1, which we write (i = 0, 1, ... N)

$$m_{i0}\, w_0 + m_{i1}\, w_1 + \ldots\ldots\ m_{iN}\, w_N = y_i^* = y_0^*\, \beta^{-i} \qquad \text{(A-2.5)}$$

and we obtain

$$m_{ij}\, \beta^i = \begin{cases} A & \text{for } j = 0 \\ B & \text{for } j = 1,2, \ldots\ldots\ N \end{cases} \qquad \text{(A-2.6)}$$

The elements m_{ij} of $[\underset{\sim}{M}]^{\infty}$ are thus completely identified by Equation A-2.6, together with A-2.4. $[\underset{\sim}{M}]^n$ may be visualized as:

$$[\underset{\sim}{M}]^{\infty} = \begin{vmatrix} A & B & B \ldots\ldots\ldots & B \\ A\beta^{-1} & B\beta^{-1} & B\beta^{-1} & B\beta^{-1} \\ A\beta^{-2} & B\beta^{-2} & B\beta^{-2} & B\beta^{-2} \\ \vdots & & & \\ & & & \\ \vdots & & & \\ A\beta^{-N} & B\beta^{-N} & B\beta^{-N} & B\beta^{-N} \end{vmatrix} \qquad \text{(A-2.7)}$$

It may be verified that this matrix is invariant on multiplication on the left or on the right by $[\underset{\sim}{M}]$.

It is interesting to look at the other possible approach starting from Equation 3.17

$$[\underset{\sim}{M}]^{\infty} = [\underset{\sim}{S}][\underset{\sim}{D}]^{\infty}[\underset{\sim}{S}]^{-1} \qquad \text{(A-2.8)}$$

Clearly $[\underset{\sim}{D}]^{\infty}$ reduces to the single element 1 in the last row and last column, all other element being zero. The product $[\underset{\sim}{S}][\underset{\sim}{D}]^{\infty}$ is a matrix with the first N columns of zeroes, the last column being the last column of $[\underset{\sim}{S}]$, that is the eigenvector belong to $\lambda = 1$, Multiplying this matrix by $[\underset{\sim}{S}]^{-1}$, we should recover the result of Equation A-2.7. It is easy to show that the only elements of $[\underset{\sim}{S}]^{-1}$ that appear in the product are the elements of the last row, which are the cofactors of the above mentioned eigenvector in $[\underset{\sim}{S}]$. Identifying with Equation A-2.7, we conclude that the last row of $[\underset{\sim}{S}]^{-1}$ is (A B B B ..... B).

Exhibit A-3

EXAMPLE OF ANALYSIS OF TRANSIENT REGIME

The equilibrium constants (Figure 21) are charaterized by the following values of the slopes

at Low pH: $k^l = 0.70$

at High pH: $k^h = 0.52$

$$\beta = \frac{k^l}{k^h} = 1.346$$

This corresponds to the experimental conditions of Run . We also have:

$$\rho = \frac{V}{\bar{V}} = \frac{26 \text{ cc fluid}}{26 \text{ cc solid}} = 1$$

and N = 5 since there are five stages. With these values, the matrix $[\underset{\sim}{M}]$ is written (Equations 3.12 and 3.13)

$$[\underset{\sim}{M}] = \frac{1}{2.584} \begin{vmatrix} 1.70 & 1.19 & 0 & 0 & 0 & 0 \\ 0.52 & 1.364 & 0.70 & 0 & 0 & 0 \\ 0 & 0.52 & 1.364 & 0.70 & 0 & 0 \\ 0 & 0 & 0.52 & 1.364 & 0.70 & 0 \\ 0 & 0 & 0 & 0.52 & 1.364 & 0.70 \\ 0 & 0 & 0 & 0 & 0.52 & 1.884 \end{vmatrix}$$

The eigenvalues, calculated as outlined in Chapter III, are:

$$\lambda_0 = 0.1050 \qquad \lambda_3 = 0.7336$$

$$\lambda_1 = 0.2522 \qquad \lambda_4 = 0.9239$$

$$\lambda_2 = 0.4838 \qquad \lambda_5 = 1.0000$$

(NOTE: Calculations were performed with 8 significant figures)

In Run , the initial condition is that all fluid fractions are identical, and in equilibrium with the solid phase fractions with the following protein concentrations (i = 0, 1, ... N):

$$\left.\begin{aligned} w = y_i(0) &= 1.18 \text{ gm/liter} \\ x_i(0) &= 0.826 \text{ gm/liter} \end{aligned}\right\} \quad \text{equilibrium at Low pH}$$

so that the total amount of protein is:

$$W = 0.290 \text{ gm of protein}$$

then from Equation 3.28, we calculate

$$y_0^* = 2.324 \text{ gm protein/liter}$$

The final steady state is then given by Equation 3.26

$$\underset{\sim}{Y}^1(\infty) = \begin{vmatrix} y_0^* = 2.324 \\ y_1^* = 1.726 \\ y_2^* = 1.282 \\ y_3^* = 0.953 \\ y_4^* = 0.708 \\ y_5^* = 0.526 \end{vmatrix}$$

Now, we should like to know the composition at an arbitrary cycle n. In principle, we would have to calculate $[\underset{\sim}{S}]^{-1}$ in order to use Equations 3.17 and 3.18, or calculate the $[\underset{\sim}{A}_j]$ in order to use Equations 3.19 and 3.20. We shall see that these tedious calculations may be partly avoided if only estimations are sought, and for n sufficiently large. Equation 3.17 or 3.19, when developed, lead to expressions of the form:

$$\underset{\sim}{Y}^1(n) = \left| \begin{array}{l} a_{00}\lambda_0^n + a_{01}\lambda_1^n + a_{02}\lambda_2^n + a_{03}\lambda_3^n + a_{04}\lambda_4^n + y_0^* \\ a_{10}\lambda_0^n + a_{11}\lambda_1^n + \text{----------} + y_1^* \\ a_{20}\lambda_0^n + \text{----------} + y_2^* \\ a_{30}\lambda_0^n \\ a_{40}\lambda_0^n \\ a_{50}\lambda_0^n \text{----------} + y_5^* \end{array} \right|$$

the last column corresponds to $\underset{\sim}{Y}^1(\infty)$. The examination of the successive powers of the λ's shows that the contribution of the smallest eigenvalues becomes rapidly negligible. This is illustrated on Figure A-1 where $\ln \lambda_i^n$ is plotted against n. We observe that λ_0^n becomes neligible with respect to 1 ($\lambda_0^n < 10^{-3}$) as early as the third cycle, λ_1^n around the fifth cycle, λ_2^n around the tenth cycle. The contribution of λ_3 persists until the 20^{th} cycle, and that of λ_4 until the 90^{th} cycle. These contributions are somewhat modified by the factors a_{ij}, but these actually reinforce the importance of the largest eigenvalues. Figure A-2 shows a comparison between the

rigorous curve (full line) and the approximation obtained by neglecting the contributions of λ_1, λ_2, λ_3. This relation is expressed by:

$$\underset{\sim}{Y}(n) - \underset{\sim}{Y}(\infty) = w\underset{\sim}{C}\lambda_4^n \qquad \text{(A-3.1)}$$

with

$$\underset{\sim}{C} = \begin{vmatrix} - 1.024 \\ - 0.563 \\ - 0.090 \\ + 0.339 \\ + 0.540 \\ + 0.615 \end{vmatrix} \quad \text{and } w = 1.18 \text{ gm/liter}$$

It is seen that this approximation, besides showing the correct trend; gives an estimate better than 10% for $n \geqslant 3$, better than 5% for $n \geqslant 10$, and better than 1% for $n \geqslant 20$. For all practical purposes, it seems thus sufficient to calculate the matrix $[\underset{\sim}{A}_j]$ corresponding to $\lambda_j = \lambda_4$ in Equation 3.19, or in other terms, to calculate the two lines in $[\underset{\sim}{S}]^{-1}$ that correspond to the two largest eigenvalues.

Note that Equation A-3.1 is comparable to a result established by Pigford et al (1969) for linear packed bed parapumps after a certain start-up period. Using their notation, their Equations 3.16 and 3.17 may be put in the form:

$$(y_T)_n - (y_T)_\infty = (y_B)_n - (y_B)_\infty = \left(\frac{1-b}{1+b}\right)^n y_0$$

which expresses, that the "distance" from the steady state

for top and bottom reservoir concentrations is a power function of the number of cycles.

Exhibit A-4

EXTENSION OF THE THEORETICAL APPROACH

The discussion illustrates that in any experiment, we would be faced with imperfect phase transfer, volume variations of the fractions, volume differences in the stages, and so forth. In addition, the mode of parametric pumping considered here is restrictive: it ignores partial reflux and multiple transfers per half-cycle, situations studied elsewhere (Grevillot, G., and Tondeur D. 1977, 1980) from the point of view of the cyclic steady state. We should like to give a hint on how the present approach can be extended, without major difficulty, to account for some of the situations mentioned above. For this purpose, we shall examine how the basic equations are altered separately by the different effects.

Existence of a dead volume

In the experiments presented, we mentioned that some of solute (protein) was not transferred when the apparatus was connected to the reservoirs and pH-converters. Let us characterize this "dead volume" V_T or V_B, by its ratio γ to the volume of the solid phase fractions.

$$\gamma = V_T/\bar{V} = V_B/\bar{V} \qquad \text{(A-4.1)}$$

and we assume this ratio is constant and equal for all stages, in transfer up and transfer down. When the material balances (Equation 3.1) are re-written, taking this hold up into account, one obtains the same form as Equation 3.5, but with ϱ replaced by $\varrho - \gamma$, k^l by $k^l + \gamma$ and k^h by $k^h + \gamma$; (so that the denominator $\varrho + k^h$ in Equation 3.5 is unchanged). A similar property holds for the transfer up half-cycle, and finally the whole approach outlined so far remains valid providing the substitutions indicated above are made in the elements of matrix $[\underset{\sim}{M}]$. The discontinuous lines on Figure A-4.1 show the resultof such a calcultion, made with a 10% dead volume of solvent ($\gamma = 0.1$) which slightly overestimates the reality.

Intuitively, the effect of γ can be foreseen by considering the system as having a lower ϱ, and higher k's, but a lower effective β. The effect on the cyclic steady state is seen immediately from Fenske's equation (Equation 3.25), with data given:

$$\frac{y_0^*}{y_N^*} = \beta^5_{\text{effective}} = \left[\frac{k^l+\gamma}{k^h+\gamma}\right]^5 \quad \begin{cases} = 3.29 \text{ for } \gamma = 0 \\ = 2.77 \text{ for } \gamma = 0.1 \end{cases}$$

The effect of a smaller ϱ and larger k's is favorable to speed of convergence but may be offset by the decrease in β.

Unequal volume of fractions

A simple modification accounts for the volume of solid phase being different in each stage, and the volumes of each

fluid phase fraction being different, providing these volumes are constant (same volume transferred up and down). It suffices to take a different φ_q for each stage and for equilibrations at different pH levels. In the transfer down matrix $[\underset{\sim}{\Theta}_h]$ (Equation 3.8), the unique φ will be replaced by φ_q^h, different in each line, and similarly, in matix $[\underset{\sim}{\Theta}_1]$, φ_q^1 will be introduced. The product matrix $[\underset{\sim}{M}]$ remains tridiagonal, and the methods for eigenvalues and eigenvector calculations are unchanged. All the other properties mentionned still hold.

Volume variations of phases

As discussed earlier, important variations of the volume of the fluid fractions may be caused by geometric dissymmetry of the apparatus in transferdown versus transfer up, and by dilatation of the solid phase. These effects can be quantified and accounted for in writing the material balances. Unfortunately, they will cause the value of φ to change each stage and at each cycle. Therefore, there is no unique matrix $[\underset{\sim}{M}]$ and the approach presented fails to apply.

Several transfers per half-cycle

Suppose the apparatus used in the present work was equipped with an additional reservoir in series at each end. Then seven fluid phase fractions would be used, and there would be two successive transfers in the same direction at each pH level. The cyclic steady state of such system has been extensively studied (Chen, H. T., et al 1980). The matrix formal-

ism can be used conveniently by noting that each transfer of fluid phase follwed by an equilibration is described by a bidiagonal matrix similar to $[\underset{\sim}{\Theta}_l]$ or $[\underset{\sim}{\Theta}_h]$. Let us consider for example a parapump with two stages and four fluid phase fractions thus two transfers per half cycle. The matrix $[\underset{\sim}{M}]$ describing the complete cycle is the product of successive bidiagonal matrices describing each transfer;

$$[\underset{\sim}{\Theta}_{12}]\ (\varsigma+k^l) \qquad [\underset{\sim}{\Theta}_{11}]\ (\varsigma+k^h) \qquad [\underset{\sim}{\Theta}_{h2}]\ (\varsigma+k^h) \qquad [\underset{\sim}{\Theta}_{h2}]\ (\varsigma+k^l)$$

$$\begin{vmatrix} \varsigma+k^l & 0 & 0 & 0 \\ 0 & \varsigma+k^l & 0 & 0 \\ 0 & k^h & \varsigma & 0 \\ 0 & 0 & k^h & \varsigma \end{vmatrix} \begin{vmatrix} \varsigma+k^h & 0 & 0 & 0 \\ k^h & \varsigma & 0 & 0 \\ 0 & k^h & \varsigma & 0 \\ 0 & 0 & 0 & \varsigma+k^h \end{vmatrix} \begin{vmatrix} \varsigma & k^l & 0 & 0 \\ 0 & \varsigma & k^l & 0 \\ 0 & 0 & \varsigma+k^h & 0 \\ 0 & 0 & 0 & \varsigma+k^h \end{vmatrix} \begin{vmatrix} \varsigma+k^l & 0 & 0 & 0 \\ 0 & \varsigma & k^l & 0 \\ 0 & 0 & \varsigma & k^l \\ 0 & 0 & 0 & \varsigma+k^l \end{vmatrix}$$

and

$$[\underset{\sim}{M}] = \frac{[\underset{\sim}{\Theta}_{12}]\ [\underset{\sim}{\Theta}_{11}]\ [\underset{\sim}{\Theta}_{h2}]\ [\underset{\sim}{\Theta}_{h1}]}{(\varsigma+k^l)^2\ (\varsigma+k^h)^2} \qquad \text{(A-4.2)}$$

$[\underset{\sim}{M}]$ is no longer tridiagonal, but in the present case, comprises 5 diagonals. The simple numerical methods for calculating eigenvalues and eigenvectors of tridiagonal matrices no longer apply, but otherwise the general method is unchanged. Also notice that different definitions of $[\underset{\sim}{M}]$ may be introduced, depending on how the beginning of the cycle is defined. These various forms differ from each other by a circular permutation on the bidiagonal matrices $[\underset{\sim}{\Theta}]$.

Partial reflux parapump

We adopt the description given by Chen,H.T.,et al (1980) for one transfer per half-cycle, in a pump where fresh feed is added at each half-cycle in an intermediat stage, and with a different reflux ratio at each end. The operating scheme of such a pump is summarized on Figure A-4.1. The equations describing the operation of the lower section during the low pH half-cycle, and the upper section during the high pH half-cycle are that of the simplest case, that is, Equation 3.1 to 3.10. The other equations are that for a holdup in each stage, as explained in a previous paragraph (ϱ replaced by $\varrho - \gamma$, k by $k + \gamma$). Special material balances must be written for the feed stage. The result may be written in the following form:

$$\underset{\sim}{Y}^{h}(n) = [\underset{\sim}{\Theta}_h]\ \underset{\sim}{Y}^{l}(n) + \underset{\sim}{F}_h \qquad \text{(A-4.3)}$$

and

$$\underset{\sim}{Y}^{l}(n+1) = [\underset{\sim}{\Theta}_l]\ \underset{\sim}{Y}^{h}(n) + \underset{\sim}{F}_l$$

$$= [\underset{\sim}{\Theta}_l]\ [\underset{\sim}{\Theta}_h]\ \underset{\sim}{Y}^{l}(n) + [\underset{\sim}{\Theta}_l]\ \underset{\sim}{F}_h + \underset{\sim}{F}_l \qquad \text{(A-4.4)}$$

where $[\underset{\sim}{\Theta}_h]$ and $[\underset{\sim}{\Theta}_l]$ are bidiagonal matrices and $\underset{\sim}{F}_h$ and $\underset{\sim}{F}_l$ are column vectors representing the feed contribution. Table A-4.1 give the elements of these matrices and vectors, as corresponding to the stages in which the conservation and equilibrium relations are written. All other elements are zero. y_F is the feed composition, assumed constant. The feed vec-

Table A-4.1

Index of stage	Stage or reservoir	$(\varrho+k^h)[\underset{\sim}{\Theta}_h]$		$(\varrho+k^h)\,\underset{\sim}{F}_h$	$(\varrho+k^l)[\underset{\sim}{\Theta}_l]$		$(\varrho+k^l)\,\underset{\sim}{F}_l$
		Main diagonal	Upper diagonal		Main diagonal	Lower diagonal	
	Top reservoir				$\varrho + k^l$		0
1, 2,..f-1	Stage in top section	$\varrho - \gamma_h$	$k^l + \gamma_h$	0		k^h	0
f(feed)	Feed stage	$\varrho - \gamma_h$	k^l	$\gamma_h \cdot y_F$	$\varrho - \gamma_l$	k^h	$\gamma_l \cdot y_F$
f+1, f+2, ... N	Stage in bottom section	ϱ	k^l	0	$\varrho - \gamma_l$	$k^h + \gamma_l$	0
	Bottom reservoir	$\varrho + k^h$		0			

tors $\underset{\sim}{F}_h$ and $\underset{\sim}{F}_1$ have each a single non-zero element, owing to the fact that the feed is added in a given single stage. The non-zero element is not on the line in the two vectors because the feed is mixed with a different mobile phase fraction, in the high pH and low pH steps. Note that a feed distributed over several stages can be accounted for by the same Equations A-4.3 and A-4.4, but different elements in the matrices $[\underset{\sim}{\Theta}_h]$ and $[\underset{\sim}{\Theta}_1]$, and additional non-zero elements in $\underset{\sim}{F}_1$ and $\underset{\sim}{F}_h$.

The general solution of the first-order recurrence of Equations A-4.3 and A-4.4, relating $\underset{\sim}{Y}^1(n+1)$ to $\underset{\sim}{Y}^1(n)$, is easily seen to be:

$$\underset{\sim}{Y}^1(n) = [\underset{\sim}{M}]^n \underset{\sim}{Y}^1(0) + [\underset{\sim}{I} + \underset{\sim}{M} + \underset{\sim}{M}^2 + \ldots\ldots + \underset{\sim}{M}^{n-1}][\underset{\sim}{F}_1 + [\underset{\sim}{\Theta}_1]\underset{\sim}{F}_h] \qquad \text{(A-4.5)}$$

where $[\underset{\sim}{M}] = [\underset{\sim}{\Theta}_1][\underset{\sim}{\Theta}_h]$. Equation A-4.5 is to be compared to Equation 3.14 (Note: that $[\underset{\sim}{M}]$ is not the same in these two Equations). The matrix $[\underset{\sim}{M}]$ is still tridiagonal and the methods for calculating the eigenvalues remain valid. The geometric matrix series $[\underset{\sim}{I} + \underset{\sim}{M} + \underset{\sim}{M}^2 + \ldots\ldots + \underset{\sim}{M}^{n-1}]$ can be rewritten by applying Sylvester's theorem (Equations 3.19 and 3.20) to each term, so that a scalar geometric series appears on each eigenvalue. If all λ's are different from 1, Equation A-4.5 becomes

$$\underset{\sim}{Y}(n) = \sum_{j=0}^{N} [\underset{\sim}{A}_j] \left[\lambda_j^n \underset{\sim}{Y}(0) + \frac{1 - \lambda_j^n}{1 - \lambda_j} \cdot \underset{\sim}{F}\right] \qquad \text{(A-4.6)}$$

where $[\underset{\sim}{A}_j]$ are matrices obtained from $[\underset{\sim}{M}]$ by applying Equation 3.20, and $\underset{\sim}{F}$ is the overall feed vector, given by:

$$\underset{\sim}{F} = \underset{\sim}{F}_1 + [\underset{\sim}{\Theta}_1]\,\underset{\sim}{F}_h = \frac{y_F}{(\varsigma+k^l)(\varsigma+k^h)} \begin{vmatrix} 0 \\ \vdots \\ 0 \\ \varsigma\gamma_h \\ \gamma_l(\varsigma+k^h) + \gamma_h k^h \\ 0 \\ \vdots \\ 0 \end{vmatrix}$$

the two non-zero elements of $\underset{\sim}{F}$ are on lines f and f+1.

If any eigenvalue was larger than or equal to one, the series $[\underset{\sim}{I} + \underset{\sim}{M} + \underset{\sim}{M}^2 + \ldots\ldots \underset{\sim}{M}^{n-1}]$ would not converge and no steady state would be reached. On physical grounds, we may thus state that all eigenvalues are smaller than 1. Under these conditions, the cyclic steady-state is not obtained directly as an eigenvector of $[\underset{\sim}{M}]$, but by letting $\underset{\sim}{Y}(n+1) = \underset{\sim}{Y}(n)$ in Equation A-4.4, or $n \rightarrow \infty$ in Equation A-4.6:

$$\underset{\sim}{Y}^l(\infty) = [\underset{\sim}{I}-\underset{\sim}{M}]^{-1}.\underset{\sim}{F} = \sum_{j=o}^{N} \frac{[A_j]}{1-\lambda_j}.\underset{\sim}{F} \qquad \text{(A-4.7)}$$

We know that the cyclic steady state can be geometrically represented by a Mc Cabe-Thiele diagram somewhat more complicated than that of total reflux, and that the analytical expressions for the separation factor are quite involved. There is thus little hope to bring Equation A-4.7 to a more analytical

form by simple manipulations.

Exhibit A-5

Conditions for Computational the Results with variable parameter:

Operating Variable	Cases I	Case II
Volume of Fluid Phase per Column	26.0 cm^3	30.0 cm^3
Volume of Solid Phase per Column	26.0 cm^3	30.0 cm^3
Equilibrium Constant at Low pH	0.70	0.75
Equilibrium Constant at High pH	0.52	0.60
β	1.346	1.250
ρ	1.0	1.0

Table A-5.1

PROTEIN CONCENTRATION: CASE I

Number of Cycles, n	y_0	y_1	y_2	y_3	y_4	y_5	x_1	x_2	x_3	x_4	x_5
1	1.180	1.180	1.180	1.180	1.180	1.180	1.000	0.850	0.800	0.700	0.600
11	1.824	1.351	1.218	1.095	0.955	0.785	1.013	0.863	0.763	0.662	0.550
21	2.096	1.592	1.264	1.021	0.824	0.674	1.112	0.874	0.707	0.571	0.447
30	2.214	1.652	1.275	0.982	0.763	0.600	1.157	0.882	0.686	0.524	0.400
40	2.267	1.685	1.277	0.967	0.721	0.571	1.177	0.888	0.672	0.501	0.375
50	---	---	---	---	---	---	1.185	0.889	0.664	0.491	0.361

Table A-5.2

PROTEIN CONCENTRATION: CASE II

Number of Cycles, n	y_0	y_1	y_2	y_3	y_4	y_5	x_1	x_2	x_3	x_4	x_5
1	1.200	1.202	1.201	1.204	1.203	1.200	0.800	0.800	0.800	0.800	0.800
6	1.519	1.300	1.217	1.184	1.118	0.975	0.854	0.803	0.780	0.735	0.652
11	1.683	1.401	1.238	1.135	1.034	0.872	0.923	0.815	0.751	0.677	0.577
20	1.874	1.522	1.272	1.112	0.946	0.781	1.000	0.834	0.715	0.608	0.503
30	2.010	1.583	1.284	1.055	0.882	0.702	1.046	0.846	0.697	0.563	0.454
40	2.046	1.617	1.298	1.035	0.845	0.674	1.065	0.847	0.683	0.543	0.431
50	---	---	---	---	---	---	1.071	0.851	0.677	0.532	0.419

APPENDIX B

EXPERIMENTAL DATA

Information and Condition of the experimental results are as follow.

Exhibit B-1, Sample of Calculation

Exhibit B-2, Single Column System

Exhibit B-3, Two Column System: Mode 1

Exhibit B-4, Two Column System: Mode 2

Exhibit B-5, Two Column System: Mode 3

Exhibit B-6, Two Column System: Mode 4

Exhibit B-1

Sample Calculation:

1. To calculate the concentration of Haemoglobin. (using a 0.02 weight percent solution)

a). Via 403μ Reading: (Any pH Value)

$$\frac{\text{Reading of Sample at 403 } \mu}{\text{Initial Reading of Feed at 403 } \mu} = \frac{R_s\ 403}{R_F\ 403}$$

= Concentration of Haemoglobin

b). Via 560μ, 576μ and 630μ (A_1, A_2 and A_3 respectively)

$$\text{Concentration of Haemoglobin} = \frac{\sum C \text{ Sample}}{\sum C \text{ Initial}}$$

pH = $P_3(4.0)$;

$$C_{4.0} = (2.593\text{x}10^{-5})A_1 + (4.483\text{x}10^{-5})A_2 + (1.741\text{x}10^{-4})A_3$$

pH = $P_2(6.2)$;

$$C_{6.2} = (2.505\text{x}10^{-5})A_1 + (4.525\text{x}10^{-5})A_2 + (1.808\text{x}10^{-4})A_3$$

pH = $P_1(8.5)$

$$C_{8.5} = (1.958\text{x}10^{-5})A_1 + (4.789\text{x}10^{-5})A_2 + (2.214\text{x}10^{-4})A_3$$

Thus, the concentration of Haemoglobin (y_H) can express as:

$$y_H = \frac{C_{pH} \text{ of Sample at } 560\mu, 576\mu \text{ and } 630\mu}{C_{pH} \text{ of Initial Feed Reading of } 560\mu, 576\mu \text{ and } 630\mu}$$

2. To calculate the concentration of Albumin.
(using a 0.02 weight percent solution)

Reading at 595μ = Total Protein Concentration (y_{AH})
= Albumin Concentration (y_A) +
Haemoglobin Concentration (y_H)

Thus:

$$y_A = y_{AH} - y_H$$

Let, R_{S595} = Reading of Sample at 595μ
R_{B595} = Dye Reading at 595μ
R_{F595} = Reading of Feed at 595μ
R_{S403} = Reading of Sample at 403$^*\mu$
R_{F403} = Reading of Feed at 403$^*\mu$
0.04 = Total weight percent of Haemoglobin and Albumin
0.02 = Component weight percent

$$y_A = \frac{\left|\frac{R_{S595} - R_{B595}}{R_{F595} - R_{B595}}\right|(0.04) - \left|\frac{R_{S403}}{R_{F403}}\right|(0.02)}{(0.02)}$$

* NOTE: One can use the calculated value of Haemoglobin via 560μ, 576μ and 630μ also.

Exhibit B-2

Experimental Parameters For Single Column-Two Reservoirs

Run	Feed (wt %) Haemoglobin	Albumin	Displacement rate,Q cm^3/s	Feed volume (cm^3) Bottom Qt_{II}	Top Qt_{IV}	Reservoir Displacement $Qt_I=Qt_{III}$ (cm^3)	Ionic cone molarity,M Bottom Buffer	NaCl	Top Buffer
1	0.02	0.02	$8.33x10^{-3}$	10	10	22.5	0.2	0.1	0.05
2	0.02	0.02	$8.33x10^{-3}$	5	10	22.5	0.2	0.1	0.05
3	0.02	0.02	$8.33x10^{-3}$	15	10	22.5	0.2	0.1	0.05
4	0.02	0.02	$8.33x10^{-3}$	20	10	22.5	0.2	0.1	0.05
5	0.02	0.02	$8.33x10^{-3}$	10	10	22.5	0.2	0.1	0.05
6	0.02	0.02	$16.67x10^{-3}$	10	10	22.5	0.2	0.1	0.05
7	0.02	0.02	$25.00x10^{-3}$	10	10	22.5	0.2	0.1	0.05
8	0.02	0.02	$8.33x10^{-3}$	15	10	22.5	0.05	0.65	0.05
9	0.02	0.02	$8.33x10^{-3}$	15	10	22.5	0.25	___	0.025
10	0.02	0.02	$8.33x10^{-3}$	15	10	22.5	0.05	0.25	0.05
11	0.02	0.02	$16.67x10^{-3}$	10	10	35	0.2	0.1	0.05
12	0.02	0.02	$8.33x10^{-3}$	10	10	10	0.2	0.1	0.05
13	0.01	0.01	$8.33x10^{-3}$	15	10	22.5	0.25	___	0.025

For all run: column length=0.15m; $V_T=V_B=10cm^3$; $P_1=8.5$ and $P_2=6.2$

TABLE B-1

Run #1

Anion Column, Haemoglobin

TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle,n	403 μ	y_H	403 μ	y_H
I.C.	0.003	0.00	0.433	1.00
1	0.091	0.21	0.283	0.53
2	0.161	0.37	0.166	0.38
3	0.167	0.39	0.117	0.27
4	0.189	0.44	0.058	0.13
5	0.198	0.46	0.033	0.08
6	0.184	0.43	0.024	0.06
7	0.191	0.44	0.014	0.03
8	0.195	0.45	0.105	0.24
9	0.169	0.39	0.022	0.05
10	0.171	0.40	0.013	0.03

Run #2

Anion Column, Albumin

TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle,n	595$^*\mu$	y_A	595$^*\mu$	y_A
I.C.	0.998	1.00	1.059	1.00
1	0.755	0.62	0.901	0.80
2	0.903	0.91	0.960	0.84
3	0.941	1.02	1.250	1.18
4	0.989	1.11	1.255	1.19
5	1.019	1.18	1.528	1.24
6	1.039	1.29	1.206	1.13
7	1.059	1.33	1.214	1.14
8	1.039	1.26	0.976	0.96
9	0.989	1.16	1.131	1.06
10	0.975	1.16	1.127	1.05

595$^*\mu$: 0.1 cc Sample/3.0 cc Dye

TABLE B-2

Run #3

Anion Column, Haemoglobin

TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle,n	403μ	y_H	403μ	y_H
I.C.	0.002	0.00	0.408	1.00
1	0.065	0.16	0.254	0.62
2	0.162	0.40	0.152	0.37
3	0.187	0.46	0.093	0.23
4	0.219	0.54	0.080	0.20
5	0.215	0.53	0.052	0.13
6	0.199	0.49	0.030	0.07
7	0.200	0.49	0.022	0.05
8	0.174	0.43	0.017	0.04
9	0.164	0.40	0.019	0.05
10	0.159	0.39	0.018	0.04

Run #4

Cation Column, Haemoglobin

TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle,n	403μ	y_H	403μ	y_H
I.C.	0.778	1.00	0.009	0.00
1	0.525	0.68	0.440	0.57
2	0.420	0.54	0.609	0.78
3	0.321	0.41	0.623	0.82
4	0.166	0.21	0.648	0.83
5	0.123	0.16	0.670	0.86
6	0.096	0.12	0.657	0.85
7	0.076	0.10	0.661	0.85
8	0.062	0.08	0.644	0.85
9	0.058	0.07	0.598	0.77
10	0.060	0.08	0.596	0.77

TABLE B-3

Run #5

Cation Column, Albumin

TR (P_2) = 6.2, BR (P_3) = 4.0

Cycle,n	$595^{*}\mu$	y_A	$595^{*}\mu$	y_A
I.C.	1.141	1.00	0.471	0.00
1	0.954	0.77	0.920	0.49
2	0.876	0.68	1.061	0.98
3	0.762	0.47	1.017	0.82
4	0.626	0.27	1.083	0.99
5	0.584	0.20	1.087	0.98
6	0.581	0.22	1.057	0.91
7	0.559	0.19	1.066	0.93
8	0.532	0.13	1.046	0.87
9	0.550	0.19	1.024	0.88
10	0.569	0.24	1.015	0.86

Run #6

Anion Column, Albumin

TR (P_2) = 6.2, BR (P_3) = 4.0

Cycle,n	$595^{*}\mu$	y_A	$595^{*}\mu$	y_A
I.C.	0.487	0.00	1.117	1.00
1	0.678	0.50	0.906	0.73
2	0.877	0.87	0.736	0.46
3	0.888	0.84	0.637	0.31
4	0.970	1.01	0.630	0.32
5	1.051	1.26	0.564	0.19
6	1.072	1.36	0.525	0.12
7	1.039	1.26	0.502	0.07
8	0.981	1.15	0.487	0.04
9	0.951	1.09	0.479	0.01
10	0.947	1.08	0.477	0.01

$595^{*}\mu$: 0.1 cc Sample/3.0 cc Dye

TABLE B-4

Run #7

Cation Column, Haemoglobin

TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle,n	403 μ	y_H	403 μ	y_H
I.C.	0.439	1.00	0.000	0.00
1	0.189	0.43	0.093	0.21
2	0.078	0.18	0.127	0.29
3	0.026	0.06	0.156	0.36
4	0.018	0.04	0.168	0.37
5	0.014	0.03	0.161	0.37
6	0.017	0.04	0.158	0.36
7	0.015	0.03	0.147	0.33
8	0.015	0.03	0.159	0.36
9	0.013	0.03	0.155	0.35
10	0.014	0.03	0.151	0.34

Run #8

Cation Column, Albumin

TR (P_2) = 6.2, BR (P_3) = 4.0

Cycle,n	595* μ	y_A	595* μ	y_A
I.C.	1.090	1.00	0.478	0.00
1	0.778	0.58	0.725	0.52
2	0.584	0.22	0.871	1.02
3	0.481	0.01	0.930	1.15
4	0.485	0.00	0.983	1.29
5	0.454	0.00	0.966	1.25
6	0.461	0.00	0.943	1.18
7	0.450	0.00	0.921	1.14
8	0.454	0.00	0.973	1.28
9	0.453	0.00	0.956	1.23
10	0.449	0.00	0.958	1.25

595* μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-5

Run #9

Cation Column, Haemoglobin

TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle,n	403 μ	y_H	403 μ	y_H
I.C.	0.827	1.00	0.003	0.00
1	0.405	0.49	0.405	0.49
2	0.256	0.31	0.782	0.95
3	0.193	0.23	0.901	1.09
4	0.088	0.11	0.816	0.99
5	0.056	0.07	0.812	0.98
6	0.048	0.06	0.865	1.05
7	0.037	0.45	0.877	1.06
8	0.039	0.05	0.802	0.97
9	0.033	0.04	0.759	0.92
10	0.030	0.04	0.749	0.91

Run #10

Anion Column, Albumin

TR (P_2) = 6.2, BR (P_3) = 4.0

Cycle,n	595* μ	y_A	595* μ	y_A
I.C.	0.459	0.00	1.137	1.00
1	0.995	1.05	0.831	0.60
2	1.089	0.92	0.677	0.33
3	1.292	1.35	0.622	0.25
4	1.232	1.28	0.509	0.05
5	1.250	1.32	0.488	0.02
6	1.225	1.20	0.497	0.05
7	1.228	1.20	0.479	0.02
8	1.228	1.47	0.490	0.05
9	1.126	1.04	0.496	0.07
10	1.119	1.03	0.499	0.08

595* μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-6

Run #11

Cation Column, Haemoglobin

TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle,n	403μ	y_H	403μ	y_H
I.C.	0.415	1.00	0.002	0.00
1	0.172	0.42	0.063	0.15
2	0.092	0.22	0.103	0.25
3	0.053	0.13	0.111	0.27
4	0.032	0.08	0.105	0.25
5	0.023	0.06	0.108	0.26
6	0.018	0.04	0.088	0.21
7	0.015	0.04	0.098	0.24
8	0.024	0.06	0.120	0.29
9	0.032	0.08	0.106	0.26
10	0.029	0.07	0.112	0.27

Run #12

Anion Column, Albumin

TR (P_2) = 6.2, BR (P_3) = 4.0

Cycle,n	595*μ	y_A	595*μ	y_A
I.C.	0.436	0.00	1.113	1.00
1	0.598	0.25	0.737	0.87
2	0.722	0.54	0.635	0.29
3	0.752	0.61	0.588	0.24
4	0.762	0.66	0.525	0.17
5	0.776	0.69	0.493	0.02
6	0.739	0.63	0.495	0.03
7	0.826	0.87	0.483	0.01
8	0.932	1.15	0.489	0.00
9	0.866	0.98	0.492	0.00
10	0.873	0.98	0.487	0.00

595*μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-7

Run #13

Cation Column, Haemoglobin
TR (P_1) = 8.5, BR (P_2) = 6.2

Cycle, n	403 μ	y_H	403 μ	y_H
I.C.	0.871	1.00	0.003	0.00
1	0.584	0.67	0.475	0.55
2	0.432	0.50	0.798	0.92
3	0.336	0.39	0.906	1.04
4	0.264	0.30	0.890	1.02
5	0.200	0.23	0.889	1.02
6	0.167	0.19	0.829	0.95
7	0.111	0.13	0.826	0.95
8	0.078	0.09	0.853	0.98
9	0.056	0.06	0.780	0.90
10	0.041	0.05	0.769	0.88

Table Ex-B-2.1

SUMMARY OF THE EXPERIMENTAL RESULTS

ONE-COLUMN: BATCH OPERATION

Haemoglobin-Albumin System

	Haemoglobin		Albumin	
Number of Cycles, n	Top y_H	Bottom y_H	Top y_A	Bottom y_A
1	0.881	1.053	---	---
2	0.840	1.122	---	---
3	0.797	1.131	---	---
4	0.775	1.140	---	---
5	0.763	1.165	0.996	---
6	0.754	1.145	0.998	0.955
7	0.744	1.160	---	---

Table Ex-B-2.2

SUMMARY OF THE EXPERIMENTAL RESULTS

ONE-COLUMN: BATCH OPERATION

Number of Cycles, n	Haemoglobin-Buffer		Albumin-Buffer	
	Anion-Column	Cation-Column	Anion-Column	Cation-Column
	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$	$\frac{\langle y_B \rangle_n}{\langle y_T \rangle_n}$	$\frac{\langle y_B \rangle_n}{\langle y_T \rangle_n}$
1	2.951	1.354	1.132	3.157
2	6.220	2.740	1.985	6.247
3	8.832	3.630	2.642	7.081
4	9.973	4.426	3.212	8.253
5	11.244	4.813	4.233	9.108

Table Ex-B-2.3

SUMMARY OF THE EXPERIMENTAL RESULTS

ONE-COLUMN: SEMI-CONTINUOUS OPERATION

Haemoglobin-Anion-Column

Case I. $P_1 = 8.0$ and $P_2 = 6.0$ Case II. $P_1 = 8.5$ and $P_2 = 6.2$

Number of Cycles, n	Solute at P_1 $\frac{y_{H\ n}}{y_{0\ n}}$	Solute at P_2 $\frac{y_{H\ n}}{y_{0\ n}}$	Solute at P_1 $\frac{y_{H\ n}}{y_{0\ n}}$	Solute at P_2 $\frac{y_{H\ n}}{y_{0\ n}}$
1	1.058	0.904	1.238	0.880
2	1.071	0.968	1.196	0.741
3	1.102	0.937	1.281	0.734
4	1.076	0.938	1.182	0.602

Table Ex-B-2.4

SUMMARY OF THE EXPERIMENTAL RESULTS

ONE-COLUMN: SEMI-CONTINUOUS OPERATION

Albumin-Anion-Column

	Case I. $P_2 = 6.0$ and $P_3 = 4.0$		Case II. $P_2 = 6.2$ and $P_3 = 4.0$	
Number of Cycles, n	Solute at P_2 $\frac{\langle y_A \rangle_n}{\langle y_0 \rangle_n}$	Solute at P_3 $\frac{\langle y_A \rangle_n}{\langle y_0 \rangle_n}$	Solute at P_2 $\frac{\langle y_A \rangle_n}{\langle y_0 \rangle_n}$	Solute at P_3 $\frac{\langle y_A \rangle_n}{\langle y_0 \rangle_n}$
1	1.265	0.941	1.620	0.906
2	1.350	0.942	1.870	0.793
3	1.440	0.925	1.651	0.622
4	1.490	1.470	1.810	0.842

Table Ex-B-2.5

SUMMARY OF THE EXPERIMENTAL RESULTS

ONE-COLUMN: SEMI-CONTINUOUS OPERATION

Haemoglobin-Cation-Column

Number of Cycles, n	1. $P_1 = 8.0$, $P_2 = 6.0$ $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$	2. $P_1 = 8.5$, $P_2 = 6.2$ $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	0.852	0.245
2	---	0.314
3	0.764	0.323
4	0.631	0.310
5	0.586	0.312
6	---	---
7	0.495	---
8	0.502	0.267
9	0.514	---

Exhibit B-3

Experimental Parameters For Two-Column System: Mode 1

Run	Feed (wt %)		Ionic conc. molarity, M					
			Top		Middle		Bottom	
	Haemoglobin	Albumin	Buffer	NaCl	Buffer	NaCl	Buffer	NaCl
14	0.02	0.02	0.2	0.1	0.1	0.05	0.1	0.05
15	0.01	0.01	0.2	0.1	0.1	0.05	0.1	0.05
16	0.01	0.01	0.2	0.1	0.1	0.05	0.1	0.05
17	0.02	0.02	0.25	0.1	0.1	0.05	0.1	0.05
18	0.02	0.02	0.2	0.1	0.1	0.05	0.1	0.05
19	0.02	0.02	0.2	0.1	0.1	0.05	0.1	0.05

For all run: Dead Volume $V_T = V_B = V_M = 30$ cc, Displacement 12 cc, Flow Rate 1 cc/min
Feed time 8 min, Circulation time 24 min.

NOTE: Run 18 and 19 are Semi-Continuous Process

TABLE B-8

Run #14

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.868	0.865	1.00	1.00	0.868	0.865	1.00	1.00	1.00	1.00
1	0.962	1.022	1.11	1.66	1.315	0.986	1.52	1.00	0.73	0.60
2	1.094	0.809	1.26	0.47	1.117	0.843	1.29	0.48	0.98	1.02
3	1.079	0.797	1.24	0.42	0.973	0.838	1.12	0.42	1.11	1.00
4	1.129	0.872	1.30	0.73	1.253	0.879	1.44	0.69	0.90	0.95
5	1.060	0.878	1.22	0.84	1.235	0.939	1.42	0.71	0.86	0.85
6	1.086	0.853	1.25	0.69	1.239	0.977	1.42	0.66	0.88	0.96
7	1.096	0.890	1.26	0.86	1.225	0.946	1.41	0.79	0.89	0.92

595*μ : 0.1 cc Sample/5.0 cc Dye

TABLE B-9

Run #15

TR (P_1) = 8.5 BR (P_1) = 8.5

Cycle,n	403μ	595$^*\mu$	y_H	y_A	403μ	595$^*\mu$	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.722	0.641	1.00	1.00	0.722	0.641	1.00	1.00	1.00	1.00
1	1.899	0.825	2.63	0.20	0.685	0.668	0.95	0.22	2.77	1.10
2	1.430	0.766	1.98	0.44	0.906	0.661	1.26	0.28	1.57	0.64
3	1.254	0.717	1.74	0.33	1.017	0.589	1.41	0.35	1.23	1.06
4	1.187	0.677	1.64	0.37	0.883	0.550	1.22	0.27	1.34	0.73
5	1.082	0.619	1.50	0.43	0.895	0.574	1.24	0.35	1.21	0.81
6	1.020	0.602	1.41	0.34	0.843	0.556	1.17	0.55	0.82	1.62
7	1.012	0.618	1.40	0.38	0.690	0.587	0.96	0.39	1.46	1.03

595$^*\mu$: 0.1 cc Sample/5.0 cc Dye

TABLE B-10

Run #16

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403μ	595$^*\mu$	y_H	y_A	403μ	595$^*\mu$	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.788	0.674	1.00	1.00	0.390	0.640	1.00	1.00	1.00	1.00
1	0.691	0.784	1.22	0.38	0.148	0.596	0.79	0.48	1.54	1.26
2	0.910	0.798	1.16	0.28	0.110	0.541	0.54	0.43	2.15	1.54
3	0.907	0.714	1.15	0.49	0.191	0.566	0.61	0.99	1.89	2.02
4	0.793	0.868	1.01	0.26	0.101	0.513	0.41	0.28	2.46	1.08
5	0.727	0.665	0.92	0.24	0.094	0.501	0.35	0.23	2.63	0.96
6	0.778	0.687	0.99	0.20	0.078	0.473	0.22	0.34	4.50	1.70
7	0.721	0.722	0.92	0.20	0.079	0.488	0.29	0.28	3.17	1.40

595$^*\mu$: 0.1 cc Sample/5.0 cc Dye

TABLE B-11

Run #17

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403μ	595$^*\mu$	y_H	y_A	403μ	595$^*\mu$	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.003	0.472	0.00	1.00	0.889	1.192	1.00	1.00	0.00	1.00
1	0.475	0.901	0.53	0.48	0.506	0.855	0.57	0.55	0.93	1.15
2	0.506	1.062	0.57	0.76	0.364	0.903	0.41	0.30	1.39	0.39
3	0.629	1.060	0.71	0.23	0.293	0.682	0.33	0.23	2.15	1.00
4	0.726	1.200	0.82	0.09	0.193	0.591	0.22	0.15	3.73	1.67
5	0.759	1.229	0.85	0.12	0.146	0.586	0.16	0.10	5.31	0.83
6	0.727	1.217	0.82	0.17	0.121	0.593	0.13	0.11	6.31	0.65
7	0.696	1.279	0.78	0.20	0.103	0.596	0.12	0.13	6.50	0.65
8	0.610	1.178	0.69	0.10	0.064	0.546	0.07	0.09	9.86	0.13
9	0.512	1.096	0.58	0.10	0.049	0.539	0.06	0.12	9.67	1.20
10	0.503	1.078	0.57	0.10	0.031	0.533	0.04	0.12	14.25	1.20

595$^*\mu$: 0.1 cc Sample/5.0 cc Dye

TABLE B-12

Run #18

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.434	1.492	1.00	0.00	0.589	1.411	1.00	1.00	1.00	1.00
1	0.351	1.520	0.81	0.64	0.138	0.830	0.23	1.02	3.52	1.59
2	0.348	1.508	0.80	1.05	0.064	0.663	0.11	1.01	7.27	0.96
3	0.269	1.462	0.62	0.91	0.025	0.521	0.04	0.95	15.50	1.04
4	0.268	1.411	0.62	1.19	0.026	0.491	0.04	0.78	15.50	0.66
5	0.242	1.355	0.56	1.23	0.015	0.456	0.03	0.87	18.67	0.71
6	0.220	1.423	0.51	1.24	0.013	0.488	0.02	1.09	25.50	0.88
7	0.239	1.456	0.55	1.44	0.027	0.524	0.04	0.89	13.37	0.62
8	0.227	1.167	0.52	1.25	0.024	0.524	0.04	0.89	13.00	0.71
9	0.235	1.358	0.54	1.01	0.026	0.505	0.04	0.95	13.50	0.94
10	0.229	1.298	0.53	1.09	0.021	0.493	0.04	0.95	13.25	0.87

595*μ : 0.1 cc Sample/5.0 cc Dye

TABLE B-13

Run #19

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403μ	595$^*\mu$	y_H	y_A	403μ	595$^*\mu$	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	1.072	0.840	1.00	1.00	1.072	0.840	1.00	1.00	1.00	1.00
1	1.918	1.088	1.79	1.51	0.335	0.710	0.31	1.50	5.77	0.99
2	1.392	0.908	1.30	0.00	0.364	0.756	0.34	1.06	3.82	-
3	1.171	0.821	1.09	0.12	0.274	0.946	0.25	0.81	4.36	6.75
4	1.019	0.726	0.95	0.00	0.179	0.771	0.17	0.46	5.59	-
5	0.886	0.639	0.83	0.22	0.170	0.791	0.16	0.13	5.19	0.59
6	0.889	0.692	0.83	0.28	0.201	0.813	0.19	0.34	4.37	1.21
7	0.872	0.673	0.813	0.23	0.227	0.846	0.21	0.32	3.87	1.39

595$^*\mu$: 0.1 cc Sample/5.0 cc Dye

Table Ex-B-3.1

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 1

BATCH OPERATION

Haemoglobin and Albumin

Number of Cycles, n	P_1= 8.5, P_2= 6.2 Haemoglobin $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$	P_1= 8.5, P_2= 6.2 Albumin $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$	P_2= 6.2, P_3= 4.0 Albumin $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	2.122	1.014	1.803
2	4.514	1.201	3.980
3	8.163	0.988	5.877
4	10.930	0.765	6.243
5	12.281	1.302	10.355

Exhibit B-4

Experimental Parameters For Four-Column System: Mode 2

Run	Feed (wt %) Haemoglobin	Albumin	Ionic Concentration in Molarity, M: Top (P_2) Buffer	Top (P_2) NaCl	Middle (P_1) Buffer	Middle (P_1) NaCl	Middle (P_3) Buffer	Middle (P_3) NaCl	Bottom (P_2) Buffer	Bottom (P_2) NaCl
20	0.02	0.02	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
21	0.02	0.02	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
22	0.01	0.01	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
23	0.02	0.02	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
24	0.01	0.01	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05

For all run: Dead Volume $V_T = V_B = V_M = 30$ cc, Displacement 12 cc, Flow Rate 1 cc/min
Feed time 8 min, Circulation time 24 min

NOTE: Run #23 and 24 are Semi-Continuous Process.

TABLE B-14

Run #20

TR (P_2) = 6.2 BR (P_2) = 6.2

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.003	0.451	0.00	0.00	0.003	0.451	0.00	0.00	----	-----
1	0.184	0.652	0.14	0.38	0.065	0.665	0.05	0.49	2.80	1.29
2	0.219	0.595	0.16	0.21	0.071	1.133	0.05	1.70	3.20	0.12
3	0.212	0.600	0.16	0.23	0.097	0.719	0.07	0.62	2.29	2.70
4	0.166	0.573	0.12	0.19	0.120	0.787	0.09	0.78	1.33	4.11
5	0.205	0.622	0.15	0.29	0.092	0.712	0.07	0.60	2.14	2.07
6	0.204	0.640	0.15	0.34	0.088	0.725	0.07	0.64	2.14	1.88
7	0.182	0.669	0.14	0.43	0.048	0.775	0.04	0.80	3.90	1.86
8	0.135	0.637	0.10	0.38	0.108	0.789	0.08	0.79	1.25	2.08
9	0.130	0.640	0.10	0.39	0.091	0.791	0.07	0.81	1.43	2.08
10	0.132	0.637	0.10	0.38	0.080	0.778	0.06	0.78	1.67	2.05

595* μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-14 (Cont'd)

Run #20

MR (P_1) = 8.5 MR (P_3) = 4.0

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A
I.C.	0.753	1.003	1.00	1.00	0.741	1.105	1.00	1.00
1	0.601	0.936	0.80	0.96	0.386	1.238	0.52	1.89
2	0.601	1.066	0.80	1.44	0.203	0.868	0.27	0.99
3	0.583	0.982	0.77	1.16	0.116	0.766	0.16	0.80
4	0.368	0.524	0.49	0.61	0.448	0.612	0.61	0.00
5	0.427	0.751	0.57	0.51	0.069	0.647	0.09	0.50
6	0.135	1.060	0.18	1.58	0.329	0.906	0.44	0.86
7	0.158	0.796	0.21	1.05	0.00	0.618	0.07	0.44
8	0.190	0.744	0.25	0.81	0.045	0.585	0.06	0.34
9	0.171	0.752	0.23	0.87	0.041	0.592	0.06	0.42
10	0.183	0.772	0.24	0.93	0.044	0.581	0.06	0.39

595* μ : 0.1 cc Sample/3.0 cc Dye

TABLE B-15

Run #21

TR (P_2) = 6.2 BR (P_2) = 6.2

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.002	0.459	0.00	0.00	0.002	0.459	0.00	0.00	----	----
1	0.181	0.541	0.19	0.14	0.091	0.603	0.10	0.55	1.90	3.93
2	0.206	0.581	0.23	0.36	0.115	0.593	0.12	0.40	1.92	1.54
3	0.220	0.600	0.23	0.32	0.109	0.589	0.12	0.40	1.92	1.25
4	0.212	0.624	0.23	0.41	0.120	0.676	0.13	0.70	1.77	1.71
5	0.192	0.592	0.21	0.32	0.093	0.604	0.10	0.46	2.10	1.44

595* μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-15 (Cont'd)

Run #21

MR (P_1) = 8.5 MR (P_3) = 4.0

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A
I.C.	0.712	1.029	1.00	1.00	0.607	0.988	1.00	1.00
1	0.620	0.944	0.87	0.84	0.157	0.779	0.26	0.96
2	0.514	0.887	0.72	0.79	0.099	0.689	0.16	0.72
3	0.449	0.815	0.63	0.63	0.074	0.596	0.12	0.43
4	0.322	0.748	0.45	0.58	0.072	0.601	0.12	0.45
5	0.302	0.763	0.42	0.66	0.051	0.591	0.08	0.44

595* μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-16

Run #22

TR (P_2) = 6.2 BR (P_2) = 6.2

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.778	1.540	1.00	1.00	0.894	1.420	1.00	1.00	1.00	1.00
1	1.031	1.658	1.33	0.58	0.776	1.313	0.87	1.39	1.53	2.40
2	1.128	1.694	1.45	0.37	0.605	1.178	0.68	1.63	2.13	4.41
3	1.092	1.672	1.40	0.14	0.404	0.956	0.45	1.63	3.11	3.98
4	1.060	1.597	1.36	0.08	0.357	0.980	0.40	1.70	3.40	21.25
5	0.833	1.535	1.07	0.05	0.301	0.909	0.34	1.79	3.15	35.80
6	9.839	1.527	1.08	0.10	0.258	0.864	0.29	1.63	3.72	16.30
7	0.642	1.420	0.83	0.15	0.173	0.789	0.19	1.58	4.37	10.53
8	0.549	1.349	0.71	0.15	0.191	0.900	0.21	1.56	3.38	10.40
9	0.466	1.278	0.60	0.11	0.202	0.938	0.23	1.48	2.61	13.45
10	0.454	1.259	0.58	0.10	0.197	0.925	0.22	1.46	2.64	14.60

595*μ: 0.2 cc Sample/3.0 cc Dye

TABLE B-16 (Cont'd)

Run #22

MR (P_1) = 8.5 MR (P_3) = 4.0

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A
I.C.	1.086	1.565	1.00	1.00	0.346	1.282	1.00	1.00
1	0.603	1.382	0.56	1.11	0.207	1.277	0.60	0.91
2	0.683	1.433	0.63	1.13	0.186	1.355	0.54	0.86
3	0.481	1.372	0.44	1.02	0.187	1.349	0.54	0.87
4	0.467	1.317	0.43	1.12	0.176	1.372	0.51	0.78
5	0.392	1.281	0.36	1.15	0.170	1.402	0.49	0.93
6	0.378	1.236	0.35	1.16	0.159	1.321	0.46	0.91
7	0.263	1.121	0.24	0.97	0.155	1.293	0.45	0.95
8	0.267	1.100	0.25	0.92	0.150	1.280	0.43	0.94
9	0.250	1.139	0.23	1.01	0.166	1.264	0.48	1.27
10	0.246	1.117	0.23	0.97	0.149	1.257	0.43	0.80

595* μ: 0.2 cc Sample/3.0 cc Dye

TABLE B-17

Run # 23

TR (P_2) = 6.2 BR (P_2) = 6.2

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.001	0.488	0.00	1.00	0.393	1.563	1.00	1.00	0.00	1.00
1	0.052	0.706	0.13	0.89	0.212	1.154	0.56	0.91	0.23	1.02
2	0.068	0.831	0.17	0.68	0.139	0.917	0.35	0.83	0.49	1.22
3	0.092	1.002	0.23	0.59	0.092	0.766	0.23	0.60	1.00	1.02
4	0.097	0.977	0.25	0.52	0.061	0.687	0.16	0.70	1.56	1.35
5	0.094	0.988	0.24	0.40	0.044	0.627	0.11	0.62	2.18	1.55
6	0.092	0.942	0.23	0.46	0.036	0.607	0.09	0.58	2.56	1.26
7	0.092	0.887	0.23	0.35	0.037	0.596	0.09	0.51	2.56	1.46
8	0.086	0.828	0.23	0.35	0.034	0.604	0.09	0.71	2.56	2.03
9	0.070	0.740	0.18	0.36	0.038	0.621	0.10	0.77	1.80	2.14
10	0.064	0.734	0.16	0.34	0.035	0.618	0.09	0.76	1.78	2.24

595*μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-17 (Cont'd)

Run # 23

MR (P_1) = 8.5 MR (P_3) = 4.0

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A
I.C.	1.151	1.589	1.00	1.00	0.923	1.603	1.00	0.00
1	0.509	1.215	0.41	0.85	0.794	1.465	0.86	0.28
2	0.302	1.006	0.25	0.66	0.733	1.311	0.79	0.47
3	0.245	0.938	0.20	0.58	0.612	1.184	0.66	0.73
4	0.209	0.891	0.17	0.53	0.526	1.095	0.57	0.67
5	0.185	0.899	0.15	0.56	0.426	0.969	0.46	0.70
6	0.215	0.860	0.18	0.47	0.350	0.954	0.38	0.62
7	0.191	0.863	0.16	0.50	0.307	0.867	0.33	0.52
8	0.184	---	0.15	---	0.252	0.867	0.27	0.42
9	0.158	0.825	0.13	0.46	0.211	0.816	0.23	0.30
10	0.154	0.821	0.13	0.45	0.209	0.802	0.23	0.30

595*μ: 0.1 cc Sample/3.0 cc Dye

TABLE B-18

Run #24

TR (P_2) = 6.2

BR (P_2) = 6.2

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$	$\frac{\langle y_A \rangle_B}{\langle y_A \rangle_T}$
I.C.	0.890	1.510	1.00	1.00	0.890	1.510	1.00	1.00	----	----
1	1.212	1.679	1.30	0.68	0.850	1.529	0.91	1.15	1.43	1.69
2	1.340	1.744	1.44	0.45	0.687	1.339	0.74	0.92	1.95	2.04
3	1.409	1.749	1.51	0.29	0.543	1.207	0.58	0.82	2.60	2.83
4	1.561	1.742	1.67	0.22	0.447	1.117	0.48	0.75	3.48	3.41
5	1.502	1.756	1.61	0.15	0.381	1.052	0.41	0.70	3.93	4.67
6	1.337	1.748	1.43	0.13	0.308	1.062	0.33	0.84	4.33	6.46
7	1.295	1.721	1.25	0.11	0.256	0.929	0.27	0.60	4.63	5.45
8	1.171	1.588	1.25	0.14	0.199	0.832	0.21	0.48	5.95	3.43
9	1.057	1.561	1.13	0.16	0.143	0.762	0.15	0.40	7.53	2.50
10	1.034	1.542	1.11	0.16	0.137	0.703	0.15	0.30	7.40	1.88

595*μ: 0.2 cc Sample/3.0 cc Dye

TABLE B-18 (Cont'd)

Run #24

TR (P_1) = 8.5 BR (P_3) = 4.0

Cycle,n	403μ	595$^*\mu$	y_H	y_A	403μ	595$^*\mu$	y_H	y_A
I.C.	1.300	1.604	1.00	1.00	0.434	1.492	1.00	1.00
1	0.606	1.450	0.46	1.22	0.222	1.171	0.51	0.96
2	0.275	0.987	0.21	0.68	0.134	0.864	0.31	0.52
3	0.202	0.970	0.15	0.71	0.082	0.726	0.19	0.35
4	0.169	0.930	0.13	0.67	0.043	0.573	0.09	0.13
5	0.168	0.815	0.13	0.47	0.031	0.552	0.07	0.11
6	0.180	0.869	0.14	0.55	0.027	0.537	0.06	0.09
7	0.173	0.897	0.13	0.60	0.023	0.518	0.05	0.06
8	0.176	0.799	0.13	0.44	0.032	0.548	0.07	0.10
9	0.154	0.832	0.12	0.51	0.026	0.509	0.06	0.03
10	0.147	0.815	0.11	0.49	0.024	0.521	0.06	0.07

595$^*\mu$: 0.2 cc Sample/3.0 cc Dye

Table Ex-B-4.1

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 2 BATCH OPERATION

Haemoglobin-Albumin

Number of Cycles, n	Case I. P_1=8.0, P_2=6.2, P_3=4.0				Case II. P_1=8.0, P_2=6.0, P_3=4.0				Case III. P_1=8.5, P_2=6.2, P_3=4.0			
	Top		Bottom		Top		Bottom		Top		Bottom	
	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_o}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$
1	0.483	0.374	0.132	0.051	0.554	0.251	0.141	0.108	0.842	0.601	0.364	0.152
2	0.798	---	0.151	0.048	0.398	0.262	0.167	0.130	0.804	0.784	0.442	0.354
3	0.774	0.751	0.152	0.152	0.434	0.393	0.150	0.125	---	---	---	---
4	0.603	0.613	0.197	0.110	0.463	0.402	0.145	0.123	---	---	---	---
5	---	---	0.145	0.085	0.458	0.434	0.205	0.109	0.943	0.808	0.382	0.341
6	0.851	0.796	0.170	0.143	---	---	---	---	---	---	---	---
7	0.775	0.692	0.217	0.187	---	---	---	---	---	---	---	---
8	0.824	0.801	0.102	0.078	---	---	---	---	0.948	0.930	0.478	0.352
9	0.875	0.770	0.215	0.096	---	---	---	---	---		---	---
10	0.915	0.771	0.224	0.082	---	---	---	---	---		---	---

Table Ex-B-4.2

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 2 SEMI-CONTINUOUS OPERATION

Haemoglobin

	Case I.				Case II.			
	1. P_1=8.0, P_2=6.0		2. P_1=8.0, P_2= 6.2		1. P_1=8.5, P_2=6.0		2. P_1=8.5, P_2=6.2	
Number of Cycles, n	Top $\frac{\langle y \rangle_n}{y_0}$	Bottom $\frac{\langle y \rangle_n}{y_0}$	Top $\frac{\langle y \rangle_n}{y_0}$	Bottom $\frac{\langle y \rangle_n}{y_0}$	Top $\frac{\langle y \rangle_n}{y_0}$	Bottom $\frac{\langle y \rangle_n}{y_0}$	Top $\frac{\langle y \rangle_n}{y_0}$	Bottom $\frac{\langle y \rangle_n}{y_0}$
1	0.985	0.560	1.200	0.305	1.425	0.754	---	0.555
2	0.945	0.235	1.201	0.301	1.042	0.521	1.470	0.450
3	0.810	0.202	1.135	0.115	0.865	0.497	1.631	0.323
4	---	---	---	---	1.133	0.305	1.354	0.115
5	---	---	---	---	0.804	0.325	1.313	0.144
6	---	---	---	---	0.815	0.172	1.325	0.185
7	---	---	---	---	0.741	---	1.010	0.023

Table Ex-B-4.3

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 2 SEMI-CONTINUOUS OPERATION

Albumin

Number of Cycles, n	1. P_2= 6.0, P_3= 4.0 Top $\frac{\langle y \rangle_n}{y_0}$	1. P_2= 6.0, P_3= 4.0 Bottom $\frac{\langle y \rangle_n}{y_0}$	2. P_2= 6.2, P_3= 4.0 Top $\frac{\langle y \rangle_n}{y_0}$	2. P_2= 6.2, P_3= 4.0 Bottom $\frac{\langle y \rangle_n}{y_0}$
1	0.955	1.144	1.210	1.335
2	0.987	1.530	0.685	1.650
3	0.835	1.734	0.716	1.643
4	0.940	1.835	0.740	1.871
5	0.624	1.510	0.501	1.442
6	0.774	1.614	0.451	1.116
7	0.671	1.533	0.450	1.175
8	0.698	1.555	0.482	---

Table Ex-B-4.4

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 2 SEMI-CONTINUOUS OPERATION

Haemoglobin-Albumin

1. P_1=8.0, P_2=6.0 and P_3=4.0 ; 2. P_1=8.5, P_2=6.2 and P_3=4.0

Number of Cycles, n	Top		Bottom		Top		Bottom	
	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$	$\frac{y_H}{y_0}$	$\frac{y_A}{y_0}$
1	1.193	0.765	0.713	1.179	1.495	0.545	0.440	1.312
2	1.315	0.785	0.733	1.165	1.565	0.395	0.415	1.490
3	1.390	0.572	0.430	1.345	1.602	0.282	0.201	---
4	1.435	0.497	0.389	1.363	---	0.221	0.278	---
5	1.405	0.379	0.415	1.372	1.485	0.095	---	1.432
6	1.315	0.307	0.351	1.361	1.589	0.174	0.154	1.504
7	1.494	0.255	0.343	1.501	1.732	0.144	0.211	1.673

Exhibit B-5

Experimental Parameters For Two-Column System: Mode 3

Run	Feed (wt %) Haemoglobin	Albumin	Ionic Concentration in Molarity, M Top (P_1) Buffer	NaCl	Top (P_3) Buffer	NaCl	Middle (P_2) Buffer	NaCl	Bottom (P_1) Buffer	NaCl	Bottom (P_3) Buffer	NaCl
25	0.02	0.02	0.15	0.05	0.15	0.05	0.15	0.05	0.15	0.05	0.15	0.05
26	0.02	0.02	0.20	0.05	0.20	0.05	0.20	0.05	0.20	0.05	0.20	0.05
27	0.01	0.01	0.20	0.05	0.20	0.40	0.20	0.05	0.20	0.05	0.20	0.05
28	0.02	0.02	0.05	0.05	0.20	0.40	0.15	0.05	0.20	0.40	0.05	0.05

For all run: Dead Volume $V_T = V_B = V_M = 30$ cc, Displacement 12 cc, Flow Rate 1 cc/min
Circulation time 24 min.

TABLE B-19

Run #25

TR (P_1) = 8.5 BR (P_1) = 8.5

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A	$\frac{\langle y_H \rangle_B}{\langle y_H \rangle_T}$
I.C.	0.967	1.237	1.00	1.00	0.967	1.237	1.00	1.00	1.00
1	0.793	0.999	0.85	0.61	1.122	1.365	1.30	1.19	1.53
2	1.073	0.624	1.14	0.00	1.382	1.478	1.47	1.34	1.29
3	0.443	0.868	0.47	0.60	1.472	1.530	1.57	1.39	3.34
4	0.550	0.828	0.59	0.38	1.488	1.529	1.59	1.37	2.69
5	0.331	0.808	0.37	0.55	1.592	1.520	1.70	1.24	4.59
6	0.363	0.759	0.39	0.38	1.585	1.518	1.69	1.24	4.33
7	0.258	0.741	0.28	0.44	1.517	1.432	1.62	1.07	5.79
8	0.205	0.593	0.21	0.09	1.380	1.341	1.47	0/96	7.00

595* μ: 0.2 cc Sample/5.0 cc Dye

TABLE B-19 (Cont'd)

Run #25

TR (P_3) = 4.0

BR (P_3) = 4.0

Cycle,n	403μ	595$^*\mu$	y_H	y_A	403μ	595$^*\mu$	y_H	y_A	$\frac{\langle y_A \rangle_T}{\langle y_A \rangle_B}$
I.C.	0.475	1.294	1.00	1.00	0.475	1.294	1.00	1.00	1.00
1	0.339	1.151	0.71	0.94	0.294	0.744	0.62	0.01	94.00
2	0.269	1.252	0.57	1.34	0.035	0.431	0.07	0.001	1340.00
3	0.199	1.240	0.48	1.40	0.011	0.515	0.02	0.05	28.00
4	0.182	1.202	0.48	1.40	0.014	0.499	0.03	0.0001	14000
5	0.174	1.014	0.37	1.40	0.011	0.459	0.02	0.0001	14000
6	0.310	1.320	0.65	1.42	0.123	0.490	0.03	0.0001	14200
7	0.188	1.308	0.40	1.65	1.013	0.443	0.03	0.0001	16500
8	0.183	0.807	0.39	0.05	0.012	0.486	0.03	0.0001	500.00

595$^*\mu$: 0.2 cc Sample/5.00 cc Dye

TABLE B-19 (Cont'd)

Run #25

MR (P_2) = 6.2

Cycle,n	403μ	595$^{*}\mu$	y_H	y_A
I.C.	1.203	1.259	1.00	1.00
1	0.566	1.243	0.51	1.64
2	0.691	1.246	0.63	1.55
3	0.245	0.842	0.22	0.78
4	0.190	0.896	0.17	0.98
5	0.238	0.882	0.22	0.091
6	0.150	0.677	0.14	0.41
7	0.129	0.669	0.12	0.37
8	0.081	0.612	0.07	0.28

595$^{*}\mu$: 0.2 cc Sample/5.0 cc Dye

TABLE B-20

Run #26

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403μ	595$^*\mu$	y_H	y_A	403μ	595$^*\mu$	y_H	y_A	$\frac{\langle y_H \rangle_B}{\langle y_H \rangle_T}$
I.C.	0.892	1.239	1.00	1.00	0.892	1.239	1.00	1.00	1.00
1	0.851	1.182	0.95	0.90	1.278	1.517	1.43	1.29	1.51
2	0.744	1.112	0.83	0.85	1.442	1.581	1.62	1.27	1.95
3	0.632	1.012	0.71	0.71	1.496	1.498	1.68	1.01	2.37
4	0.555	0.912	0.62	0.54	1.440	1.657	1.61	1.46	2.60
5	0.485	0.869	0.51	0.54	1.438	1.652	1.61	1.45	3.16
6	0.438	0.845	0.49	0.50	1.356	1.572	1.52	1.34	3.10
7	0.392	0.767	0.44	0.35	1.291	1.445	1.45	1.10	3.30
8	0.350	0.804	0.39	0.49	1.185	1.481	1.33	1.29	3.41
9	0.281	0.755	0.33	0.43	1.110	1.422	1.24	1.22	3.76
10	0.284	0.749	0.32	0.42	1.107	1.420	1.24	1.22	3.88

595$^*\mu$: 0.2 cc Sample/5.0 cc Dye

TABLE B-20 (Cont'd)

Run #26

TR (P_3) = 4.0

BR (P_3) = 4.0

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_A \rangle_T}{\langle y_A \rangle_B}$
I.C.	0.530	1.222	1.00	1.00	0.530	1.222	1.00	1.00	1.00
1	0.347	1.192	0.66	1.26	0.236	0.897	0.45	0.70	1.80
2	0.277	1.183	0.52	1.37	0.077	0.673	0.15	0.42	3.26
3	0.245	1.156	0.46	1.36	0.043	0.531	0.08	0.11	12.36
4	0.220	1.150	0.42	1.39	0.029	0.541	0.06	0.16	8.69
5	0.196	1.072	0.37	1.23	0.020	0.511	0.04	0.10	12.30
6	0.186	1.085	0.35	1.28	0.013	0.503	0.03	0.09	14.22
7	0.171	1.026	0.32	1.16	0.012	0.508	0.02	0.11	10.55
8	0.131	0.941	0.25	1.01	0.013	0.499	0.03	0.08	12.63
9	0.149	0.921	0.28	0.93	0.012	0.493	0.02	0.07	13.29
10	0.135	0.919	0.26	0.95	0.012	0.495	0.02	0.07	13.57

595*μ : 0.2 cc Sample/5.0 cc Dye

TABLE B-20 (Cont'd)

Run #26

MR (P_2) = 6.2

Cycle,n	403μ	595$^{*}\mu$	y_H	y_A
I.C.	1.222	1.128	1.00	1.00
1	0.454	0.908	0.36	1.06
2	0.197	0.774	0.16	0.86
3	0.154	0.649	0.12	0.64
4	0.156	0.663	0.12	0.53
5	0.154	0.691	0.12	0.63
6	0.153	0.645	0.12	0.48
7	0.157	0.660	0.12	0.52
8	0.173	0.741	0.14	0.78
9	0.132	0.687	0.11	0.64
10	0.142	0.650	0.11	0.50

595$^{*}\mu$: 0.2 cc Sample/5.0 cc Dye

TABLE B-21

Run #27

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A	$\frac{\langle y_H \rangle_B}{\langle y_H \rangle_T}$
I.C.	0.823	1.243	1.00	1.00	0.818	0.851	1.00	0.00	1.00
1	0.766	1.227	0.93	1.03	1.067	1.354	1.30.	1.00	1.40
2	0.629	1.156	0.76	1.01	1.093	1.362	1.33	0.97	1.75
3	0.512	1.082	0.62	0.95	1.349	1.427	1.64	0.85	2.65
4	0.418	0.950	0.51	0.71	1.277	1.412	1.55	0.90	3.04
5	0.357	0.982	0.43	0.87	1.331	1.436	1.62	0.90	3.77
6	0.311	0.908	0.38	0.72	1.283	1.455	1.56	1.01	4.11
7	0.261	0.734	0.32	0.33	1.220	1.444	1.48	1.05	4.63
8	-----	0.827	----	----	1.158	1.364	1.41	0.92	-----
9	0.194	0.797	0.24	0.58	1.096	1.287	1.33	0.79	5.54
10	0.180	0.789	0.22	0.57	0.889	1.236	1.08	0.90	4.91

595* μ: 0.2 cc Sample/5.0 cc Dye

TABLE B-21 (Cont'd)

Run #27

TR (P_3) = 4.0

BR (P_3) = 4.0

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_A \rangle_T}{\langle y_A \rangle_B}$
I.C.	0.010	0.832	0.00	1.00	0.408	1.214	1.00	1.00	1.00
1	0.050	0.874	0.12	0.89	0.157	0.793	0.39	1.54	0.58
2	0.066	0.819	0.16	0.71	0.082	0.649	0.20	0.23	3.09
3	0.062	0.607	0.15	0.17	0.040	0.510	0.10	0.32	0.53
4	0.062	0.804	0.15	0.68	0.015	0.521	0.04	0.05	13.60
5	0.058	0.753	0.14	0.56	0.011	0.509	0.03	0.02	28.00
6	0.062	0.761	0.15	0.57	0.014	0.507	0.03	0.01	57.00
7	0.054	0.875	0.13	0.89	0.011	0.508	0.03	0.01	89.00
8	0.044	0.734	0.11	0.53	0.010	0.499	0.03	0.01	53.00
9	0.049	0.671	0.12	1.14	0.009	0.501	0.02	0.01	114.00
10	0.049	0.692	0.12	0.79	0.011	0.495	0.03	0.00	------

595*μ: 0.2 cc Sample/5.0 cc Dye

TABLE B-21(Cont'd)

Run #27

MR (P_2) = 6.2

Cycle,n	403μ	595$^*\mu$	y_H	y_A
I.C.	1.276	1.353	1.00	1.00
1	0.349	0.877	0.27	0.63
2	0.260	0.793	0.20	0.50
3	0.269	0.588	0.21	0.01
4	0.193	0.736	0.15	0.43
5	0.181	0.821	0.14	0.64
6	0.141	0.748	0.11	0.49
7	0.122	0.762	0.10	0.66
8	0.124	0.747	0.10	0.50
9	0.111	0.751	0.09	0.52
10	0.103	0.687	0.08	0.38

595$^*\mu$: 0.2 cc Sample/5.0 cc Dye

TABLE B-22

Run #28

TR (P_1) = 8.5

BR (P_1) = 8.5

Cycle,n	403 μ	595* μ	y_H	y_A	403 μ	595* μ	y_H	y_A	$\frac{\langle y_H \rangle_B}{\langle y_H \rangle_T}$
I.C	0.860	1.556	1.00	1.00	0.828	1.490	1.00	1.00	1.00
1	0.573	1.254	0.67	0.78	0.831	1.565	1.00	1.14	1.49
2	0.519	1.202	0.60	0.75	0.959	1.572	1.16	1.00	1.93
3	0.413	1.050	0.48	0.60	1.079	1.671	1.30	1.05	2.71
4	0.337	0.965	0.39	0.54	1.165	1.715	1.40	1.04	3.59
5	0.276	0.921	0.32	0.53	1.101	1.696	1.33	1.07	4.16
6	0.248	0.864	0.29	0.46	1.099	1.706	1.33	1.09	4.59
7	0.207	0.852	0.24	0.48	0.968	1.587	1.17	1.02	4.88
8	0.174	0.762	0.20	0.36	0,945	1.588	1.14	1.05	5.70
9	0.156	0.813	0.18	0.46	0.803	1.541	0.97	1.12	5.39
10	0.147	0.775	0.17	0.41	0.725	1.549	0.88	1.23	5.18

595* μ: 0.2 cc Sample/3.0 cc Dye

TABLE B-22 (Cont'd)

Run #28

TR (P_3) = 4.0

BR (P_3) = 4.0

Cycle,n	403μ	595*μ	y_H	y_A	403μ	595*μ	y_H	y_A	$\frac{\langle y_H \rangle_B}{\langle y_H \rangle_T}$
I.C.	0.447	1.549	1.00	1.00	0.494	1.525	1.00	1.00	1.00
1	0.337	1.465	0.75	1.09	0.264	1.147	0.53	0.77	1.42
2	0.298	1.476	0.67	1.19	0.128	0.811	0.26	0.42	2.83
3	0.257	1.460	0.58	1.25	0.053	0.647	0.11	0.27	4.63
4	0.233	1.378	0.52	1.67	0.021	0.498	0.04	0.06	27.83
5	0.213	1.384	0.48	1.21	0.024	0.525	0.05	0.10	12.10
6	0.194	1.344	0.43	1.19	0.019	0.491	0.04	0.05	23.80
7	0.186	1.255	0.42	1.04	0.024	0.504	0.05	0.06	17.33
8	0.174	1.288	0.39	1.13	0.018	0.478	0.04	0.03	37.67
9	0.160	1.250	0.36	1.02	0.019	0.465	0.04	0.00	---
10	0.121	1.162	0.27	1.02	0.019	0.485	0.04	0.04	25.50

595*μ: 0.2 cc Sample/3.0 cc Dye

TABLE B-22 (Cont'd)

Run #28

MR (P_2) = 6.2

Cycle,n	403μ	595$^*\mu$	y_H	y_A
I.C.	1.168	1.530	1.00	1.00
1	0.763	1.541	0.65	1.36
2	0.440	1.360	0.38	1.30
3	0.325	1.206	0.28	1.11
4	0.220	0.977	0.19	0.79
5	0.239	1.063	0.21	0.93
6	0.221	1.013	0.19	0.85
7	0.128	0.862	0.11	0.65
8	0.130	0.918	0.11	0.75
9	0.158	0.963	0.14	0.95
10	0.131	0.842	0.11	0.68

595$^*\mu$: 0.2 cc Sample/3.0 cc Dye

Table Ex-B-5.1

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 3 BATCH OPERATION

Haemoglobin-Albumin: P_1=8.5, P_2=6.2 and P_3=4.0

Number of Cycles, n	Case I. Dead Vol. = 0 cc. Top $\frac{y_H}{y_0}$	Case I. Top $\frac{y_A}{y_0}$	Case I. Bottom $\frac{y_H}{y_0}$	Case I. Bottom $\frac{y_A}{y_0}$	Case II. Dead Vol. = 30 cc. Top $\frac{y_H}{y_0}$	Case II. Top $\frac{y_A}{y_0}$	Case II. Bottom $\frac{y_H}{y_0}$	Case II. Bottom $\frac{y_A}{y_0}$	Case III. Dead Vol. = 40 cc. Top $\frac{y_H}{y_0}$	Case III. Top $\frac{y_A}{y_0}$	Case III. Bottom $\frac{y_H}{y_0}$	Case III. Bottom $\frac{y_A}{y_0}$
1	0.715	1.193	1.296	0.843	0.694	1.270	1.431	0.992	0.403	0.922	1.303	0.889
2	0.570	1.344	1.469	1.140	0.502	1.265	1.514	0.821	0.202	0.972	1.334	0.760
3	0.481	1.381	1.564	0.592	0.443	1.373	1.668	1.012	0.150	0.851	1.637	0.622
4	0.498	1.403	1.593	0.741	0.415	1.451	1.603	1.359	0.151	0.893	1.538	0.514
5	0.384	1.383	1.690	0.679	0.361	1.450	1.595	1.385	0.045	0.901	1.613	0.572
6	0.650	1.411	1.677	---	0.341	1.395	1.526	1.233	0.044	1.002	1.564	0.571
7	0.502	1.640	1.494	---	0.294	1.292	1.454	1.105	0.152	1.048	1.472	0.585
8	0.484	---	---	---	0.385	1.284	1.333	0.773	0.101	0.920	1.405	0.580
9	---	---	---	---	0.280	1.022	1.238	0.882	0.040	1.001	1.325	0.531
10	---	---	---	---	0.256	1.204	1.235	0.964	0.115	0.904	1.144	0.567

Exhibit B-6

Experimental Parameters For Two-Column System: Mode 4

Run	Feed (wt %) Haemoglobin	Albumin	Ionic Concentration in Molarity, M pH (P_1) = 8.5 Buffer	NaCl	pH (P_2) = 6.2 Buffer	NaCl	pH (P_3) = 4.0 Buffer	NaCl
29	0.015	0.015	0.10	0.05	0.15	0.05	----	----
30	0.010	0.010	0.10	0.00	0.15	0.05	----	----
31	0.020	0.020	0.15	0.05	0.20	0.05	----	----
32	0.020	0.020	0.15	0.00	0.20	0.05	----	----
33	0.020	0.020	----	----	0.15	0.05	0.15	0.05
34	0.020	0.020	----	----	0.15	0.05	0.15	0.05
35	0.020	0.020	----	----	0.15	0.05	0.15	0.05

For all run: Dead Volume $V_T = V_B = V_M$ = 30 cc, Displacement 12 cc, Flow Rate 1 cc/min, Feed time 8 min, Circulation time 24 min.

Note: Run #32 and #35 are Semi Countinuous Process.

TABLE B-23

Run #29

TR (P_1) = 8.5

BR (P_2) = 6.2

Cycle,n	403 μ	y_H	403 μ	y_H	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$
I.C.	0.000	0.00	0.797	1.00	0.00
1	0.445	0.57	0.606	0.76	0.75
2	0.698	0.88	0.411	0.52	1.69
3	0.847	1.06	0.305	0.38	2.79
4	0.858	1.08	0.244	0.31	3.48
5	0.711	0.89	0.173	0.22	4.05
6	0.838	1.05	0.134	0.17	6.18
7	0.729	0.92	0.133	0.17	5.41
8	0.706	0.89	0.111	0.14	6.36
9	0.605	0.76	0.097	0.12	6.33
10	0.598	0.75	0.084	0.11	6.82

TABLE B -24

Run #30

TR (P_1) = 8.5

BR (P_2) = 6.2

Cycle,n	403 μ	y_H	403 μ	y_H	$\frac{\langle y_H \rangle_B}{\langle y_H \rangle_B}$
I.C.	0.005	1.00	1.096	1.00	1.00
1	0.353	0.88	0.197	0.20	4.40
2	0.480	0.52	0.146	0.15	3.47
3	0.620	0.67	0.129	0.13	5.15
4	0.699	0.76	0.115	0.12	6.33
5	0.678	0.74	0.095	0.10	7.40
6	0.537	0.58	0.090	0.09	6.44
7	0.541	0.58	0.099	0.10	5.80
8	0.543	0.59	0.103	0.10	5.90
9	0.415	0.45	0.103	0.10	4.50
10	0.398	0.43	0.101	0.10	4.30

TABLE B-25

Run #31

TR (P_1) = 8.5 BR (P_2) = 6.2

Cycle,n	403 μ	y_H	403 μ	y_H	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$
I.C.	0.920	1.00	0.920	1.00	1.00
1	1.200	1.30	0.514	0.56	2.32
2	1.137	1.24	0.208	0.23	5.39
3	1.072	1.17	0.149	0.16	7.31

TABLE B-26

Run #32

TR (P_1) = 8.5 BR (P_2) = 6.2

Cycle,n	403 μ	y_H	403 μ	y_H	$\frac{\langle y_H \rangle_T}{\langle y_H \rangle_B}$
I.C.	0.751	1.00	0.920	1.00	1.00
1	1.210	1.61	0.256	0.28	5.75
2	1.250	1.66	0.196	0.21	7.90
3	0.962	1.28	0.090	0.10	12.80

TABLE B-27

Run #33

TR (P_2) = 6.2

BR (P_3) = 4.0

Cycle,n	$595^{*}\mu$	y_A	$595^{*}\mu$	y_A	$\frac{\langle y_A \rangle_T}{\langle y_A \rangle_B}$
I.C.	0.657	1.00	0.657	1.00	1.00
1	0.807	1.54	0.646	0.96	1.60
2	0.651	0.98	0.567	0.67	1.46
3	0.614	0.84	0.576	0.71	1.18
4	0.640	0.94	0.586	0.74	1.27
5	0.548	0.61	0.518	0.50	1.22
6	0.590	0.76	0.507	0.46	1.65
7	0.569	0.68	0.507	0.46	1.48
8	0.571	0.69	0.513	0.48	1.44

$595^{*}\mu$: 0.1 cc Sample/5.0 cc Dye

TABLE B-28

Run #34

TR (P_2) = 6.2

BR (P_3) = 4.0

Cycle,n	$595^{*}\mu$	y_A	$595^{*}\mu$	y_A	$\frac{\langle y_A \rangle_T}{\langle y_A \rangle_B}$
I.C.	0.657	1.00	0.657	1.00	-----
1	0.696	1.14	0.718	0.76	1.50
2	0.836	1.65	0.802	0.56	2.95
3	0.837	1.65	0.870	0.46	3.59
4	0.949	2.06	0.890	0.18	11.44
5	0.802	1.53	0.775	0.41	3.73
6	0.825	1.61	0.687	0.33	4.88
7	0.807	1.54	0.705	0.35	4.40
8	0.815	1.57	-----	0.23	6.83

$595^{*}\mu$: 0.1 cc Sample/5.0 cc Dye

TABLE B-29

Run #35

TR (P_2) = 6.2 BR (P_3) = 4.0

Cycle, n	$595^{*}\mu$	y_A	$595^{*}\mu$	y_A	$\frac{\langle y_A \rangle_T}{\langle y_A \rangle_B}$
I.C.	0.631	1.00	0.631	1.00	1.00
1	0.534	0.61	0.442	0.24	2.54
2	0.427	0.18	0.372	0.00	--
3	0.517	0.54	0.437	0.22	2.45
4	0.543	0.65	0.438	0.23	2.83
5	0.493	0.45	0.427	0.18	2.50
6	0.499	0.47	0.442	0.24	1.96
7	0.530	0.60	0.448	0.27	2.22
8	0.514	0.53	0.439	0.23	2.30

$595^{*}\mu$: 0.1 cc Sample/5.0 cc Dye

Table Ex-B-6.1

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 4 BATCH OPERATION

Haemoglobin and Albumin

Number of Cycles, n	Haemoglobin P_1= 8.5, P_2= 6.2 $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$	Albumin P_2= 6.2, P_3= 4.0 $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	3.817	3.051
2	8.779	7.224
3	13.258	11.258
4	14.269	11.650
5	14.901	12.572

Table Ex-B-6.2

SUMMARY OF THE EXPERIMENTAL RESULTS

TWO-COLUMN SYSTEM: MODE 4 SEMI-CONTINUOUS OPERATION

Haemoglobin and Albumin

$P_1 = 8.5$ and $P_2 = 6.2$

Number of Cycles, n	Haemoglobin $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$	Albumin $\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	3.452	1.022
2	8.320	1.734
3	13.214	0.347
4	14.904	0.510
5	15.153	0.908

APPENDIX C

COMPUTER PROGRAM ON EQUILIBRIUM THEORY: SEPARATION OF MULTI-COMPONENT VIA pH-PARAMETRIC PUMPING

The following material is appended to this work to detail the computational operations discussed in Chapters II and V. There follow, in order,

Table C-1, Nomenclature for Computer Program Input and Output

Exhibit C-1, The Computer Program

Exhibit C-2, Sample Input

Exhibit C-3, Sample Output, First 5 Cycles of Operation

TABLE C-1

NOMENCLATURE FOR COMPUTER PROGRAM INPUT AND OUTPUT

Program Symbol	Text Symbol	Destination
YHCOL1	--	average solute concentration of a higher isoelectric point in the fluid phase for a column, duration t_I
YACOL2	--	average solute concentration of a lower isoelectric point in the fluid phase for a column, duration t_{II}
YHRS3	--	average solute concentration of a higher isoelectric point in the reservoir, duration t_{III}
YARS4	--	average solute concentration of a lower isoelectric point in the reservoir, duration t_{IV}
YHRSL5	y_{ML}	average solute concentration of a higher isoelectric point in the middle reservoir, ML, duration t_V
YARSL6	y_{ML}	average solute concentration of a lower isoelectric point in the middle reservoir, ML, duration t_{VI}
YHRSR7	y_{MR}	average solute concentration of a higher isoelectric point in the middle reservoir, MR, duration t_{VII}
YARSR8	y_{MR}	average solute concentration of a lower isoelectric point in the middle reservoir, MR, duration t_{VIII}
YHAO	y_0	initial solute concentration of a higher isoelectric point in an anion exchanger column
YAAO	y_0	initial solute concentration of a lower isoelectric point in an anion exchanger column
YHCO	y_0	initial solute concentration of a higher isoelectric point in a cation exchanger column

TABLE C-1 (Cont'd)

Program Symbol	Text Symbol	Designation
YACO	y_0	initial solute concentration of a lower isoelectric point in a cation exchanger column
YHO	y_0	solute concentration of a higher isoelectric point in the feed
YAO	y_0	solute concentration of a lower isoelectric point in the feed
V	V	volume of fluid phase in the column
VDEAD	V_B, V_T	reservoir dead volume
V_B	V	volume of solid phase in the column
HAKP1	$k^-_{p_1}$	anion exchanger equilibrium constant for a higher isoelectric point solute at pH = P_1 (high pH level)
HCKP1	$k^+_{p_1}$	cation exchanger equilibrium constant for a higher isoelectric point solute at pH = P_1 (high pH level)
AAKP2	$k^-_{p_2}$	anion exchanger equilibrium constant for a lower isoelectric point solute at pH = P_2 (middle pH level)
ACKP3	$k^+_{p_3}$	cation exchanger equilibrium constant for a lower isoelectric point solute at pH = P_3 (low pH level)
YHOO	y_0	initial solute concentration of a higher isoelectric point in the middle reservoir, MR
YAOO	y_0	initial solute concentration of a lower isoelectric point in the middle reservoir, MR
YHRS1(I=1) YARS1(I=1)	$\langle y_T \rangle_n$	the product(s) in the top reservoir
YHRS1(I=M) YARS1(I=M)	$\langle y_B \rangle_n$	the product(s) in the bottom reservoir

EXHIBIT C-1

```
$JOB
C       MULTI-COMPONENT VIA PH PARAMETRIC PUMPING



1           DIMENSION
           1YHCOL1(10,200),YHCOL2(10,200),YHCOL3(10,200),YHCOL4(10,200),
           2YHCOL5(10,200),YHCOL6(10,200),YHCOL7(10,200),YHCOL8(10,200),
           3YACOL1(10,200),YACOL2(10,200),YACOL3(10,200),YACOL4(10,200),
           4YACOL5(10,200),YACOL6(10,200),YACOL7(10,200),YACOL8(10,200),
2           DIMENSION
           1YHRS1(10,200),YHRS2(10,200),YHRS3(10,200),YHRS4(10,200),
           2YHRS5(10,200),YHRS6(10,200),YHRS7(10,200),YHRS8(10,200),
           3YARS1(10,200),YARS2(10,200),YARS3(10,200),YARS4(10,200),
           4YARS5(10,200),YARS6(10,200),YARS7(10,200),YARS8(10,200),
3           DIMENSION
           1YHRSL1(10,200),YHRSL2(10,200),YHRSL3(10,200),YHRSL4(10,200),
           2YHRSL5(10,200),YHRSL6(10,200),YHRSL7(10,200),YHRSL8(10,200),
           3YARSL1(10,200),YARSL2(10,200),YARSL3(10,200),YARSL4(10,200),
           4YARSL5(10,200),YARSL6(10,200),YARSL7(10,200),YARSL8(10,200),
4           DIMENSION
           1YHRSR1(10,200),YHRSR2(10,200),YHRSR3(10,200),YHRSR4(10,200),
           2YHRSR5(10,200),YHRSR6(10,200),YHRSR7(10,200),YHRSR8(10,200),
           3YARSR1(10,200),YARSR2(10,200),YARSR3(10,200),YARSR4(10,200),
           4YARSR5(10,200),YARSR6(10,200),YARSR7(10,200),YARSR8(10,200),
5           READ(5,10)N,NCYCL
```

```
      10 FORMAT(7I10)
         READ(5,20)YHAO,YAAO,YHCO,YACO,V,VDEAD,VB,
        1HAKP1,HCKP1,AAKP1,ACKP1,
        2HAKP2,HCKP2,AAKP2,ACKP2,
        3HAKP3,HCKP3,AAKP3,ACKP3,
      20 FORMAT(7F10.3)
         YHRS1(1,1)=1.0
         YARS1(1,1)=1.0
         YHCOL1(1,1)=YHAO
         YACOL1(1,1)=YAAO
         DO 50  I=2,M
         A=(-1)**I
         IF(A)51,51,52
      52 YHRSL1(I,1)=YHCOL1(I-1,1)
         YARSL1(I,1)=YACOL1(I-1,1)
         YHRSR1(I,1)=1.0
         YARSR1(I,1)=1.0
         YHCOL1(I,1)=YHAO
         YACOL1(I,1)=YACO
         GO TO 50
      51 YHRS1(I,1)=YHCOL1(I-1,1)
         YARS1(I,1)=YACOL1(I-1,1)
         YHCOL1(I,1)=YHAO
         YACOL1(I,1)=YAAO
      50 CONTINUE
         MM=M+1
         YHRS1(MM,1)=YHCO
         YARS1(MM,1)=YACO
         J=1
    2001 WRITE(6,3333)J
```

```
 3333 FORMAT(2X,'***J=',I10)
      YHRS2(1,J)=YHRS1(1,J)
      YARS2(1,J)=YARS1(1,J)
      I=1
      WRITE(6,2222)I
      WRITE(6,222)YHRS2(I,J),YARS2(I,J)
      DO 150 I=1,M
      A=(-1)**I
      IF(A)151,151,152
  151 YHCOL2(I,J)=(V*YHRS1(I,J)+VB*YHCOL1(I,J)*HAKP1)/(V+VB*HAKP2)
      YACOL2(I,J)=(V*YARS1(I,J)+VB*YACOL1(I,J)*AAKP1)/(V+VB*AAKP2)
      YHRSL2(I+1,J)=(VDEAD*YHRSL1(I+1,J)+V*YHCOL1(I,J))/(VDEAD+V)
      YARSL2(I+1,J)=(VDEAD*YARSL1(I+1,J)+V*YACOL1(I,J))/(VDEAD+V)
      YHRSR2(I+1,J)=YHRSR1(I+1,J)
      YARSR2(I+1,J)=YARSR1(I+1,J)
      II=I+1
      WRITE(6,2222)II
 2222 FORMAT(2X,'I=',I10)
      WRITE(6,111)YHRSL2(I+1,J),YARSL2(I+1,J),YHRSR2(I+1,J),
     XYARSR2(I+1,J)
  111 FORMAT(5X,'YHRSL2=',E20.5,'YARSL2=',E20.5,'YHRSR2=',E20.5,'
     @YARSR2=',E20.5)
      GO TO 150
  152 YHCOL2(I,J)=(V*YHRSR1(I,J)+VB*YHCOL1(I,J)*HCKP2)/(V+VB*HCKP3)
      YACOL2(I,J)=(V*YARSR1(I,J)+VB*YACOL1(I,J)*ACKP2)/(V+VB*ACKP3)
      YHRS2(I+1,J)=(VDEAD*YHRS1(I+1,J)+V*YHCOL1(I,J))/(VDEAD+V)
      YARS2(I+1,J)=(VDEAD*YARS1(I+1,J)+V*YACOL1(I,J))/(VDEAD+V)
      II=I+1
      WRITE(6,2222)II
      WRITE(6,222)YHRS2(I+1,J),YARS2(I+1,J)
  222 FORMAT(5X,'YHRS2=',E25.5,'YARS2=',E25.5)
```

```
150 CONTINUE
    DO 250 I=1,M
    A=(-1)**I
    IF(A)251,251,252
251 YHCOL3(I,J)=(VDEAD*YHRS2(I,J)+V*YHCOL2(I,J)+VB*HAKP2*YHCOL2(I,J))
   X/(VDEAD+V+VB*HAKP2)
    YACOL3(I,J)=(VDEAD*YARS2(I,J)+V*YACOL2(I,J)+VB*AAKP2*YACOL2(I,J))
   X/(VDEAD+V+VB*AAKP2)
    YHRS3(I,J)=YHCOL3(I,J)
    YARS3(I,J)=YACOL3(I,J)
    WRITE(6,2222)I
    WRITE(6,444)YHRS3(I,J),YARS3(I,J)
444 FORMAT(5X,'YHRS3=',E25.5,'YARS3=',E25.5)
    GO TO 250
252 YHCOL3(I,J)=(VDEAD*YHRSR2(I,J)+V*YHCOL2(I,J)+VB*HCKP3*YHCOL2(I,J))
   1/(VDEAD+V+VB*HCKP3)
    YACOL3(I,J)=(VDEAD*YARSR2(I,J)+V*YACOL2(I,J)+VB*ACKP3*YACOL2(I,J))
   1/(VDEAD+V+VB*ACKP3)
    YHRSR3(I,J)=YHCOL3(I,J)
    YARSR3(I,J)=YACOL3(I,J)
    YHRSL3(I,J)=YHRSL2(I,J)
    YARSL3(I,J)=YARSL2(I,J)
    WRITE(6,2222)I
    WRITE(6,333)YHRSR3(I,J),YARSR3(I,J),YHRSL3(I,J),YARSL3(I,J)
333 FORMAT(5X,'YHRSR3=',E20.5,'YARSR3=',E20.5,'YHRSL3=',E20.5,'YARSL3
   @=',E20.5)
250 CONTINUE
    YHRS3(MM,J)=YHRS2(MM,J)
    YARS3(MM,J)=YHRS2(MM,J)
```

```
      WRITE(6,2222)MM
      WRITE(6,555)YHRS3(MM,J),YARS3(MM,J)
  555 FORMAT(5X,'YHRS3=',E25.5,'YARS3=',E25.5)
      DO 350 I=1,M
      A=(-1)**I
      IF(A)351,351,352
  351 YHCOL4(I,J)=(V*YHRSR3(I+1,J)+VB*HAKP2*YHCOL3(I,J))/(V+VB*HAKP3)
      YACOL4(I,J)=(V*YARSR3(I+1,J)+VB*AAKP2*YACOL3(I,J))/(V+VB*AAKP3)
      YHRS4(I,J)=(YHRS3(I,J)*VDEAD+V*YHCOL3(I,J))/(V+VDEAD)
      YARS4(I,J)=(YHRS3(I,J)*VDEAD+V*YHCOL3(I,J))/(V+VDEAD)
      WRITE(6,2222)I
      WRITE(6,1111)YHRS4(I,J),YARS4(I,J)
 1111 FORMAT(5X,'YHRS4=',E25.5,'YARS4=',E25.5)
      GO TO 350
  352 YHCOL4(I,J)=(V*YHRSR3(I+1,J)+VB*HCKP3*YHCOL3(I,J))/(V+VB*HCKP2)
      YACOL4(I,J)=(V*YARS3(I+1,J)+VB*ACKP3*YACOL3(I,J))/(V+VB*ACKP2)
      YHRSR4(I,J)=YHRSR3(I,J)
      YARSR4(I,J)=YARSR3(I,J)
      YHRSL4(I,J)=YHRSL3(I,J)
      YARSL4(I,J)=YARSL3(I,J)
      WRITE(6,2222)I
      WRITE(6,666)YHRSR4(I,J),YARSR4(I,J),YHRSL4(I,J),YARSL4(I,J)
  666 FORMAT(5X,'YHRSR4=',E20.5,'YARSR4=',E20.5,'YHRSL4=',E20.5,'
     @YARSL4=',E20.5)
  350 CONTINUE
      YHRS4(MM,J)=YHRS3(MM,J)
      YARS4(MM,J)=YARS3(MM,J)
      WRITE(6,2222)MM
      WRITE(6,777)YHRS4(MM,J),YARS4(MM,J)
  777 FORMAT(5X,'YHRS4=',E25.5,'YARS4=',E25.5)
      DO 450 I=1,M
```

```
      A=(-1)**I
      IF(A)451,451,452
  451 YHRS5(I,J)=YHRS4(I,J)
      YARS5(I,J)=YARS4(I,J)
      IF(I-1)4510,4510,4520
 4510 WRITE(6,2222)I
      WRITE(6,999)YHRS5(I,J),YARS5(I,J)
 4520 YHCOL5(I,J)=(VDEAD*YHRSR4(I+1,J)+V*YHCOL4(I,J)+VB*HAKP3*YHCOL4
     @(I,J)/(V+VDEAD+VB*HAKP3)
      YACOL5(I,J)=(VDEAD*YARSR4(I+1,J)+V*YACOL4(I,J)+VB*AAKP3*YACOL4
     @(I,J))/(VDEAD+VB*AAKP3)
      YHRSR5(I+1,J)=YHCOL5(I,J)
      YARSR5(I+1,J)=YACOL5(I,J)
      YHRSL5(I+1,J)=YHRSL4(I+1,J)
      YARSL5(I+1,J)=YARSL4(I+1,J)
      II=I+1
      WRITE(6,2222)II
      WRITE(6,888)YHRSR5(I+1,J),YARSR5(I+1,J),
     @YHRSL5(I+1,J),YARSL5(I+1,J)
  888 FORMAT(5X,'YHRSR5=',E20.5,'YARSR5=',E20.5,'YHRSL5=',
     @E20.5,'YARSL5=',E20.5)
      GO TO 450
  452 YHCOL5(I,J)=(VDEAD*YHRS4(I+1,J)+V*YHCOL4(I,J)+VB*HCKP2*YHCOL4(I,J)
     @)/V+VDEAD+VB*HCKP2)
      YACOL5(I,J)=(VDEAD)*YARS4(I+1,J)+V*YACOL4(I,J)+VB*ACKP2*YACOL4(I,J)
     @)/(V+VDEAD+VB*ACKP2)
      YHRS5(I+1,J)=YHCOL5(I,J)
      YARS5(I+1,J)=YHCOL5(I,J)
      II=I+1
      WRITE(6,2222)II
      WRITE(6,999)YHRS5(I+1,J),YARS5(I+1,J)
```

```
141    999 FORMAT(5X,'YHRS5=',E25.5,'YARS5=',E25.5)
142    450 CONTINUE
143        DO 550 I=1,M
144        A=(-1)**I
145        IF(A)551,551,552
146    551 YHRS6(I,J)=YHRS5(I,J)
147        YARS6(I,J)=YARS5(I,J)
148        IF(I-1)5510,5510,5520
149   5510 WRITE(6,2222)I
150        WRITE(6,1002)WHRS6(I,J),YARS6(I,J)
151   5520 YHCOL6(I,J)=(V*YHRS5(I,J)+VB*HAKP3*YHCOL5(I,J)/(V+VB*HAKP2)
152        YACOL6(I,J)=(V*YARS5(I,J)+VB*AAKP3*YACOL5(I,J)/(V+VB*AAKP2)
153        YHRSR6(I+1,J)=(YHRSR5(I+1,J)*VDEAD+V*YHCOL5(I,J))(V+VDEAD)
154        YARSR6(I+1,J)=(YARSR5(I+1,J)*VDEAD+V*YACOL5(I,J))(V+VDEAD)
155        YHRSL6(I+1,J)=YHRSL5(I+1,J)
156        YARSL6(I+1,J)=YARSL5(I+1,J)
157        II=I+1
158        WRITE(6,2222)II
159        WRITE(6,1001)YHRSR6(I+1,J),YARSR6(I+1,J),
          @YHRSL6(I+1,J),YARSL6(I+1,J)
160   1001 FORMAT(5X,'YHRSR6='E20.5,
          @'YARSR6=',E20.5'YHRSL6='E20.5,'YARSL6=',E20.5)
161        GO TO 550
162    552 YHCOL6(I,J)=(V*YHRSL5(I,J)+VB*HCKP2*YHCOL5(I,J)/(V+VB*HCKP1)
163        YACOL6(I,J)=(V*YARSL5(I,J)+VB*ACKP2*YACOL5(I,J)/(V+VB*ACKP1)
164        YHRS6(I+1,J)=(VDEAD*YHRS5(I+1,J)+V*YHCOL5(I,J))(V+VDEAD)
165        YARS6(I+1,J)=(YARS5(I+1,J)*VDEAD+V*YACOL5(I,J))(V+VDEAD)
166        II=I+1
167        WRITE(6,2222)II
168        WRITE(6,1002)YHRS6(I+1,J),YARS6(I+1,J)
```

```
1002 FORMAT(5X,'YHRS6='E25.5,'YARS6=',E25.5
 550 CONTINUE
     DO 650 I=1,M
     A=(-1)**I
     IF(A)651,651,652
 651 YHCOL7(I,J)=(VDEAD*YHRS6(I,J)+V*YHCOL6(I,J)+VB*HAKP2*YHCOL6(I,J))/
    @(V+VDEAD+VB*HAKP2)
 651 YACOL7(I,J)=(VDEAD*YARS6(I,J)+V*YACOL6(I,J)+VB*AAKP2*YACOL6(I,J))/
    @(V+VDEAD+VB*AAKP2)
     YHRS7(I,J)=YHCOL7(I,J)
     YARS7(I,J)=YACOL7(I,J)
     WRITE(6,2222)I
     WRITE(6,1003)YHRS7(I,J),YARS7(I,J)
1003 FORMAT(5X,'YHRS7='E25.5,'YARS7='E25.5)
     GO TO 650
 652 YHCOL7(I,J)=(VDEAD*YHRSL6(I,J)+V*YHCOL6(I,J)+VB*HCKP1*YHCOL6(I,J))
    @/(V+VDEAD+VB*HCKP1)
 652 YACOL7(I,J)=(VDEAD*YARSL6(I,J)+V*YACOL6(I,J)+VB*ACKP1*YACOL6(I,J))
    @/(V+VDEAD+VB*ACKP1)
     YHRSL7(I,J)=YHCOL7(I,J)
     YARSL7(I,J)=YACOL7(I,J)
     YHRSR7(I,J)=YHRSR6(I,J)
     YHRSR7(I,J)=YARSR6(I,J)
     WRITE(6,2222)I
     WRITE(6,1004)YHRSL7(I,J),YARSL7(I,J),YHRSR7(I,J),YARSR7(I,J)
1004 FORMAT(5X,'YHRSL7=',E20.5,'YARSL7=',E20.5,'YHRSR7=',E20.5,
    @'YARSR7=',E20.5)
 650 CONTINUE
     YHRS7(MM,J)=YHRS6(MM,J)
     YARS7(MM,J)=YARS6(MM,J)
     WRITE(6,2222)MM
```

```
195         WRITE(6,1005)YHRS7(MM,J),YARS7(MM,J)
196    1005 FORMAT(5X,'YHRS7=',E25.5,'YARS7=',E25.5)
197         DO 750 I=1,M
198         A=(-1)**I
199         IF(A)751,751,752
200     751 YHCOL8(I,J)=(V*YHRSL7(I+1,J)+VB*HAKP2*YHCOL7(I,J))/V+VB*HAKP1)
201         YACOL8(I,J)=(V*YARSL7(I+1,J)+VB*AAKP2*YACOL7(I,J))/V+VB*AAKP1)
202         YHRS8(I,J)=(VDEAD*YHRS7(I,J)+V*YHCOL7(I,J))/(VDEAD+V)
203         YARS8(I,J)=(VDEAD*YARS7(I,J)+V*YACOL7(I,J))/(VDEAD+V)
204         WRITE(6,2222)I
205         WRITE(6,1006)YHRS8(I,J),YARS8(I,J)
206     1006 FORMAT(5X,'YHRS8=',E25.5,'YARS8=',E25.5)
207          GO TO 750
208      752 YHCOL8(I,J)=(V*YHRS7(I+1,J)+VB*HCKP1*YHCOL7(I,J))/(V+VB*HCKP2)
209         YACOL8(I,J)=(V*YARS7(I+1,J)+VB*ACKP1*YACOL7(I,J))/(V+VB*ACKP2)
210         YHRSL8(I,J)=YHRSL7(I,J)
211         YARSL8(I,J)=YARSL7(I,J)
212         YHRSR8(I,J)=YHRSR7(I,J)
213         YARSR8(I,J)=YARSR7(I,J)
214         WRITE(6,2222)I
215         WRITE(6,1007)YHRSL8(I,J),YARSL8(I,J),YHRSR8(I,J),YARSR8(I,J)
216     1007 FORMAT(5X,'YHRSL8=',E20.5,'YARSL8=',E20.5,'YHRSR8=',E20.5,
            @'YARSR8=',E20.5)
217      750 CONTINUE
218         YHRS8(MM,J)=YHRS7(MM,J)
219         YARS8(MM,J)=YARS7(MM,J)
```

```
      WRITE(6,2222)MM
      WRITE(6,1008)YHRS8(MM,J),YARS8(MM,J)
1008  FORMAT(5X,'YHRS8=',E25.5,'YARS8',E25.5)
      L=J
      J=J+1
      DO 850 I=1,M
      A=(-1)**I
      IF(A)851,851,852
 851  YHRS1(I,J)=YHRS8(I,L)
      YARS1(I,J)=YARS8(I,L)
      IF(I-1)8510,8510,8520
8510  WRITE(6,2222)I
      WRITE(6,1010)YHRS1(I,J),YARS1(I,J)
8520  YHCOL1(I,J)=(VDEAD*YHRSL8(I+1,L)+VB*HAKP1*YHCOL8(I,L)+V*YHCOL8(I,
     @L))/(V+VDEAD+VB*HAKP1)
      YACOL1(I,J)=(VDEAD*YARSL8(I+1,L)+VB*AAKP1*YACOL8(I,L)+V*YACOL8(I,
     @L))/(V+VDEAD+VB*AAKP1)
      YHRSL1(I+1,J)=YHCOL1(I,J)
      YARSL1(I+1,J)=YACOL1(I,J)
      YHRSR1(I+1,J)=YHRSR8(I+1,L)
      YARSR1(I+1,J)=YARSR8(I+1,L)
      II=I+1
      WRITE(6,2222)II
      WRITE(6,1009)YHRSL1(I+1,J),YARSL1(I+1,J),
     1YHRSR1(I+1,J),YARSR1(I+1,J)
1009  FORMAT(5X,'YHRSL1=',E20.5,
     @'YARSL1=',E20.5,'YHRSR1=',E20.5,'YARSR1=',E20.5)
      GO TO 850
 852  YHCOL1(I,J)=(VDEAD*YHRS8(I+1,L)+V*YHCOL8(I,L)+VB*HCKP2*YHCOL8(I,L)
     1)/(VDEAD+V+VB*HCKP2)
      YACOL1(I,J)=(VDEAD*YARS8(I+1,L)+V*YACOL8(I,L)+VB*ACKP2*YACOL8(I,J)
```

```
     1)/(VDEAD+V+VB*ACKP2)
246        YHRS1(I+1,J)=YHCOL1(I,J)
247        YARS1(I+1,J)=YACOL1(I,J)
248        II=I+1
249        WRITE(6,2222)II
250        WRITE(6,1010)YHRS1(I+1,J),YARS1(I+1,J)
251   1010 FORMAT(5X,'YHRS1=',E25.5,'YARS1=',E25.5)
252    850 CONTINUE
253        IF(J-NCYCL)2001,2000,2000
254   2000 STOP
255        END
```

EXHIBIT C-2

SAMPLE INPUT

M	NCYCL
4	30

YHAO	YAAO	YHCO	YACO	YHO	YAO	V	VDEAD	VB
1.000	1.000	0.497	0.660	0.692	0.941	30.000	30.000	20.000

HAKP1	HCKP1	AAKP1	ACKP1
2.500	1.500	4.000	1.300

HAKP2	HCKP2	AAKP2	ACKP2
0.600	3.000	4.000	1.500

HAKP3	HCKP3	AAKP3	ACKP3
0.300	4.000	2.000	4.500

M = 2

```
      $ENTRY
***J=          1
I=          1
   YHRS2=              0.10000E 01YARS2=              0.10000E 01
I=          2
   YHRSL2=          0.10000E 01YARSL2=          0.84100E 00YHRSR2=          0.10000E 01        YARSR2=          0.10000E 01
I=          3
   YHRS2=              0.85100E 00YARS2=              0.11670E 01
I=          1
   YHRS3=              0.12000E 01YARS3=              0.11269E 01
I=          2
   YHRSR3=          0.86949E 00YARSR3=          0.89463E 00YHRSL3=          0.10000E 01YARSL3 =          0.84100E 00
I=          3
   YHRS3=              0.85100E 00YARS3=              0.11670E 01
I=          1
   YHRS4=              0.12000E 01YARS4=              0.11269E 01
I=          2
   YHRSR4=          0.86949E 00YARSR4=          0.89463E 00YHRSL4=          0.10000E 01        YARSL4=          0.84100E 00
I=          3
   YHRS4=              0.85100E 00YARS4=              0.11670E 01
I=          1
   YHRS5=              0.12000E 01YARS5=              0.11269E 01
I=          2
   YHRSR5=          0.74753E 00YARSR5=          0.94551E 00YHRSL5=          0.10000E 01YARSL5=          0.84100E 00
I=          3
   YHRS5=              0.95813E 00YARS5=              0.12636E 01
I=          1
   YHRS6=              0.12000E 01YARS6=              0.11269E 01
I=          2
   YHRSR6=          0.74753E 00YARSR6=          0.94550E 00YHRSL6=          0.10000E 01YARSL6=          0.84100E 00
I=          3
   YHRS6=              0.95813E 00YARS6=              0.12636E 01
I=          1
   YHRS7=              0.13118E 01YARS7=              0.10803E 01
I=          2
```

```
   YHRSL7=          0.11666E 01YARSL7=          0.76193E 00YHRSR7=          0.74753E 00YARSR7=          0.94550E 00
I=          3
   YHRS7=               0.95813E 00YARS7=               0.12636E 01
I=          1
   YHRS8=               0.13118E 01YARS8=               0.10803E 01
I=          2
   YHRSL8=          0.11666E 01YARSL8=          0.76193E 00YHRSR8=          0.74753E 00YARSR8=          0.94550E 00
I=          3
   YHRS8=               0.95813E 00YARS8=               0.12636E 01
I=          1
   YHRS1=               0.13118E 01YARS1=               0.10803E 01
I=          2
   YHRSL1=          0.98227E 00YARSL1=          0.67582E 00YHRSR1=          0.74753E 00YARSR1=          0.94550E 00
I=          3
   YHRS1=               0.82135E 00YARS1=               0.13361E 01
***I=          2
I=          1
   YHRS2=               0.13118E 01YARS2=               0.10803E 01
I=          2
   YHRSL2=          0.98227E 00YARSL2=          0.67582E 00YHRSR2=          0.74753E 00       YARSR2=          0.94550E 00
I=          3
   YHRS2=               0.82135E 00YARS2=               0.13361E 01
I=          1
   YHRS3=               0.14808E 01YARS3=               0.11592E 01
I=          2
   YHRSR3=          0.67960E 00YARSR3=          0.86299E 00YHRSL3=          0.98227E 00YARSL3 =          0.67582E 00
I=          3
   YHRS3=               0.82135E 00YARS3=               0.13361E 01
I=          1
   YHRS4=               0.14808E 01YARS4=               0.11592E 01
I=          2
   YHRSR4=          0.67960E 00YARSR4=          0.86299E 00YHRSL4=          0.98227E 00       YARSL4=          0.67582E 00
I=          3
   YHRS4=               0.82135E 00YARS4=               0.13361E 01
I=          1
   YHRS5=               0.14808E 01YARS5=               0.11592E 01
I=          2
   YHRSR5=          0.62197E 00YARSR5=          0.91828E 00YHRSL5=          0.98227E 00YARSL5=          0.67582E 00
I=          3
   YHRS5=               0.87711E 00YARS5=               0.14117E 01
I=          1
   YHRS6=               0.14808E 01YARS6=               0.11592E 01
I=          2
   YHRSR6=          0.62197E 00YARSR6=          0.91828E 00YHRSL6=          0.98227E 00YARSL6=          0.67582E 00
I=          3
   YHRS6=               0.87711E 00YARS6=               0.14117E 01
I=          1
   YHRS7=               0.15336E 01YARS7=               0.11085E 01
I=          2
   YHRSL7=          0.11325E 01YARSL7=          0.64099E 00YHRSR7=          0.62197E 00YARSR7=          0.91828E 00
I=          3
   YHRS7=               0.87711E 00YARS7=               0.14117E 01
I=          1
   YHRS8=               0.15336E 01YARS8=               0.11085E 01
I=          2
   YHRSL8=          0.11325E 01YARSL8=          0.64099E 00YHRSR8=          0.62197E 00YARSR8=          0.91828E 00
I=          3
   YHRS8=               0.87711E 00YARS8=               0.14117E 01
```

```
I=          1
   YHRS1=          0.15336F 01YARS1=          0.11085F 01
I=          2
   YHRSL1=      0.97157E 00YARSL1=      0.58262E 00YHRSR1=      0.62197E 00YARSR1=      0.91828E 00
I=          3
   YHRS1=          0.75382F 00YARS1=          0.14436E 01
***J=       3
I=          1
   YHRS2=          0.15336F 01YARS2=          0.11085E 01
I=          2
   YHRSL2=      0.97157E 00YARSL2=      0.58282E 00YHRSR2=      0.62197E 00      YARSR2=      0.91828E 00
I=          3
   YHRS2=          0.75382E 00YARS2=          0.14436E 01
I=          1
   YHRS3=          0.16811E 01YARS3=          0.11618E 01
I=          2
   YHRSR3=      0.57672E 00YARSR3=      0.84887E 00YHRSL3=      0.97157E 00YARSL3 =      0.58282E 00
I=          3
   YHRS3=          0.75382E 00YARS3=          0.14436E 01
I=          1
   YHRS4=          0.16811E 01YARS4=          0.11618E 01
I=          2
   YHRSR4=      0.57672E 00YARSR4=      0.84887E 00YHRSL4=      0.97157E 00      YARSL4=      0.58282E 00
I=          3
   YHRS4=          0.75382E 00YARS4=          0.14436E 01
I=          1
   YHRS5=          0.16811E 01YARS5=          0.11618E 01
I=          2
   YHRSR5=      0.55730E 00YARSR5=      0.90513E 00YHRSL5=      0.97157E 00YARSL5=      0.58282E 00
I=          3
   YHRS5=          0.79096E 00YARS5=          0.15072E 01
I=          1
   YHRS6=          0.16811E 01YARS6=          0.11618E 01
I=          2
   YHRSR6=      0.55730E 00YARSR6=      0.90513E 00YHRSL6=      0.97157E 00YARSL6=      0.58282E 00
I=          3
   YHRS6=          0.79096E 00YARS6=          0.15072E 01
I=          1
   YHRS7=          0.16989E 01YARS7=          0.11103E 01
I=          2
   YHRSL7=      0.11040E 01YARSL7=      0.57379E 00YHRSR7=      0.55730E 00YARSR7=      0.90513E 00
I=          3
   YHRS7=          0.79096E 00YARS7=          0.15072E 01
I=          1
   YHRS8=          0.16989E 01YARS8=          0.11103E 01
I=          2
   YHRSL8=      0.11040E 01YARSL8=      0.57379E 00YHRSR8=      0.55730E 00YARSR8=      0.90513E 00
I=          3
   YHRS8=          0.79096E 00YARS8=          0.15072E 01
I=          1
   YHRS1=          0.16989F 01YARS1=          0.11103E 01
I=          2
   YHRSL1=      0.96131E 00YARSL1=      0.53014E 00YHRSR1=      0.55730E 00YARSR1=      0.90513E 00
I=          3
   YHRS1=          0.68225E 00YARS1=          0.15155E 01
***J=       4
I=          1
   YHRS2=          0.16989E 01YARS2=          0.11103E 01
```

```
I=          2
   YHRSL2=          0.96131E 00YARSL2=          0.53014E 00YHRSR2=          0.55730E 00        YARSR2=          0.90513E 00
I=          3
   YHRS2=               0.68225E 00YARS2=               0.15155E 01
I=          1
   YHRS3=               0.18297E 01YARS3=               0.11503E 01
I=          2
   YHRSR3=          0.51784E 00YARSR3=          0.84352E 00YHRSL3=          0.96131E 00YARSL3 =          0.53014E 00
I=          3
   YHRS3=               0.66225E 00YARS3=               0.15155E 01
I=          1
   YHRS4=               0.18297E 01YARS4=               0.11503E 01
I=          2
   YHRSR4=          0.51784E 00YARSR4=          0.84352E 00YHRSL4=          0.96131E 00        YARSL4=          0.53014E 00
I=          3
   YHRS4=               0.68225E 00YARS4=               0.15155E 01
I=          1
   YHRS5=               0.18297E 01YARS5=               0.11503E 01
I=          2
   YHRSR5=          0.52264E 00YARSR5=          0.89905E 00YHRSL5=          0.96131E 00YARSL5=          0.53014E 00
I=          3
   YHRS5=               0.71464E 00YARS5=               0.15720E 01
I=          1
   YHRS6=               0.18297E 01YARS6=               0.11503E 01
I=          2
   YHRSR6=          0.52264E 00YARSR6=          0.89905E 00YHRSL6=          0.96131E 00YARSL6=          0.53014E 00
I=          3
   YHRS6=               0.71464E 00YARS6=               0.15720E 01
I=          1
   YHRS7=               0.18253E 01YARS7=               0.10994E 01
I=          2
   YHRSL7=          0.10780E 01YARSL7=          0.53649E 00YHRSR7=          0.52264E 00YARSR7=          0.89905E 00
I=          3
   YHRS7=               0.71464E 00YARS7=               0.15720E 01
I=          1
   YHRS8=               0.18253E 01YARS8=               0.10994E 01
I=          2
   YHRSL8=          0.10780E 01YARSL8=          0.53649E 00YHRSR8=          0.52264E 00YARSR8=          0.89905E 00
I=          3
   YHRS8=               0.71464E 00YARS8=               0.15720E 01
I=          1
   YHRS1=               0.18253E 01YARS1=               0.10994E 01
I=          2
   YHRSL1=          0.95025E 00YARSL1=          0.50005E 00YHRSR1=          0.52264E 00YARSR1=          0.89905E 00
I=          3
   YHRS1=               0.61882E 00YARS1=               0.15662E 01
***J=          5
I=          1
   YHRS2=               0.18253E 01YARS2=               0.10994E 01
I=          2
   YHRSL2=          0.95025E 00YARSL2=          0.50005E 00YHRSR2=          0.52264E 00        YARSR2=          0.89905E 00
I=          3
   YHRS2=               0.61882E 00YARS2=               0.15662E 01
I=          1
   YHRS3=               0.19424E 01YARS3=               0.11328E 01
I=          2
   YHRSR3=          0.48228E 00YARSR3=          0.84233E 00YHRSL3=          0.95025E 00YARSL3 =          0.50005E 00
I=          3
```

```
I=          1
   YHRS4=          0.19424E 01YARS4=          0.11328E 01
I=          2
   YHRSR4=      0.48228E 00YARSR4=      0.84233E 00YHRSL4=      0.95025E 00      YARSL4=      0.50005E 00
I=          3
   YHRS4=          0.61882E 00YARS4=          0.15662E 01
I=          1
   YHRS5=          0.19424E 01YARS5=          0.11328E 01
I=          2
   YHRSR5=      0.50330E 00YARSR5=      0.89642E 00YHRSL5=      0.95025E 00YARSL5=      0.50005E 00
I=          3
   YHRS5=          0.65195E 00YARS5=          0.16182E 01
I=          1
   YHRS6=          0.19424E 01YARS6=          0.11328E 01
I=          2
   YHRSR6=      0.50330E 00YARSR6=      0.89642E 00YHRSL6=      0.95025E 00YARSL6=      0.50005E 00
I=          3
   YHRS6=          0.65195E 00YARS6=          0.16182E 01
I=          1
   YHRS7=          0.19231E 01YARS7=          0.10832E 01
I=          2
   YHRSL7=      0.10542E 01YARSL7=      0.51585E 00YHRSR7=      0.50330E 00YARSR7=      0.89642E 00
I=          3
   YHRS7=          0.65195E 00YARS7=          0.16182E 01
I=          1
   YHRS8=          0.19231E 01YARS8=          0.10832E 01
I=          2
   YHRSL8=      0.10542E 01YARSL8=      0.51585E 00YHRSR8=      0.50330E 00YARSR8=      0.89642E 00
I=          3
   YHRS8=          0.65195E 00YARS8=          0.16182E 01
I=          1
   YHRS1=          0.19231E 01YARS1=          0.10832E 01
I=          2
   YHRSL1=      0.93881E 00YARSL1=      0.48268E 00YHRSR1=      0.50330E 00YARSR1=      0.89642E 00
I=          3
   YHRS1=          0.56665E 00YARS1=          0.16037E 01
***J=       6
I=          1
   YHRS2=          0.19231E 01YARS2=          0.10832E 01
I=          2
   YHRSL2=      0.93881E 00YARSL2=      0.48268E 00YHRSR2=      0.50330E 00      YARSR2=      0.89642E 00
I=          3
   YHRS2=          0.56665E 00YARS2=          0.16037E 01
I=          1
   YHRS3=          0.20289E 01YARS3=          0.11136E 01
I=          2
   YHRSR3=      0.45975E 00YARSR3=      0.84296E 00YHRSL3=      0.93881E 00YARSL3 =      0.48268E 00
I=          3
   YHRS3=          0.56665E 00YARS3=          0.16037E 01
I=          1
   YHRS4=          0.20289E 01YARS4=          0.11136E 01
I=          2
   YHRSR4=      0.45975E 00YARSR4=      0.84296E 00YHRSL4=      0.93881E 00      YARSL4=      0.48268E 00
I=          3
   YHRS4=          0.56665E 00YARS4=          0.16037E 01
I=          1
   YHRS5=          0.20289E 01YARS5=          0.11136E 01
```

M = 4

```
      SENTRY
***J=          1
I=          1
   YHRS2=                0.10000E 01YARS2=                0.10000E 01
I=          2
   YHRSL2=          0.10000E 01YARSL2=          0.10000E 01YHRSR2=          0.10000E 01        YARSR2=          0.10000E 01
I=          3
   YHRS2=                0.10000E 01YARS2=                0.10000E 01
I=          4
   YHRSL2=          0.10000E 01YARSL2=          0.10000E 01YHRSR2=          0.10000E 01        YARSR2=          0.10000E 01
I=          5
   YHRS2=                0.10000E 01YARS2=                0.10000E 01
I=          1
   YHRS3=                0.11667E 01YARS3=                0.10000E 01
I=          2
   YHRSR3=          0.10000E 01YARSR3=          0.85714E 00YHRSL3=          0.10000E 01YARSL3 =          0.10000E 01
I=          3
   YHRS3=                0.11667E 01YARS3=                0.10000E 01
I=          4
   YHRSR3=          0.10000E 01YARSR3=          0.85714E 00YHRSL3=          0.10000E 01YARSL3 =          0.10000E 01
I=          5
   YHRS3=                0.10000E 01YARS3=                0.10000E 01
I=          1
   YHRS4=                0.11667E 01YARS4=                0.10000E 01
I=          2
   YHRSR4=          0.10000E 01YARSR4=          0.85714E 00YHRSL4=          0.10000E 01        YARSL4=          0.10000E 01
I=          3
   YHRS4=                0.11667E 01YARS4=                0.10000E 01
I=          4
   YHRSR4=          0.10000E 01YARSR4=          0.85714E 00YHRSL4=          0.10000E 01        YARSL4=          0.10000E 01
I=          5
   YHRS4=                0.10000E 01YARS4=                0.10000E 01
I=          1
   YHRS5=                0.11667E 01YARS5=                0.10000E 01
I=          2
   YHRSR5=          0.10139E 01YARSR5=          0.10357E 01YHRSL5=          0.10000E 01YARSL5=          0.10000E 01
I=          3
   YHRS5=                0.11309E 01YARS5=                0.11310E 01
I=          4
   YHRSR5=          0.10139E 01YARSR5=          0.10357E 01YHRSL5=          0.10000E 01YARSL5=          0.10000E 01
I=          5
   YHRS5=                0.10000E 01YARS5=                0.11310E 01
I=          1
   YHRS6=                0.11667E 01YARS6=                0.10000E 01
I=          2
   YHRSR6=          0.10139E 01YARSR6=          0.10357E 01YHRSL6=          0.10000E 01YARSL6=          0.10000E 01
```

```
I=           3
   YHRS6=              0.11472E 01YARS6=              0.10714E 01
I=           4
   YHRSR6=          0.10139E 01YARSR6=          0.10357E 01YHRSL6=          0.10000E 01YARSL6=          0.10000E 01
I=           5
   YHRS6=              0.10000E 01YARS6=              0.11310E 01
I=           1
   YHRS7=              0.11539E 01YARS7=              0.85969E 00
I=           2
   YHRSL7=          0.11994E 01YARSL7=          0.10109E 01YHRSR7=          0.10139E 01YARSR7=          0.10357E 01
I=           3
   YHRS7=              0.11539E 01YARS7=              0.85969E 00
I=           4
   YHRSL7=          0.11667E 01YARSL7=          0.10109E 01YHRSR7=          0.10139E 01YARSR7=          0.10357E 01
I=           5
   YHRS7=              0.10000E 01YARS7=              0.11310E 01
I=           1
   YHRS8=              0.11539E 01YARS8=              0.85969E 00
I=           2
   YHRSL8=          0.11994E 01YARSL8=          0.10109E 01YHRSR8=          0.10139E 01YARSR8=          0.10357E 01
I=           3
   YHRS8=              0.11539E 01YARS8=              0.85969E 00
I=           4
   YHRSL8=          0.11667E 01YARSL8=          0.10109E 01YHRSR8=          0.10139E 01YARSR8=          0.10357E 01
I=           5
   YHRS8=              0.10000E 01YARS8=              0.11310E 01
I=           1
   YHRS1=              0.11539E 01YARS1=              0.85969E 00
I=           2
   YHRSL1=          0.10248E 01YARSL1=          0.97851E 00YHRSR1=          0.10139E 01YARSR1=          0.10357E 01
I=           3
   YHRS1=              0.99233E 00YARS1=              0.87229E 00
I=           4
   YHRSL1=          0.99909E 00YARSL1=          0.97851E 00YHRSR1=          0.10139E 01YARSR1=          0.10357E 01
I=           5
   YHRS1=              0.86905E 00YARS1=              0.11209E 01
***J=        2
I=           1
   YHRS2=              0.11539E 01YARS2=              0.85969E 00
I=           2
   YHRSL2=          0.10248E 01YARSL2=          0.97851E 00YHRSR2=          0.10139E 01       YARSR2=          0.10357E 01
I=           3
   YHRS2=              0.10658E 01YARS2=              0.86657E 00
I=           4
   YHRSL2=          0.99909E 00YARSL2=          0.97851E 00YHRSR2=          0.10139E 01       YARSR2=          0.10357E 01
I=           5
   YHRS2=              0.86905E 00YARS2=              0.11209E 01
I=           1
   YHRS3=              0.13140E 01YARS3=              0.88515E 00
I=           2
   YHRSR3=          0.10093E 01YARSR3=          0.87608E 00YHRSL3=          0.10248E 01YARSL3 =          0.97851E 00
I=           3
   YHRS3=              0.12708E 01YARS3=              0.88761E 00
I=           4
   YHRSR3=          0.98285E 00YARSR3=          0.89384E 00YHRSL3=          0.99909E 00YARSL3 =          0.97851E 00
I=           5
   YHRS3=              0.86905E 00YARS3=              0.11209E 01
I=           1
```

```
 YHRS4=          0.13140E 01YARS4=          0.88515E 00
I=       2
 YHRSR4=        0.10093E 01YARSR4=         0.87608E 00YHRSL4=         0.10248E 01       YARSL4=         0.97851E 00
I=       3
 YHRS4=          0.12708E 01YARS4=          0.88761E 00
I=       4
 YHRSR4=        0.98285E 00YARSR4=         0.89384E 00YHRSL4=         0.99909E 00       YARSL4=         0.97851E 00
I=       5
 YHRS4=          0.86905E 00YARS4=          0.11209E 01
I=       1
 YHRS5=          0.13140E 01YARS5=          0.88515E 00
I=       2
 YHRSR5=        0.10347E 01YARSR5=         0.10244E 01YHRSL5=         0.10248E 01YARSL5=         0.97851E 00
I=       3
 YHRS5=          0.12148E 01YARS5=          0.10327E 01
I=       4
 YHRSR5=        0.10068E 01YARSR5=         0.10413E 01YHRSL5=         0.99909E 00YARSL5=         0.97851E 00
I=       5
 YHRS5=          0.89343E 00YARS5=          0.12510E 01
I=       1
 YHRS6=          0.13140E 01YARS6=          0.88515E 00
I=       2
 YHRSR6=        0.10347E 01YARSR6=         0.10244E 01YHRSL6=         0.10248E 01YARSL6=         0.97851E 00
I=       3
 YHRS6=          0.12402E 01YARS6=          0.96673E 00
I=       4
 YHRSR6=        0.10068E 01YARSR6=         0.10413E 01YHRSL6=         0.99909E 00YARSL6=         0.97851E 00
I=       5
 YHRS6=          0.89343E 00YARS6=          0.12510E 01
I=       1
 YHRS7=          0.12907E 01YARS7=          0.76865E 00
I=       2
 YHRSL7=        0.12431E 01YARSL7=         0.98302E 00YHRSR7=         0.10347E 01YARSR7=         0.10244E 01
I=       3
 YHRS7=          0.12488E 01YARS7=          0.77178E 00
I=       4
 YHRSL7=        0.11392E 01YARSL7=         0.10012E 01YHRSR7=         0.10068E 01YARSR7=         0.10413E 01
I=       5
 YHRS7=          0.89343E 00YARS7=          0.12510E 01
I=       1
 YHRS8=          0.12907E 01YARS8=          0.76864E 00
I=       2
 YHRSL8=        0.12431E 01YARSL8=         0.98302E 00YHRSR8=         0.10347E 01YARSR8=         0.10244E 01
I=       3
 YHRS8=          0.12488E 01YARS8=          0.77178E 00
I=       4
 YHRSL8=        0.11392E 01YARSL8=         0.10012E 01YHRSR8=         0.10068E 01YARSR8=         0.10413E 01
I=       5
 YHRS8=          0.89343E 00YARS8=          0.12510E 01
I=       1
 YHRS1=          0.12907E 01YARS1=          0.76864E 00
I=       2
 YHRSL1=        0.10689E 01YARSL1=         0.93708E 00YHRSR1=         0.10347E 01YARSR1=         0.10244E 01
I=       3
 YHRS1=          0.10700E 01YARS1=          0.78938E 00
I=       4
 YHRSL1=        0.98428E 00YARSL1=         0.95205E 00YHRSR1=         0.10068E 01YARSR1=         0.10413E 01
I=       5
```

```
   YHRS1=          0.78335E 00YARS1=          0.12302E 01
***J=      3
I=      1
   YHRS2=          0.12907E 01YARS2=          0.76864E 00
I=      2
   YHRSL2=      0.10689E 01YARSL2=      0.93708E 00YHRSR2=      0.10347E 01      YARSR2=      0.10244E 01
I=      3
   YHRS2=          0.11513E 01YARS2=          0.78138E 00
I=      4
   YHRSL2=      0.98428E 00YARSL2=      0.95205E 00YHRSR2=      0.10068E 01      YARSR2=      0.10413E 01
I=      5
   YHRS2=          0.78335E 00YARS2=          0.12302E 01
I=      1
   YHRS3=          0.14504E 01YARS3=          0.80474E 00
I=      2
   YHRSR3=      0.10422E 01YARSR3=      0.86124E 00YHRSL3=      0.10689E 01YARSL3 =      0.93708E 00
I=      3
   YHRS3=          0.13502E 01YARS3=          0.81384E 00
I=      4
   YHRSR3=      0.95895E 00YARSR3=      0.90600E 00YHRSL3=      0.98428E 00YARSL3 =      0.95205E 00
I=      5
   YHRS3=          0.78335E 00YARS3=          0.12302E 01
I=      1
   YHRS4=          0.14504E 01YARS4=          0.80474E 00
I=      2
   YHRSR4=      0.10422E 01YARSR4=      0.86124E 00YHRSL4=      0.10689E 01      YARSL4=      0.93708E 00
I=      3
   YHRS4=          0.13502E 01YARS4=          0.81384E 00
I=      4
   YHRSR4=      0.95895E 00YARSR4=      0.90600E 00YHRSL4=      0.98428E 00      YARSL4=      0.95205E 00
I=      5
   YHRS4=          0.78335E 00YARS4=          0.12302E 01
I=      1
   YHRS5=          0.14504E 01YARS5=          0.80474E 00
I=      2
   YHRSR5=      0.10762E 01YARSR5=      0.99065E 00YHRSL5=      0.10689E 01YARSL5=      0.93708E 00
I=      3
   YHRS5=          0.12842E 01YARS5=          0.96133E 00
I=      4
   YHRSR5=      0.99155E 00YARSR5=      0.10340E 01YHRSL5=      0.98428E 00YARSL5=      0.95205E 00
I=      5
   YHRS5=          0.82098E 00YARS5=          0.13542E 01
I=      1
   YHRS6=          0.14504E 01YARS6=          0.80474E 00
I=      2
   YHRSR6=      0.10762E 01YARSR6=      0.99065E 00YHRSL6=      0.10689E 01YARSL6=      0.93708E 00
I=      3
   YHRS6=          0.13142E 01YARS6=          0.89429E 00
I=      4
   YHRSR6=      0.99155E 00YARSR6=      0.10340E 01YHRSL6=      0.98428E 00YARSL6=      0.95205E 00
I=      5
   YHRS6=          0.82098E 00YARS6=          0.13542E 01
I=      1
   YHRS7=          0.14192E 01YARS7=          0.70305E 00
I=      2
   YHRSL7=      0.13009E 01YARSL7=      0.93910E 00YHRSR7=      0.10762E 01YARSR7=      0.99065E 00
I=      3
   YHRS7=          0.13203E 01YARS7=          0.71330E 00
```

```
I=          4
 YHRSL7=        0.11075E 01YARSL7=        0.98556E 00YHRSR7=        0.99155E 00YARSR7=        0.10340E 01
I=          5
 YHRS7=             0.82098E 00YARS7=             0.13542E 01
I=          1
 YHRS8=             0.14192E 01YARS8=             0.70305E 00
I=          2
 YHRSL8=        0.13009E 01YARSL8=        0.93910E 00YHRSR8=        0.10762E 01YARSR8=        0.99065E 00
I=          3
 YHRS8=             0.13203E 01YARS8=             0.71330E 00
I=          4
 YHRSL8=        0.11075E 01YARSL8=        0.98556E 00YHRSR8=        0.99155E 00YARSR8=        0.10340E 01
I=          5
 YHRS8=             0.82098E 00YARS8=             0.13542E 01
I=          1
 YHRS1=             0.14192E 01YARS1=             0.70305E 00
I=          2
 YHRSL1=        0.11235E 01YARSL1=        0.88852E 00YHRSR1=        0.10762E 01YARSR1=        0.99065E 00
I=          3
 YHRS1=             0.11303E 01YARS1=             0.73211E 00
I=          4
 YHRSL1=        0.96448E 00YARSL1=        0.92721E 00YHRSR1=        0.99155E 00YARSR1=        0.10340E 01
I=          5
 YHRS1=             0.72416E 00YARS1=             0.13234E 01
***J=        4
I=          1
 YHRS2=             0.14192E 01YARS2=             0.70305E 00
I=          2
 YHRSL2=        0.11235E 01YARSL2=        0.88852E 00YHRSR2=        0.10762E 01       YARSR2=        0.99065E 00
I=          3
 YHRS2=             0.12166E 01YARS2=             0.72356E 00
I=          4
 YHRSL2=        0.96448E 00YARSL2=        0.92721E 00YHRSR2=        0.99155E 00       YARSR2=        0.10340E 01
I=          5
 YHRS2=             0.72416E 00YARS2=             0.13234E 01
I=          1
 YHRS3=             0.15818E 01YARS3=             0.74280E 00
I=          2
 YHRSR3=        0.10878E 01YARSR3=        0.83066E 00YHRSL3=        0.11235E 01YARSL3 =       0.88852E 00
I=          3
 YHRS3=             0.14082E 01YARS3=             0.76280E 00
I=          4
 YHRSR3=        0.93425E 00YARSR3=        0.90692E 00YHRSL3=        0.96448E 00YARSL3 =       0.92721E 00
I=          5
 YHRS3=             0.72416E 00YARS3=             0.13234E 01
I=          1
 YHRS4=             0.15818E 01YARS4=             0.74280E 00
I=          2
 YHRSR4=        0.10878E 01YARSR4=        0.83066E 00YHRSL4=        0.11235E 01       YARSL4=        0.88852E 00
I=          3
 YHRS4=             0.14082E 01YARS4=             0.76280E 00
I=          4
 YHRSR4=        0.93425E 00YARSR4=        0.90692E 00YHRSL4=        0.96448E 00       YARSL4=        0.92721E 00
I=          5
 YHRS4=             0.72416E 00YARS4=             0.13234E 01
I=          1
 YHRS5=             0.15818E 01YARS5=             0.74280E 00
I=          2
```

```
   YHRSR5=          0.11290E 01YARSR5=          0.94714E 00YHRSL5=          0.11235E 01YARSL5=          0.88852E 00
I=         3
   YHRS5=               0.13395E 01YARS5=               0.90690E 00
I=         4
   YHRSR5=          0.97375E 00YARSR5=          0.10220E 01YHRSL5=          0.96448E 00YARSL5=          0.92721E 00
I=         5
   YHRS5=               0.76918E 00YARS5=               0.14399E 01
I=         1
   YHRS6=               0.15818E 01YARS6=               0.74280E 00
I=         2
   YHRSR6=          0.11290E 01YARSR6=          0.94714E 00YHRSL6=          0.11235E 01YARSL6=          0.88852E 00
I=         3
   YHRS6=               0.13707E 01YARS6=               0.84140E 00
I=         4
   YHRSR6=          0.97375E 00YARSR6=          0.10220E 01YHRSL6=          0.96448E 00YARSL6=          0.92721E 00
I=         5
   YHRS6=               0.76918E 00YARS6=               0.14399E 01
I=         1
   YHRS7=               0.15441E 01YARS7=               0.65128E 00
I=         2
   YHRSL7=          0.13647E 01YARSL7=          0.89005E 00YHRSR7=          0.11290E 01YARSR7=          0.94714E 00
I=         3
   YHRS7=               0.13720E 01YARS7=               0.67235E 00
I=         4
   YHRSL7=          0.10764E 01YARSL7=          0.96994E 00YHRSR7=          0.97375E 00YARSR7=          0.10220E 01
I=         5
   YHRS7=               0.76918E 00YARS7=               0.14399E 01
I=         1
   YHRS8=               0.15441E 01YARS8=               0.65128E 00
I=         2
   YHRSL8=          0.13647E 01YARSL8=          0.89005E 00YHRSR8=          0.11290E 01YARSR8=          0.94714E 00
I=         3
   YHRS8=               0.13720E 01YARS8=               0.67235E 00
I=         4
   YHRSL8=          0.10764E 01YARSL8=          0.96994E 00YHRSR8=          0.97375E 00YARSR8=          0.10220E 01
I=         5
   YHRS8=               0.76918E 00YARS8=               0.14399E 01
I=         1
   YHRS1=               0.15441E 01YARS1=               0.65128E 00
I=         2
   YHRSL1=          0.11826E 01YARSL1=          0.83888E 00YHRSR1=          0.11290E 01YARSR1=          0.94714E 00
I=         3
   YHRS1=               0.11755E 01YARS1=               0.69049E 00
I=         4
   YHRSL1=          0.94374E 00YARSL1=          0.90617E 00YHRSR1=          0.97375E 00YARSR1=          0.10220E 01
I=         5
   YHRS1=               0.68124E 00YARS1=               0.14007E 01
***J=       5
I=         1
   YHRS2=               0.15441E 01YARS2=               0.65128E 00
I=         2
   YHRSL2=          0.11826E 01YARSL2=          0.83888E 00YHRSR2=          0.11290E 01       YARSR2=          0.94714E 00
I=         3
   YHRS2=               0.12648E 01YARS2=               0.68224E 00
I=         4
   YHRSL2=          0.94374E 00YARSL2=          0.90617E 00YHRSR2=          0.97375E 00       YARSR2=          0.10220E 01
I=         5
   YHRS2=               0.68124E 00YARS2=               0.14007E 01
```

```
I=  1
 YHRS3=      0.17110E 01YARS3=      0.69148E 00
I=  2
 YHRSR3=    0.11389E 01YARSR3=     0.79350E 00YHRSL3=     0.11826E 01YARSL3 =     0.83888E 00
I=  3
 YHRS3=      0.14489E 01YARS3=      0.72598E 00
I=  4
 YHRSR3=    0.91107E 00YARSR3=     0.90309E 00YHRSL3=     0.94374E 00YARSL3 =     0.90617E 00
I=  5
 YHRS3=      0.68124E 00YARS3=      0.14007E 01
I=  1
 YHRS4=      0.17110E 01YARS4=      0.69148E 00
I=  2
 YHRSR4=    0.11389E 01YARSR4=     0.79350E 00YHRSL4=     0.11826E 01     YARSL4=     0.83888E 00
I=  3
 YHRS4=      0.14489E 01YARS4=      0.72598E 00
I=  4
 YHRSR4=    0.91107E 00YARSR4=     0.90309E 00YHRSL4=     0.94374E 00     YARSL4=     0.90617E 00
I=  5
 YHRS4=      0.68124E 00YARS4=      0.14007E 01
I=  1
 YHRS5=      0.17110E 01YARS5=      0.69148E 00
I=  2
 YHRSR5=    0.11866E 01YARSR5=     0.90024E 00YHRSL5=     0.11826E 01YARSL5=     0.83888E 00
I=  3
 YHRS5=      0.13825E 01YARS5=      0.86386E 00
I=  4
 YHRSR5=    0.95589E 00YARSR5=     0.10093E 01YHRSL5=     0.94374E 00YARSL5=     0.90617E 00
I=  5
 YHRS5=      0.73049E 00YARS5=      0.15098E 01
I=  1
 YHRS6=      0.17110E 01YARS6=      0.69148E 00
I=  2
 YHRSR6=    0.11866E 01YARSR6=     0.90024E 00YHRSL6=     0.11826E 01YARSL6=     0.83888E 00
I=  3
 YHRS6=      0.14127E 01YARS6=      0.80119E 00
I=  4
 YHRSR6=    0.95589E 00YARSR6=     0.10093E 01YHRSL6=     0.94374E 00YARSL6=     0.90617E 00
I=  5
 YHRS6=      0.73049E 00YARS6=      0.15098E 01
I=  1
 YHRS7=      0.16673E 01YARS7=      0.60761E 00
I=  2
 YHRSL7=    0.14297E 01YARSL7=     0.84096E 00YHRSR7=     0.11866E 01YARSR7=     0.90024E 00
I=  3
 YHRS7=      0.14078E 01YARS7=      0.64251E 00
I=  4
 YHRSL7=    0.10477E 01YARSL7=     0.95647E 00YHRSR7=     0.95589E 00YARSR7=     0.10093E 01
I=  5
 YHRS7=      0.73049E 00YARS7=      0.15098E 01
I=  1
 YHRS8=      0.16673E 01YARS8=      0.60761E 00
I=  2
 YHRSL8=    0.14297E 01YARSL8=     0.84096E 00YHRSR8=     0.11866E 01YARSR8=     0.90024E 00
I=  3
 YHRS8=      0.14078E 01YARS8=      0.64251E 00
I=  4
 YHRSL8=    0.10477E 01YARSL8=     0.95647E 00YHRSR8=     0.95589E 00YARSR8=     0.10093E 01
```

```
I=          5
   YHRS8=          0.73049E 00YARS8=          0.15098E 01
I=          1
   YHRS1=          0.16673E 01YARS1=          0.60761E 00
I=          2
   YHRSL1=      0.12424E 01YARSL1=      0.79096E 00YHRSR1=      0.11866E 01YARSR1=      0.90024E 00
I=          3
   YHRS1=          0.12083E 01YARS1=          0.65905E 00
I=          4
   YHRSL1=      0.92377E 00YARSL1=      0.88919E 00YHRSR1=      0.95589E 00YARSR1=      0.10093E 01
I=          5
   YHRS1=          0.64879E 00YARS1=          0.14637E 01
***J=       6
I=          1
   YHRS2=          0.16673E 01YARS2=          0.60761E 00
I=          2
   YHRSL2=      0.12424E 01YARSL2=      0.79096E 00YHRSR2=      0.11866E 01      YARSR2=      0.90024E 00
I=          3
   YHRS2=          0.12990E 01YARS2=          0.65153E 00
I=          4
   YHRSL2=      0.92377E 00YARSL2=      0.88919E 00YHRSR2=      0.95589E 00      YARSR2=      0.10093E 01
I=          5
   YHRS2=          0.64879E 00YARS2=          0.14637E 01
I=          1
   YHRS3=          0.18390E 01YARS3=          0.64690E 00
I=          2
   YHRSR3=      0.11913E 01YARSR3=      0.75441E 00YHRSL3=      0.12424E 01YARSL3 =      0.79096E 00
I=          3
   YHRS3=          0.14761E 01YARS3=          0.69859E 00
I=          4
   YHRSR3=      0.89008E 00YARSR3=      0.89759E 00YHRSL3=      0.92377E 00YARSL3 =      0.88919E 00
I=          5
   YHRS3=          0.64879E 00YARS3=          0.14637E 01
I=          1
   YHRS4=          0.18390E 01YARS4=          0.64690E 00
I=          2
   YHRSR4=      0.11913E 01YARSR4=      0.75441E 00YHRSL4=      0.12424E 01      YARSL4=      0.79096E 00
I=          3
   YHRS4=          0.14761E 01YARS4=          0.69859E 00
I=          4
   YHRSR4=      0.89008E 00YARSR4=      0.89759E 00YHRSL4=      0.92377E 00      YARSL4=      0.88919E 00
I=          5
   YHRS4=          0.64879E 00YARS4=          0.14637E 01
I=          1
   YHRS5=          0.18390E 01YARS5=          0.64690E 00
I=          2
   YHRSR5=      0.12452E 01YARSR5=      0.85327E 00YHRSL5=      0.12424E 01YARSL5=      0.79096E 00
I=          3
   YHRS5=          0.14151E 01YARS5=          0.82898E 00
I=          4
   YHRSR5=      0.93892E 00YARSR5=      0.99743E 00YHRSL5=      0.92377E 00YARSL5=      0.88919E 00
I=          5
   YHRS5=          0.70050E 00YARS5=          0.15661E 01
I=          1
   YHRS6=          0.18390E 01YARS6=          0.64690E 00
I=          2
   YHRSR6=      0.12452E 01YARSR6=      0.85326E 00YHRSL6=      0.12424E 01YARSL6=      0.79096E 00
I=          3
```

M = 6

```
        SENTRY
***J=          1
I=          1
   YHRS2=              0.10000E 01YARS2=              0.10000E 01
I=          2
   YHRSL2=          0.10000E 01YARSL2=          0.10000E 01YHRSR2=          0.10000E 01        YARSR2=          0.10000E 01
I=          3
   YHRS2=              0.10000E 01YARS2=              0.10000E 01
I=          4
   YHRSL2=          0.10000E 01YARSL2=          0.10000E 01YHRSR2=          0.10000E 01        YARSR2=          0.10000E 01
I=          5
   YHRS2=              0.10000E 01YARS2=              0.10000E 01
I=          6
   YHRSL2=          0.10000E 01YARSL2=          0.10000E 01YHRSR2=          0.10000E 01        YARSR2=          0.10000E 01
I=          7
   YHRS2=              0.10000E 01YARS2=              0.10000E 01
I=          1
   YHRS3=              0.11667E 01YARS3=              0.10000E 01
I=          2
   YHRSR3=          0.10000E 01YARSR3=          0.85714E 00YHRSL3=          0.10000E 01YARSL3 =          0.10000E 01
I=          3
   YHRS3=              0.11667E 01YARS3=              0.10000E 01
I=          4
   YHRSR3=          0.10000E 01YARSR3=          0.85714E 00YHRSL3=          0.10000E 01YARSL3 =          0.10000E 01
I=          5
   YHRS3=              0.11667E 01YARS3=              0.10000E 01
I=          6
   YHRSR3=          0.10000E 01YARSR3=          0.85714E 00YHRSL3=          0.10000E 01YARSL3 =          0.10000E 01
I=          7
   YHRS3=              0.10000E 01YARS3=              0.10000E 01
I=          1
   YHRS4=              0.11667E 01YARS4=              0.10000E 01
I=          2
   YHRSR4=          0.10000E 01YARSR4=          0.85714E 00YHRSL4=          0.10000E 01        YARSL4=          0.10000E 01
I=          3
   YHRS4=              0.11667E 01YARS4=              0.10000E 01
I=          4
   YHRSR4=          0.10000E 01YARSR4=          0.85714E 00YHRSL4=          0.10000E 01        YARSL4=          0.10000E 01
I=          5
   YHRS4=              0.11667E 01YARS4=              0.10000E 01
I=          6
   YHRSR4=          0.10000E 01YARSR4=          0.85714E 00YHRSL4=          0.10000E 01        YARSL4=          0.10000E 01
I=          7
   YHRS4=              0.10000E 01YARS4=              0.10000E 01
I=          1
   YHRS5=              0.11667E 01YARS5=              0.10000E 01
```

```
I=           2
 YHRSR5=      0.10139E 01YARSR5=      0.10357E 01YHRSL5=      0.10000E 01YARSL5=      0.10000E 01
I=           3
 YHRS5=           0.11309E 01YARS5=           0.11310E 01
I=           4
 YHRSR5=      0.10139E 01YARSR5=      0.10357E 01YHRSL5=      0.10000E 01YARSL5=      0.10000E 01
I=           5
 YHRS5=           0.11309E 01YARS5=           0.11310E 01
I=           6
 YHRSR5=      0.10139E 01YARSR5=      0.10357E 01YHRSL5=      0.10000E 01YARSL5=      0.10000E 01
I=           7
 YHRS5=           0.10000E 01YARS5=           0.11310E 01
I=           1
 YHRS6=           0.11667E 01YARS6=           0.10000E 01
I=           2
 YHRSR6=      0.10139E 01YARSR6=      0.10357E 01YHRSL6=      0.10000E 01YARSL6=      0.10000E 01
I=           3
 YHRS6=           0.11472E 01YARS6=           0.10714E 01
I=           4
 YHRSR6=      0.10139E 01YARSR6=      0.10357E 01YHRSL6=      0.10000E 01YARSL6=      0.10000E 01
I=           5
 YHRS6=           0.11472E 01YARS6=           0.10714E 01
I=           6
 YHRSR6=      0.10139E 01YARSR6=      0.10357E 01YHRSL6=      0.10000E 01YARSL6=      0.10000E 01
I=           7
 YHRS6=           0.10000E 01YARS6=           0.11310E 01
I=           1
 YHRS7=           0.11539E 01YARS7=           0.85969E 00
I=           2
 YHRSL7=      0.11994E 01YARSL7=      0.10109E 01YHRSR7=      0.10139E 01YARSR7=      0.10357E 01
I=           3
 YHRS7=           0.11539E 01YARS7=           0.85969E 00
I=           4
 YHRSL7=      0.11994E 01YARSL7=      0.10109E 01YHRSR7=      0.10139E 01YARSR7=      0.10357E 01
I=           5
 YHRS7=           0.11539E 01YARS7=           0.85969E 00
I=           6
 YHRSL7=      0.11667E 01YARSL7=      0.10109E 01YHRSR7=      0.10139E 01YARSR7=      0.10357E 01
I=           7
 YHRS7=           0.10000E 01YARS7=           0.11310E 01
I=           1
 YHRS8=           0.11539E 01YARS8=           0.85969E 00
I=           2
 YHRSL8=      0.11994E 01YARSL8=      0.10109E 01YHRSR8=      0.10139E 01YARSR8=      0.10357E 01
I=           3
 YHRS8=           0.11539E 01YARS8=           0.85969E 00
I=           4
 YHRSL8=      0.11994E 01YARSL8=      0.10109E 01YHRSR8=      0.10139E 01YARSR8=      0.10357E 01
I=           5
 YHRS8=           0.11539E 01YARS8=           0.85969E 00
I=           6
 YHRSL8=      0.11667E 01YARSL8=      0.10109E 01YHRSR8=      0.10139E 01YARSR8=      0.10357E 01
I=           7
 YHRS8=           0.10000E 01YARS8=           0.11310E 01
I=           1
 YHRS1=           0.11539E 01YARS1=           0.85969E 00
I=           2
 YHRSL1=      0.10248E 01YARSL1=      0.97851E 00YHRSR1=      0.10139E 01YARSR1=      0.10357E 01
```

```
I=           3
   YHRS1=              0.99233E 00YARS1=              0.87229E 00
I=           4
   YHRSL1=          0.10248E 01YARSL1=          0.97851E 00YHRSR1=          0.10139E 01YARSR1=          0.10357E 01
I=           5
   YHRS1=              0.99233E 00YARS1=              0.87229E 00
I=           6
   YHRSL1=          0.99909E 00YARSL1=          0.97851E 00YHRSR1=          0.10139E 01YARSR1=          0.10357E 01
I=           7
   YHRS1=              0.86905E 00YARS1=              0.11209E 01
***J=        2
I=           1
   YHRS2=              0.11539E 01YARS2=              0.85969E 00
I=           2
   YHRSL2=          0.10248E 01YARSL2=          0.97851E 00YHRSR2=          0.10139E 01       YARSR2=          0.10357E 01
I=           3
   YHRS2=              0.10658E 01YARS2=              0.86657E 00
I=           4
   YHRSL2=          0.10248E 01YARSL2=          0.97851E 00YHRSR2=          0.10139E 01       YARSR2=          0.10357E 01
I=           5
   YHRS2=              0.10658E 01YARS2=              0.86657E 00
I=           6
   YHRSL2=          0.99909E 00YARSL2=          0.97851E 00YHRSR2=          0.10139E 01       YARSR2=          0.10357E 01
I=           7
   YHRS2=              0.86905E 00YARS2=              0.11209E 01
I=           1
   YHRS3=              0.13140E 01YARS3=              0.88515E 00
I=           2
   YHRSR3=          0.10093E 01YARSR3=          0.87608E 00YHRSL3=          0.10248E 01YARSL3 =          0.97851E 00
I=           3
   YHRS3=              0.12772E 01YARS3=              0.88761E 00
I=           4
   YHRSR3=          0.10093E 01YARSR3=          0.87608E 00YHRSL3=          0.10248E 01YARSL3 =          0.97851E 00
I=           5
   YHRS3=              0.12708E 01YARS3=              0.88761E 00
I=           6
   YHRSR3=          0.98285E 00YARSR3=          0.89384E 00YHRSL3=          0.99909E 00YARSL3 =          0.97851E 00
I=           7
   YHRS3=              0.86905E 00YARS3=              0.11209E 01
I=           1
   YHRS4=              0.13140E 01YARS4=              0.88515E 00
I=           2
   YHRSR4=          0.10093E 01YARSR4=          0.87608E 00YHRSL4=          0.10248E 01       YARSL4=          0.97851E 00
I=           3
   YHRS4=              0.12772E 01YARS4=              0.88761E 00
I=           4
   YHRSR4=          0.10093E 01YARSR4=          0.87608E 00YHRSL4=          0.10248E 01       YARSL4=          0.97851E 00
I=           5
   YHRS4=              0.12708E 01YARS4=              0.88761E 00
I=           6
   YHRSR4=          0.98285E 00YARSR4=          0.89384E 00YHRSL4=          0.99909E 00       YARSL4=          0.97851E 00
I=           7
   YHRS4=              0.86905E 00YARS4=              0.11209E 01
I=           1
   YHRS5=              0.13140E 01YARS5=              0.88515E 00
I=           2
   YHRSR5=          0.10347E 01YARSR5=          0.10244E 01YHRSL5=          0.10248E 01YARSL5=          0.97851E 00
I=           3
```

```
 YHRS5=          0.12198E 01YARS5=          0.10327E 01
I=       4
 YHRSR5=        0.10316E 01YARSR5=         0.10250E 01YHRSL5=         0.10248E 01YARSL5=         0.97851E 00
I=       5
 YHRS5=          0.12148E 01YARS5=          0.10327E 01
I=       6
 YHRSR5=        0.10068E 01YARSR5=         0.10413E 01YHRSL5=         0.99909E 00YARSL5=         0.97851E 00
I=       7
 YHRS5=          0.89343E 00YARS5=          0.12510E 01
I=       1
 YHRS6=          0.13140E 01YARS6=          0.88515E 00
I=       2
 YHRSR6=        0.10347E 01YARSR6=         0.10244E 01YHRSL6=         0.10248E 01YARSL6=         0.97851E 00
I=       3
 YHRS6=          0.12459E 01YARS6=          0.96673E 00
I=       4
 YHRSR6=        0.10316E 01YARSR6=         0.10250E 01YHRSL6=         0.10248E 01YARSL6=         0.97851E 00
I=       5
 YHRS6=          0.12402E 01YARS6=          0.96673E 00
I=       6
 YHRSR6=        0.10068E 01YARSR6=         0.10413E 01YHRSL6=         0.99909E 00YARSL6=         0.97851E 00
I=       7
 YHRS6=          0.89343E 00YARS6=          0.12510E 01
I=       1
 YHRS7=          0.12907E 01YARS7=          0.76865E 00
I=       2
 YHRSL7=        0.12444E 01YARSL7=         0.98302E 00YHRSR7=         0.10347E 01YARSR7=         0.10244E 01
I=       3
 YHRS7=          0.12568E 01YARS7=          0.77062E 00
I=       4
 YHRSL7=        0.12431E 01YARSL7=         0.98302E 00YHRSR7=         0.10316E 01YARSR7=         0.10250E 01
I=       5
 YHRS7=          0.12488E 01YARS7=          0.77178E 00
I=       6
 YHRSL7=        0.11392E 01YARSL7=         0.10012E 01YHRSR7=         0.10068E 01YARSR7=         0.10413E 01
I=       7
 YHRS7=          0.89343E 00YARS7=          0.12510E 01
I=       1
 YHRS8=          0.12907E 01YARS8=          0.76864E 00
I=       2
 YHRSL8=        0.12444E 01YARSL8=         0.98302E 00YHRSR8=         0.10347E 01YARSR8=         0.10244E 01
I=       3
 YHRS8=          0.12568E 01YARS8=          0.77062E 00
I=       4
 YHRSL8=        0.12431E 01YARSL8=         0.98302E 00YHRSR8=         0.10316E 01YARSR8=         0.10250E 01
I=       5
 YHRS8=          0.12488E 01YARS8=          0.77178E 00
I=       6
 YHRSL8=        0.11392E 01YARSL8=         0.10012E 01YHRSR8=         0.10068E 01YARSR8=         0.10413E 01
I=       7
 YHRS8=          0.89343E 00YARS8=          0.12510E 01
I=       1
 YHRS1=          0.12907E 01YARS1=          0.76864E 00
I=       2
 YHRSL1=        0.10699E 01YARSL1=         0.93708E 00YHRSR1=         0.10347E 01YARSR1=         0.10244E 01
I=       3
 YHRS1=          0.10763E 01YARS1=          0.78832E 00
I=       4
```

```
 YHRSL1=        0.10665E 01YARSL1=        0.93750E 00YHRSR1=        0.10316E 01YARSR1=        0.10250E 01
I=       5
 YHRS1=             0.10700E 01YARS1=              0.78938E 00
I=       6
 YHRSL1=        0.98428E 00YARSL1=        0.95205E 00YHRSR1=        0.10068E 01YARSR1=        0.10413E 01
I=       7
 YHRS1=             0.78335E 00YARS1=              0.12302E 01
***J=    3
I=       1
 YHRS2=             0.12907E 01YARS2=              0.76864E 00
I=       2
 YHRSL2=        0.10699E 01YARSL2=        0.93708E 00YHRSR2=        0.10347E 01       YARSR2=        0.10244E 01
I=       3
 YHRS2=             0.11584E 01YARS2=              0.78027E 00
I=       4
 YHRSL2=        0.10665E 01YARSL2=        0.93750E 00YHRSR2=        0.10316E 01       YARSR2=        0.10250E 01
I=       5
 YHRS2=             0.11513E 01YARS2=              0.78138E 00
I=       6
 YHRSL2=        0.98428E 00YARSL2=        0.95205E 00YHRSR2=        0.10068E 01       YARSR2=        0.10413E 01
I=       7
 YHRS2=             0.78335E 00YARS2=              0.12302E 01
I=       1
 YHRS3=             0.14506E 01YARS3=              0.80474E 00
I=       2
 YHRSR3=        0.10436E 01YARSR3=        0.86116E 00YHRSL3=        0.10699E 01YARSL3 =       0.93708E 00
I=       3
 YHRS3=             0.13777E 01YARS3=              0.80983E 00
I=       4
 YHRSR3=        0.10398E 01YARSR3=        0.86172E 00YHRSL3=        0.10665E 01YARSL3 =       0.93750E 00
I=       5
 YHRS3=             0.13502E 01YARS3=              0.81384E 00
I=       6
 YHRSR3=        0.95895E 00YARSR3=        0.90600E 00YHRSL3=        0.98428E 00YARSL3 =       0.95205E 00
I=       7
 YHRS3=             0.78335E 00YARS3=              0.12302E 01
I=       1
 YHRS4=             0.14506E 01YARS4=              0.80474E 00
I=       2
 YHRSR4=        0.10436E 01YARSR4=        0.86116E 00YHRSL4=        0.10699E 01       YARSL4=        0.93708E 00
I=       3
 YHRS4=             0.13777E 01YARS4=              0.80983E 00
I=       4
 YHRSR4=        0.10398E 01YARSR4=        0.86172E 00YHRSL4=        0.10665E 01       YARSL4=        0.93750E 00
I=       5
 YHRS4=             0.13502E 01YARS4=              0.81384E 00
I=       6
 YHRSR4=        0.95895E 00YARSR4=        0.90600E 00YHRSL4=        0.98428E 00       YARSL4=        0.95205E 00
I=       7
 YHRS4=             0.78335E 00YARS4=              0.12302E 01
I=       1
 YHRS5=             0.14506E 01YARS5=              0.80474E 00
I=       2
 YHRSR5=        0.10775E 01YARSR5=        0.99058E 00YHRSL5=        0.10699E 01YARSL5=        0.93708E 00
I=       3
 YHRS5=             0.13061E 01YARS5=              0.95763E 00
I=       4
 YHRSR5=        0.10680E 01YARSR5=        0.99237E 00YHRSL5=        0.10665E 01YARSL5=        0.93750E 00
```

```
I=          5
 YHRS5=          0.12837E 01YARS5=          0.96145E 00
I=          6
 YHRSR5=    0.99155E 00YARSR5=    0.10340E 01YHRSL5=    0.98428E 00YARSL5=    0.95205E 00
I=          7
 YHRS5=          0.82098E 00YARS5=          0.13542E 01
I=          1
 YHRS6=          0.14506E 01YARS6=          0.60474E 00
I=          2
 YHRSR6=    0.10775E 01YARSR6=    0.99058E 00YHRSL6=    0.10699E 01YARSL6=    0.93708E 00
I=          3
 YHRS6=          0.13386E 01YARS6=          0.89045E 00
I=          4
 YHRSR6=    0.10680E 01YARSR6=    0.99237E 00YHRSL6=    0.10665E 01YARSL6=    0.93750E 00
I=          5
 YHRS6=          0.13139E 01YARS6=          0.89435E 00
I=          6
 YHRSR6=    0.99155E 00YARSR6=    0.10340E 01YHRSL6=    0.98428E 00YARSL6=    0.95205E 00
I=          7
 YHRS6=          0.82098E 00YARS6=          0.13542E 01
I=          1
 YHRS7=          0.14195E 01YARS7=          0.70305E 00
I=          2
 YHRSL7=    0.13073E 01YARSL7=    0.93879E 00YHRSR7=    0.10775E 01YARSR7=    0.99058E 00
I=          3
 YHRS7=          0.13518E 01YARS7=          0.70717E 00
I=          4
 YHRSL7=    0.12985E 01YARSL7=    0.93950E 00YHRSR7=    0.10680E 01YARSR7=    0.99237E 00
I=          5
 YHRS7=          0.13203E 01YARS7=          0.71330E 00
I=          6
 YHRSL7=    0.11075E 01YARSL7=    0.98556E 00YHRSR7=    0.99155E 00YARSR7=    0.10340E 01
I=          7
 YHRS7=          0.82098E 00YARS7=          0.13542E 01
I=          1
 YHRS8=          0.14195E 01YARS8=          0.70305E 00
I=          2
 YHRSL8=    0.13073E 01YARSL8=    0.93879E 00YHRSR8=    0.10775E 01YARSR8=    0.99058E 00
I=          3
 YHRS8=          0.13518E 01YARS8=          0.70717E 00
I=          4
 YHRSL8=    0.12985E 01YARSL8=    0.93950E 00YHRSR8=    0.10680E 01YARSR8=    0.99237E 00
I=          5
 YHRS8=          0.13203E 01YARS8=          0.71330E 00
I=          6
 YHRSL8=    0.11075E 01YARSL8=    0.98556E 00YHRSR8=    0.99155E 00YARSR8=    0.10340E 01
I=          7
 YHRS8=          0.82098E 00YARS8=          0.13542E 01
I=          1
 YHRS1=          0.14195E 01YARS1=          0.70305E 00
I=          2
 YHRSL1=    0.11285E 01YARSL1=    0.88828E 00YHRSR1=    0.10775E 01YARSR1=    0.99058E 00
I=          3
 YHRS1=          0.11555E 01YARS1=          0.72648E 00
I=          4
 YHRSL1=    0.11168E 01YARSL1=    0.88971E 00YHRSR1=    0.10680E 01YARSR1=    0.99237E 00
I=          5
 YHRS1=          0.11301E 01YARS1=          0.73215E 00
```

```
I=          6
   YHRSL1=          0.96448E 00YARSL1=          0.92721E 00YHRSR1=          0.99155E 00YARSR1=          0.10340E 01
I=          7
   YHRS1=               0.72416E 00YARS1=               0.13234E 01
***J=          4
I=          1
   YHRS2=               0.14195E 01YARS2=               0.70305E 00
I=          2
   YHRSL2=          0.11285E 01YARSL2=          0.88828E 00YHRSR2=          0.10775E 01        YARSR2=          0.99058E 00
I=          3
   YHRS2=               0.12448E 01YARS2=               0.71770E 00
I=          4
   YHRSL2=          0.11168E 01YARSL2=          0.88971E 00YHRSR2=          0.10680E 01        YARSR2=          0.99237E 00
I=          5
   YHRS2=               0.12166E 01YARS2=               0.72358E 00
I=          6
   YHRSL2=          0.96448E 00YARSL2=          0.92721E 00YHRSR2=          0.99155E 00        YARSR2=          0.10340E 01
I=          7
   YHRS2=               0.72416E 00YARS2=               0.13234E 01
I=          1
   YHRS3=               0.15834E 01YARS3=               0.74274E 00
I=          2
   YHRSR3=          0.10942E 01YARSR3=          0.83021E 00YHRSL3=          0.11285E 01YARSL3 =          0.88828E 00
I=          3
   YHRS3=               0.14738E 01YARS3=               0.75005E 00
I=          4
   YHRSR3=          0.10813E 01YARSR3=          0.83201E 00YHRSL3=          0.11168E 01YARSL3 =          0.88971E 00
I=          5
   YHRS3=               0.14082E 01YARS3=               0.76281E 00
I=          6
   YHRSR3=          0.93425E 00YARSR3=          0.90692E 00YHRSL3=          0.96448E 00YARSL3 =          0.92721E 00
I=          7
   YHRS3=               0.72416E 00YARS3=               0.13234E 01
I=          1
   YHRS4=               0.15833E 01YARS4=               0.74274E 00
I=          2
   YHRSR4=          0.10942E 01YARSR4=          0.83021E 00YHRSL4=          0.11285E 01        YARSL4=          0.88828E 00
I=          3
   YHRS4=               0.14738E 01YARS4=               0.75005E 00
I=          4
   YHRSR4=          0.10813E 01YARSR4=          0.83201E 00YHRSL4=          0.11168E 01        YARSL4=          0.88971E 00
I=          5
   YHRS4=               0.14082E 01YARS4=               0.76281E 00
I=          6
   YHRSR4=          0.93425E 00YARSR4=          0.90692E 00YHRSL4=          0.96448E 00        YARSL4=          0.92721E 00
I=          7
   YHRS4=               0.72416E 00YARS4=               0.13234E 01
I=          1
   YHRS5=               0.15833E 01YARS5=               0.74274E 00
I=          2
   YHRSR5=          0.11350E 01YARSR5=          0.94670E 00YHRSL5=          0.11285E 01YARSL5=          0.88828E 00
I=          3
   YHRS5=               0.13924E 01YARS5=               0.89510E 00
I=          4
   YHRSR5=          0.11140E 01YARSR5=          0.95019E 00YHRSL5=          0.11168E 01YARSL5=          0.88971E 00
I=          5
   YHRS5=               0.13381E 01YARS5=               0.90724E 00
I=          6
```

```
 YHRSR5= 0.97375E 00YARSR5= 0.10220E 01YHRSL5= 0.96448E 00YARSL5= 0.92721E 00
I=  7
 YHRS5= 0.76918E 00YARS5= 0.14399E 01
I=  1
 YHRS6= 0.15853E 01YARS6= 0.74274E 00
I=  2
 YHRSR6= 0.11350E 01YARSR6= 0.94670E 00YHRSL6= 0.11285E 01YARSL6= 0.88828E 00
I=  3
 YHRS6= 0.14294E 01YARS6= 0.82917E 00
I=  4
 YHRSR6= 0.11140E 01YARSR6= 0.95019E 00YHRSL6= 0.11168E 01YARSL6= 0.88971E 00
I=  5
 YHRS6= 0.13700E 01YARS6= 0.84159E 00
I=  6
 YHRSR6= 0.97375E 00YARSR6= 0.10220E 01YHRSL6= 0.96448E 00YARSL6= 0.92721E 00
I=  7
 YHRS6= 0.76918E 00YARS6= 0.14399E 01
I=  1
 YHRS7= 0.15460E 01YARS7= 0.65120E 00
I=  2
 YHRSL7= 0.13826E 01YARSL7= 0.88884E 00YHRSR7= 0.11350E 01YARSR7= 0.94670E 00
I=  3
 YHRS7= 0.14438E 01YARS7= 0.65719E 00
I=  4
 YHRSL7= 0.13583E 01YARSL7= 0.89117E 00YHRSR7= 0.11140E 01YARSR7= 0.95019E 00
I=  5
 YHRS7= 0.13720E 01YARS7= 0.67235E 00
I=  6
 YHRSL7= 0.10764E 01YARSL7= 0.96994E 00YHRSR7= 0.97375E 00YARSR7= 0.10220E 01
I=  7
 YHRS7= 0.76918E 00YARS7= 0.14399E 01
I=  1
 YHRS8= 0.15460E 01YARS8= 0.65120E 00
I=  2
 YHRSL8= 0.13826E 01YARSL8= 0.88884E 00YHRSR8= 0.11350E 01YARSR8= 0.94670E 00
I=  3
 YHRS8= 0.14438E 01YARS8= 0.65719E 00
I=  4
 YHRSL8= 0.13583E 01YARSL8= 0.89117E 00YHRSR8= 0.11140E 01YARSR8= 0.95019E 00
I=  5
 YHRS8= 0.13720E 01YARS8= 0.67235E 00
I=  6
 YHRSL8= 0.10764E 01YARSL8= 0.96994E 00YHRSR8= 0.97375E 00YARSR8= 0.10220E 01
I=  7
 YHRS8= 0.76918E 00YARS8= 0.14399E 01
I=  1
 YHRS1= 0.15460E 01YARS1= 0.65120E 00
I=  2
 YHRSL1= 0.11967E 01YARSL1= 0.83792E 00YHRSR1= 0.11350E 01YARSR1= 0.94670E 00
I=  3
 YHRS1= 0.12332E 01YARS1= 0.67650E 00
I=  4
 YHRSL1= 0.11704E 01YARSL1= 0.84104E 00YHRSR1= 0.11140E 01YARSR1= 0.95019E 00
I=  5
 YHRS1= 0.11750E 01YARS1= 0.69059E 00
I=  6
 YHRSL1= 0.94374E 00YARSL1= 0.90617E 00YHRSR1= 0.97375E 00YARSR1= 0.10220E 01
I=  7
```

```
   YHRS1=          0.68124E 00YARS1=          0.14007E 01
***J=       5
I=       1
   YHRS2=          0.15460E 01YARS2=          0.65120E 00
I=       2
   YHRSL2=      0.11967E 01YARSL2=      0.83792E 00YHRSR2=      0.11350E 01      YARSR2=      0.94670E 00
I=       3
   YHRS2=          0.13289E 01YARS2=          0.66772E 00
I=       4
   YHRSL2=      0.11704E 01YARSL2=      0.84103E 00YHRSR2=      0.11140E 01      YARSR2=      0.95019E 00
I=       5
   YHRS2=          0.12645E 01YARS2=          0.68230E 00
I=       6
   YHRSL2=      0.94374E 00YARSL2=      0.90617E 00YHRSR2=      0.97375E 00      YARSR2=      0.10220E 01
I=       7
   YHRS2=          0.68124E 00YARS2=          0.14007E 01
I=       1
   YHRS3=          0.17163E 01YARS3=          0.69121E 00
I=       2
   YHRSR3=      0.11560E 01YARSR3=      0.79216E 00YHRSL3=      0.11967E 01YARSL3 =      0.83792E 00
I=       3
   YHRS3=          0.15682E 01YARS3=          0.70035E 00
I=       4
   YHRSR3=      0.11271E 01YARSR3=      0.79590E 00YHRSL3=      0.11704E 01YARSL3 =      0.84103E 00
I=       5
   YHRS3=          0.14488E 01YARS3=          0.72601E 00
I=       6
   YHRSR3=      0.91107E 00YARSR3=      0.90309E 00YHRSL3=      0.94374E 00YARSL3 =      0.90617E 00
I=       7
   YHRS3=          0.68124E 00YARS3=          0.14007E 01
I=       1
   YHRS4=          0.17163E 01YARS4=          0.69121E 00
I=       2
   YHRSR4=      0.11560E 01YARSR4=      0.79216E 00YHRSL4=      0.11967E 01      YARSL4=      0.83792E 00
I=       3
   YHRS4=          0.15682E 01YARS4=          0.70035E 00
I=       4
   YHRSR4=      0.11271E 01YARSR4=      0.79590E 00YHRSL4=      0.11704E 01      YARSL4=      0.84103E 00
I=       5
   YHRS4=          0.14488E 01YARS4=          0.72601E 00
I=       6
   YHRSR4=      0.91107E 00YARSR4=      0.90309E 00YHRSL4=      0.94374E 00      YARSL4=      0.90617E 00
I=       7
   YHRS4=          0.68124E 00YARS4=          0.14007E 01
I=       1
   YHRS5=          0.17163E 01YARS5=          0.69121E 00
I=       2
   YHRSR5=      0.12027E 01YARSR5=      0.89895E 00YHRSL5=      0.11967E 01YARSL5=      0.83792E 00
I=       3
   YHRS5=          0.14799E 01YARS5=          0.84003E 00
I=       4
   YHRSR5=      0.11638E 01YARSR5=      0.90466E 00YHRSL5=      0.11704E 01YARSL5=      0.84103E 00
I=       5
   YHRS5=          0.13798E 01YARS5=          0.86448E 00
I=       6
   YHRSR5=      0.95587E 00YARSR5=      0.10093E 01YHRSL5=      0.94374E 00YARSL5=      0.90617E 00
I=       7
   YHRS5=          0.73049E 00YARS5=          0.15098E 01
```

```
I=          1
 YHRS6=          0.17163E 01YARS6=          0.69121E 00
I=          2
 YHRSR6=    0.12027E 01YARSR6=    0.89895E 00YHRSL6=    0.11967E 01YARSL6=    0.83792E 00
I=          3
 YHRS6=          0.15200E 01YARS6=          0.77654E 00
I=          4
 YHRSR6=    0.11638E 01YARSR6=    0.90466E 00YHRSL6=    0.11704E 01YARSL6=    0.84103E 00
I=          5
 YHRS6=          0.14112E 01YARS6=          0.80154E 00
I=          6
 YHRSR6=    0.95587E 00YARSR6=    0.10093E 01YHRSL6=    0.94374E 00YARSL6=    0.90617E 00
I=          7
 YHRS6=          0.73049E 00YARS6=          0.15098E 01
I=          1
 YHRS7=          0.16735E 01YARS7=          0.60731E 00
I=          2
 YHRSL7=    0.14670E 01YARSL7=    0.83809E 00YHRSR7=    0.12027E 01YARSR7=    0.89895E 00
I=          3
 YHRS7=          0.15345E 01YARS7=          0.61489E 00
I=          4
 YHRSL7=    0.14178E 01YARSL7=    0.84299E 00YHRSR7=    0.11638E 01YARSR7=    0.90466E 00
I=          5
 YHRS7=          0.14077E 01YARS7=          0.64253E 00
I=          6
 YHRSL7=    0.10477E 01YARSL7=    0.95647E 00YHRSR7=    0.95587E 00YARSR7=    0.10093E 01
I=          7
 YHRS7=          0.73049E 00YARS7=          0.15098E 01
I=          1
 YHRS8=          0.16735E 01YARS8=          0.60731E 00
I=          2
 YHRSL8=    0.14670E 01YARSL8=    0.83809E 00YHRSR8=    0.12027E 01YARSR8=    0.89895E 00
I=          3
 YHRS8=          0.15345E 01YARS8=          0.61489E 00
I=          4
 YHRSL8=    0.14178E 01YARSL8=    0.84299E 00YHRSR8=    0.11638E 01YARSR8=    0.90466E 00
I=          5
 YHRS8=          0.14077E 01YARS8=          0.64253E 00
I=          6
 YHRSL8=    0.10477E 01YARSL8=    0.95647E 00YHRSR8=    0.95587E 00YARSR8=    0.10093E 01
I=          7
 YHRS8=          0.73049E 00YARS8=          0.15098E 01
I=          1
 YHRS1=          0.16735E 01YARS1=          0.60731E 00
I=          2
 YHRSL1=    0.12722E 01YARSL1=    0.78864E 00YHRSR1=    0.12027E 01YARSR1=    0.89895E 00
I=          3
 YHRS1=          0.13105E 01YARS1=          0.63349E 00
I=          4
 YHRSL1=    0.12236E 01YARSL1=    0.79411E 00YHRSR1=    0.11638E 01YARSR1=    0.90466E 00
I=          5
 YHRS1=          0.12073E 01YARS1=          0.65923E 00
I=          6
 YHRSL1=    0.92576E 00YARSL1=    0.88919E 00YHRSR1=    0.95587E 00YARSR1=    0.10093E 01
I=          7
 YHRS1=          0.64879E 00YARS1=          0.14637E 01
***J=        6
I=          1
```

M = 8

```
***J=          1
I=          1
   YHRS2=              0.10000E 01YARS2=              0.10000E 01
I=          2
   YHRSL2=          0.79200E 00YARSL2=          0.84300E 00YHRSR2=          0.10000E 01          YARSR2=          0.10000E 01
I=          3
   YHRS2=              0.84300E 00YARS2=              0.96600E 00
I=          4
   YHRSL2=          0.79200E 00YARSL2=          0.84300E 00YHRSR2=          0.10000E 01          YARSR2=          0.10000E 01
I=          5
   YHRS2=              0.84300E 00YARS2=              0.96600E 00
I=          6
   YHRSL2=          0.79200E 00YARSL2=          0.84300E 00YHRSR2=          0.10000E 01          YARSR2=          0.10000E 01
I=          7
   YHRS2=              0.84300E 00YARS2=              0.96600E 00
I=          8
   YHRSL2=          0.79200E 00YARSL2=          0.84300E 00YHRSR2=          0.10000E 01          YARSR2=          0.10000E 01
I=          9
   YHRS2=              0.84300E 00YARS2=              0.96600E 00
I=          1
   YHRS3=              0.11411E 01YARS3=              0.10018E 01
I=          2
   YHRSR3=          0.90193E 00YARSR3=          0.85144E 00YHRSL3=          0.79200E 00YARSL3 =          0.84300E 00
I=          3
   YHRS3=              0.99715E 00YARS3=              0.97301E 00
I=          4
   YHRSR3=          0.90193E 00YARSR3=          0.85144E 00YHRSL3=          0.79200E 00YARSL3 =          0.84300E 00
I=          5
   YHRS3=              0.99715E 00YARS3=              0.97301E 00
I=          6
   YHRSR3=          0.90193E 00YARSR3=          0.85144E 00YHRSL3=          0.79200E 00YARSL3 =          0.84300E 00
I=          7
   YHRS3=              0.99715E 00YARS3=              0.97301E 00
I=          8
   YHRSR3=          0.90193E 00YARSR3=          0.85144E 00YHRSL3=          0.79200E 00YARSL3 =          0.84300E 00
I=          9
   YHRS3=              0.84300E 00YARS3=              0.96600E 00
I=          1
   YHRS4=              0.11411E 01YARS4=              0.10018E 01
I=          2
   YHRSR4=          0.90193E 00YARSR4=          0.85144E 00YHRSL4=          0.79200E 00          YARSL4=          0.84300E 00
```

```
I=          3
 YHRS4=          0.99715E 00YARS4=          0.97301E 00
I=          4
 YHRSR4=    0.90193E 00YARSR4=    0.85144E 00YHRSL4=    0.79200E 00    YARSL4=    0.84300E 00
I=          5
 YHRS4=          0.99715E 00YARS4=          0.97301E 00
I=          6
 YHRSR4=    0.90193E 00YARSR4=    0.85144E 00YHRSL4=    0.79200E 00    YARSL4=    0.84300E 00
I=          7
 YHRS4=          0.99715E 00YARS4=          0.97301E 00
I=          8
 YHRSR4=    0.90193E 00YARSR4=    0.85144E 00YHRSL4=    0.79200E 00    YARSL4=    0.84300E 00
I=          9
 YHRS4=          0.84300E 00YARS4=          0.96600E 00
I=          1
 YHRS5=          0.11411E 01YARS5=          0.10018E 01
I=          2
 YHRSR5=    0.97038E 00YARSR5=    0.10061E 01YHRSL5=    0.79200E 00YARSL5=    0.84300E 00
I=          3
 YHRS5=          0.10412E 01YARS5=          0.11135E 01
I=          4
 YHRSR5=    0.95775E 00YARSR5=    0.10010E 01YHRSL5=    0.79200E 00YARSL5=    0.84300E 00
I=          5
 YHRS5=          0.10412E 01YARS5=          0.11135E 01
I=          6
 YHRSR5=    0.95775E 00YARSR5=    0.10010E 01YHRSL5=    0.79200E 00YARSL5=    0.84300E 00
I=          7
 YHRS5=          0.10412E 01YARS5=          0.11135E 01
I=          8
 YHRSR5=    0.95775E 00YARSR5=    0.10010E 01YHRSL5=    0.79200E 00YARSL5=    0.84300E 00
I=          9
 YHRS5=          0.92005E 00YARS5=          0.11069E 01
I=          1
 YHRS6=          0.11411E 01YARS6=          0.10018E 01
I=          2
 YHRSR6=    0.97038E 00YARSR6=    0.10061E 01YHRSL6=    0.79200E 00YARSL6=    0.84300E 00
I=          3
 YHRS6=          0.10212E 01YARS6=          0.10497E 01
I=          4
 YHRSR6=    0.95775E 00YARSR6=    0.10010E 01YHRSL6=    0.79200E 00YARSL6=    0.84300E 00
I=          5
 YHRS6=          0.10212E 01YARS6=          0.10497E 01
I=          6
 YHRSR6=    0.95775E 00YARSR6=    0.10010E 01YHRSL6=    0.79200E 00YARSL6=    0.84300E 00
I=          7
 YHRS6=          0.10212E 01YARS6=          0.10497E 01
I=          8
 YHRSR6=    0.95775E 00YARSR6=    0.10010E 01YHRSL6=    0.79200E 00YARSL6=    0.84300E 00
I=          9
 YHRS6=          0.92005E 00YARS6=          0.11069E 01
I=          1
 YHRS7=          0.10783E 01YARS7=          0.87088E 00
I=          2
 YHRSL7=    0.10030E 01YARSL7=    0.88760E 00YHRSR7=    0.97038E 00YARSR7=    0.10061E 01
I=          3
 YHRS7=          0.94598E 00YARS7=          0.84642E 00
I=          4
 YHRSL7=    0.10030E 01YARSL7=    0.88760E 00YHRSR7=    0.95775E 00YARSR7=    0.10010E 01
```

```
I=           5
   YHRS7=          0.94598E 00YARS7=          0.84642E 00
I=           6
   YHRSL7=         0.10030E 01YARSL7=         0.88760E 00YHRSR7=         0.95775E 00YARSR7=         0.10010E 01
I=           7
   YHRS7=          0.94598E 00YARS7=          0.84642E 00
I=           8
   YHRSL7=         0.97222E 00YARSL7=         0.88724E 00YHRSR7=         0.95775E 00YARSR7=         0.10010E 01
I=           9
   YHRS7=          0.92005E 00YARS7=          0.11069E 01
I=           1
   YHRS8=          0.10783E 01YARS8=          0.87088E 00
I=           2
   YHRSL8=         0.10030E 01YARSL8=         0.88760E 00YHRSR8=         0.97038E 00YARSR8=         0.10061E 01
I=           3
   YHRS8=          0.94598E 00YARS8=          0.84642E 00
I=           4
   YHRSL8=         0.10030E 01YARSL8=         0.88760E 00YHRSR8=         0.95775E 00YARSR8=         0.10010E 01
I=           5
   YHRS8=          0.94598E 00YARS8=          0.84642E 00
I=           6
   YHRSL8=         0.10030E 01YARSL8=         0.88760E 00YHRSR8=         0.95775E 00YARSR8=         0.10010E 01
I=           7
   YHRS8=          0.94598E 00YARS8=          0.84642E 00
I=           8
   YHRSL8=         0.97222E 00YARSL8=         0.88724E 00YHRSR8=         0.95775E 00YARSR8=         0.10010E 01
I=           9
   YHRS8=          0.92005E 00YARS8=          0.11069E 01
I=           1
   YHRS1=          0.10783E 01YARS1=          0.87088E 00
I=           2
   YHRSL1=         0.84107E 00YARSL1=         0.85861E 00YHRSR1=         0.97038E 00YARSR1=         0.10061E 01
I=           3
   YHRS1=          0.80058E 00YARS1=          0.81794E 00
I=           4
   YHRSL1=         0.83188E 00YARSL1=         0.85496E 00YHRSR1=         0.95775E 00YARSR1=         0.10010E 01
I=           5
   YHRS1=          0.80058E 00YARS1=          0.81794E 00
I=           6
   YHRSL1=         0.83188E 00YARSL1=         0.85496E 00YHRSR1=         0.95775E 00YARSR1=         0.10010E 01
I=           7
   YHRS1=          0.80058E 00YARS1=          0.81794E 00
I=           8
   YHRSL1=         0.80836E 00YARSL1=         0.85467E 00YHRSR1=         0.95775E 00YARSR1=         0.10010E 01
I=           9
   YHRS1=          0.77845E 00YARS1=          0.10649E 01
***J=        2
I=           1
   YHRS2=          0.10783E 01YARS2=          0.87088E 00
I=           2
   YHRSL2=         0.84107E 00YARSL2=         0.85861E 00YHRSR2=         0.97038E 00      YARSR2=         0.10061E 01
I=           3
   YHRS2=          0.86667E 00YARS2=          0.83089E 00
I=           4
   YHRSL2=         0.83188E 00YARSL2=         0.85496E 00YHRSR2=         0.95775E 00      YARSR2=         0.10010E 01
I=           5
   YHRS2=          0.86667E 00YARS2=          0.83089E 00
I=           6
```

```
 YHRSL2=       0.83188E 00YARSL2=       0.85496E 00YHRSR2=       0.95775E 00      YARSR2=       0.10010E 01
I=       7
 YHRS2=            0.86667E 00YARS2=            0.83089E 00
I=       8
 YHRSL2=       0.80836E 00YARSL2=       0.85466E 00YHRSR2=       0.95775E 00      YARSR2=       0.10010E 01
I=       9
 YHRS2=            0.77845E 00YARS2=            0.10649E 01
I=       1
 YHRS3=            0.12268E 01YARS3=            0.89541E 00
I=       2
 YHRSR3=       0.87173E 00YARSR3=       0.84988E 00YHRSL3=       0.84107E 00YARSL3 =       0.85861E 00
I=       3
 YHRS3=            0.10698E 01YARS3=            0.86806E 00
I=       4
 YHRSR3=       0.86247E 00YARSR3=       0.84576E 00YHRSL3=       0.83188E 00YARSL3 =       0.85496E 00
I=       5
 YHRS3=            0.10698E 01YARS3=            0.86806E 00
I=       6
 YHRSR3=       0.86247E 00YARSR3=       0.84576E 00YHRSL3=       0.83188E 00YARSL3 =       0.85496E 00
I=       7
 YHRS3=            0.10631E 01YARS3=            0.86801E 00
I=       8
 YHRSR3=       0.85804E 00YARSR3=       0.85665E 00YHRSL3=       0.80836E 00YARSL3 =       0.85466E 00
I=       9
 YHRS3=            0.77845E 00YARS3=            0.10649E 01
I=       1
 YHRS4=            0.12268E 01YARS4=            0.89541E 00
I=       2
 YHRSR4=       0.87173E 00YARSR4=       0.84988E 00YHRSL4=       0.84107E 00      YARSL4=       0.85861E 00
I=       3
 YHRS4=            0.10698E 01YARS4=            0.86806E 00
I=       4
 YHRSR4=       0.86247E 00YARSR4=       0.84576E 00YHRSL4=       0.83188E 00      YARSL4=       0.85496E 00
I=       5
 YHRS4=            0.10698E 01YARS4=            0.86806E 00
I=       6
 YHRSR4=       0.86247E 00YARSR4=       0.84576E 00YHRSL4=       0.83188E 00      YARSL4=       0.85496E 00
I=       7
 YHRS4=            0.10631E 01YARS4=            0.86801E 00
I=       8
 YHRSR4=       0.85804E 00YARSR4=       0.85665E 00YHRSL4=       0.80836E 00      YARSL4=       0.85466E 00
I=       9
 YHRS4=            0.77845E 00YARS4=            0.10649E 01
I=       1
 YHRS5=            0.12268E 01YARS5=            0.89541E 00
I=       2
 YHRSR5=       0.94875E 00YARSR5=       0.98579E 00YHRSL5=       0.84107E 00YARSL5=       0.85861E 00
I=       3
 YHRS5=            0.10896E 01YARS5=            0.10137E 01
I=       4
 YHRSR5=       0.92605E 00YARSR5=       0.97694E 00YHRSL5=       0.83188E 00YARSL5=       0.85496E 00
I=       5
 YHRS5=            0.10870E 01YARS5=            0.10127E 01
I=       6
 YHRSR5=       0.92605E 00YARSR5=       0.97694E 00YHRSL5=       0.83188E 00YARSL5=       0.85496E 00
I=       7
 YHRS5=            0.10817E 01YARS5=            0.10127E 01
I=       8
```

```
 YHRSR5= 0.92119E 00YARSR5= 0.98754E 00YHRSL5= 0.80836E 00YARSL5= 0.85466E 00
I= 9
 YHRS5= 0.85679E 00YARS5= 0.12018E 01
I= 1
 YHRS6= 0.12268E 01YARS6= 0.89541E 00
I= 2
 YHRSR6= 0.94875E 00YARSR6= 0.98579E 00YHRSL6= 0.84107E 00YARSL6= 0.85861E 00
I= 3
 YHRS6= 0.10806E 01YARS6= 0.94748E 00
I= 4
 YHRSR6= 0.92605E 00YARSR6= 0.97694E 00YHRSL6= 0.83188E 00YARSL6= 0.85496E 00
I= 5
 YHRS6= 0.10792E 01YARS6= 0.94697E 00
I= 6
 YHRSR6= 0.92605E 00YARSR6= 0.97694E 00YHRSL6= 0.83188E 00YARSL6= 0.85496E 00
I= 7
 YHRS6= 0.10733E 01YARS6= 0.94692E 00
I= 8
 YHRSR6= 0.92119E 00YARSR6= 0.98754E 00YHRSL6= 0.80836E 00YARSL6= 0.85466E 00
I= 9
 YHRS6= 0.85679E 00YARS6= 0.12018E 01
I= 1
 YHRS7= 0.11562E 01YARS7= 0.78040E 00
I= 2
 YHRSL7= 0.10611E 01YARSL7= 0.89758E 00YHRSR7= 0.94875E 00YARSR7= 0.98579E 00
I= 3
 YHRS7= 0.10115E 01YARS7= 0.75706E 00
I= 4
 YHRSL7= 0.10518E 01YARSL7= 0.89394E 00YHRSR7= 0.92605E 00YARSR7= 0.97694E 00
I= 5
 YHRS7= 0.10115E 01YARS7= 0.75706E 00
I= 6
 YHRSL7= 0.10505E 01YARSL7= 0.89394E 00YHRSR7= 0.92605E 00YARSR7= 0.97694E 00
I= 7
 YHRS7= 0.10052E 01YARS7= 0.75726E 00
I= 8
 YHRSL7= 0.97138E 00YARSL7= 0.90379E 00YHRSR7= 0.92119E 00YARSR7= 0.98754E 00
I= 9
 YHRS7= 0.85679E 00YARS7= 0.12018E 01
I= 1
 YHRS8= 0.11562E 01YARS8= 0.78040E 00
I= 2
 YHRSL8= 0.10611E 01YARSL8= 0.89758E 00YHRSR8= 0.94875E 00YARSR8= 0.98579E 00
I= 3
 YHRS8= 0.10115E 01YARS8= 0.75706E 00
I= 4
 YHRSL8= 0.10518E 01YARSL8= 0.89394E 00YHRSR8= 0.92605E 00YARSR8= 0.97694E 00
I= 5
 YHRS8= 0.10115E 01YARS8= 0.75706E 00
I= 6
 YHRSL8= 0.10505E 01YARSL8= 0.89394E 00YHRSR8= 0.92605E 00YARSR8= 0.97694E 00
I= 7
 YHRS8= 0.10052E 01YARS8= 0.75726E 00
I= 8
 YHRSL8= 0.97138E 00YARSL8= 0.90379E 00YHRSR8= 0.92119E 00YARSR8= 0.98754E 00
I= 9
 YHRS8= 0.85679E 00YARS8= 0.12018E 01
I= 1
```

```
 YHRS1=          0.11562E 01YARS1=          0.78040E 00
I=       2
 YHRSL1=        0.89083E 00YARSL1=         0.85330E 00YHRSR1=         0.94875E 00YARSR1=         0.98579E 00
I=       3
 YHRS1=          0.85539E 00YARS1=          0.73338E 00
I=       4
 YHRSL1=        0.87573E 00YARSL1=         0.84683E 00YHRSR1=         0.92605E 00YARSR1=         0.97694E 00
I=       5
 YHRS1=          0.85487E 00YARS1=          0.73331E 00
I=       6
 YHRSL1=        0.87271E 00YARSL1=         0.84683E 00YHRSR1=         0.92605E 00YARSR1=         0.97694E 00
I=       7
 YHRS1=          0.84986E 00YARS1=          0.73350E 00
I=       8
 YHRSL1=        0.81184E 00YARSL1=         0.85494E 00YHRSR1=         0.92119E 00YARSR1=         0.98754E 00
I=       9
 YHRS1=          0.72870E 00YARS1=          0.11553E 01
***J=     3
I=       1
 YHRS2=          0.11562E 01YARS2=          0.78040E 00
I=       2
 YHRSL2=        0.89083E 00YARSL2=         0.85330E 00YHRSR2=         0.94875E 00   YARSR2=      0.98579E 00
I=       3
 YHRS2=          0.92636E 00YARS2=          0.74414E 00
I=       4
 YHRSL2=        0.87373E 00YARSL2=         0.84683E 00YHRSR2=         0.92605E 00   YARSR2=      0.97694E 00
I=       5
 YHRS2=          0.92607E 00YARS2=          0.74411E 00
I=       6
 YHRSL2=        0.87271E 00YARSL2=         0.84683E 00YHRSR2=         0.92605E 00   YARSR2=      0.97694E 00
I=       7
 YHRS2=          0.92049E 00YARS2=          0.74430E 00
I=       8
 YHRSL2=        0.81184E 00YARSL2=         0.85494E 00YHRSR2=         0.92119E 00   YARSR2=      0.98754E 00
I=       9
 YHRS2=          0.72870E 00YARS2=          0.11553E 01
I=       1
 YHRS3=          0.13122E 01YARS3=          0.81787E 00
I=       2
 YHRSR3=        0.86683E 00YARSR3=         0.82969E 00YHRSL3=         0.89083E 00YARSL3 =        0.85330E 00
I=       3
 YHRS3=          0.11393E 01YARS3=          0.79196E 00
I=       4
 YHRSR3=        0.85007E 00YARSR3=         0.82253E 00YHRSL3=         0.87373E 00YARSL3 =        0.84683E 00
I=       5
 YHRS3=          0.11389E 01YARS3=          0.79194E 00
I=       6
 YHRSR3=        0.84907E 00YARSR3=         0.82253E 00YHRSL3=         0.87271E 00YARSL3 =        0.84683E 00
I=       7
 YHRS3=          0.11162E 01YARS3=          0.79361E 00
I=       8
 YHRSR3=        0.82128E 00YARSR3=         0.84971E 00YHRSL3=         0.81184E 00YARSL3 =        0.85494E 00
I=       9
 YHRS3=          0.72870E 00YARS3=          0.11553E 01
I=       1
 YHRS4=          0.13122E 01YARS4=          0.81787E 00
I=       2
 YHRSR4=        0.86683E 00YARSR4=         0.82969E 00YHRSL4=         0.89083E 00   YARSL4=      0.85330E 00
```

```
I=          3
 YHRS4=          0.11393E 01YARS4=          0.79196E 00
I=          4
 YHRSR4=         0.85007E 00YARSR4=         0.82253E 00YHRSL4=         0.87373E 00        YARSL4=         0.84683E 00
I=          5
 YHRS4=          0.11389E 01YARS4=          0.79194E 00
I=          6
 YHRSR4=         0.84907E 00YARSR4=         0.82253E 00YHRSL4=         0.87271E 00        YARSL4=         0.84683E 00
I=          7
 YHRS4=          0.11162E 01YARS4=          0.79361E 00
I=          8
 YHRSR4=         0.82128E 00YARSR4=         0.84971E 00YHRSL4=         0.81184E 00        YARSL4=         0.85494E 00
I=          9
 YHRS4=          0.72870E 00YARS4=          0.11553E 01
I=          1
 YHRS5=          0.13122E 01YARS5=          0.81787E 00
I=          2
 YHRSR5=         0.95152E 00YARSR5=         0.95241E 00YHRSL5=         0.89083E 00YARSL5=         0.85330E 00
I=          3
 YHRS5=          0.11428E 01YARS5=          0.93696E 00
I=          4
 YHRSR5=         0.92018E 00YARSR5=         0.94086E 00YHRSL5=         0.87373E 00YARSL5=         0.84683E 00
I=          5
 YHRS5=          0.11377E 01YARS5=          0.93534E 00
I=          6
 YHRSR5=         0.91918E 00YARSR5=         0.94086E 00YHRSL5=         0.87271E 00YARSL5=         0.84683E 00
I=          7
 YHRS5=          0.11196E 01YARS5=          0.93692E 00
I=          8
 YHRSR5=         0.89037E 00YARSR5=         0.96761E 00YHRSL5=         0.81184E 00YARSL5=         0.85494E 00
J=          9
 YHRS5=          0.80720E 00YARS5=          0.12860E 01
I=          1
 YHRS6=          0.13122E 01YARS6=          0.81787E 00
I=          2
 YHRSR6=         0.95152E 00YARSR6=         0.95241E 00YHRSL6=         0.89083E 00YARSL6=         0.85330E 00
I=          3
 YHRS6=          0.11412E 01YARS6=          0.87105E 00
I=          4
 YHRSR6=         0.92018E 00YARSR6=         0.94086E 00YHRSL6=         0.87373E 00YARSL6=         0.84683E 00
I=          5
 YHRS6=          0.11382E 01YARS6=          0.87016E 00
I=          6
 YHRSR6=         0.91918E 00YARSR6=         0.94086E 00YHRSL6=         0.87271E 00YARSL6=         0.84683E 00
I=          7
 YHRS6=          0.11180E 01YARS6=          0.87178E 00
I=          8
 YHRSR6=         0.89037E 00YARSR6=         0.96761E 00YHRSL6=         0.81184E 00YARSL6=         0.85494E 00
I=          9
 YHRS6=          0.80720E 00YARS6=          0.12860E 01
I=          1
 YHRS7=          0.12346E 01YARS7=          0.71402E 00
I=          2
 YHRSL7=         0.11210E 01YARSL7=         0.88825E 00YHRSR7=         0.95152E 00YARSR7=         0.95241E 00
I=          3
 YHRS7=          0.10750E 01YARS7=          0.69183E 00
I=          4
 YHRSL7=         0.11037E 01YARSL7=         0.88181E 00YHRSR7=         0.92018E 00YARSR7=         0.94086E 00
```

```
I=           5
 YHRS7=              0.10746E 01YARS7=              0.69182E 00
I=           6
 YHRSL7=             0.10982E 01YARSL7=            0.88190E 00YHRSR7=          0.91918E 00YARSR7=          0.94086E 00
I=           7
 YHRS7=              0.10528E 01YARS7=              0.69384E 00
I=           8
 YHRSL7=             0.96202E 00YARSL7=            0.90856E 00YHRSR7=          0.89037E 00YARSR7=          0.96761E 00
I=           9
 YHRS7=              0.80720E 00YARS7=              0.12860E 01
I=           1
 YHRS8=              0.12346E 01YARS8=              0.71402E 00
I=           2
 YHRSL8=             0.11210E 01YARSL8=            0.88825E 00YHRSR8=          0.95152E 00YARSR8=          0.95241E 00
I=           3
 YHRS8=              0.10750E 01YARS8=              0.69183E 00
I=           4
 YHRSL8=             0.11037E 01YARSL8=            0.88181E 00YHRSR8=          0.92018E 00YARSR8=          0.94086E 00
I=           5
 YHRS8=              0.10746E 01YARS8=              0.69182E 00
I=           6
 YHRSL8=             0.10982E 01YARSL8=            0.88190E 00YHRSR8=          0.91918E 00YARSR8=          0.94086E 00
I=           7
 YHRS8=              0.10528E 01YARS8=              0.69384E 00
I=           8
 YHRSL8=             0.96202E 00YARSL8=            0.90856E 00YHRSR8=          0.89037E 00YARSR8=          0.96761E 00
I=           9
 YHRS8=              0.80720E 00YARS8=              0.12860E 01
I=           1
 YHRS1=              0.12346E 01YARS1=              0.71402E 00
I=           2
 YHRSL1=             0.94204E 00YARSL1=            0.83573E 00YHRSR1=          0.95152E 00YARSR1=          0.95241E 00
I=           3
 YHRS1=              0.90872E 00YARS1=              0.67136E 00
I=           4
 YHRSL1=             0.91779E 00YARSL1=            0.82713E 00YHRSR1=          0.92018E 00YARSR1=          0.94086E 00
I=           5
 YHRS1=              0.90741E 00YARS1=              0.67124E 00
I=           6
 YHRSL1=             0.91351E 00YARSL1=            0.82720E 00YHRSR1=          0.91918E 00YARSR1=          0.94086E 00
I=           7
 YHRS1=              0.88998E 00YARS1=              0.67316E 00
I=           8
 YHRSL1=             0.80799E 00YARSL1=            0.84939E 00YHRSR1=          0.89037E 00YARSR1=          0.96761E 00
I=           9
 YHRS1=              0.68920E 00YARS1=              0.12351E 01
***J=         4
I=           1
 YHRS2=              0.12346E 01YARS2=              0.71402E 00
I=           2
 YHRSL2=             0.94204E 00YARSL2=            0.83573E 00YHRSR2=          0.95152E 00    YARSR2=      0.95241E 00
I=           3
 YHRS2=              0.98431E 00YARS2=              0.68066E 00
I=           4
 YHRSL2=             0.91779E 00YARSL2=            0.82713E 00YHRSR2=          0.92018E 00    YARSR2=      0.94086E 00
I=           5
 YHRS2=              0.98341E 00YARS2=              0.68059E 00
I=           6
```

```
 YHRSL2=       0.91551E 00YARSL2=       0.82720E 00YHRSR2=       0.91918E 00      YARSR2=       0.94086E 00
I=       7
 YHRS2=        0.96401E 00YARS2=        0.68256E 00
I=       8
 YHRSL2=       0.80799E 00YARSL2=       0.84939E 00YHRSR2=       0.89037E 00      YARSR2=       0.96761E 00
I=       9
 YHRS2=        0.68920E 00YARS2=        0.12351E 01
I=       1
 YHRS3=        0.13986E 01YARS3=        0.75846E 00
I=       2
 YHRSR3=       0.87952E 00YARSR3=       0.79995E 00YHRSL3=       0.94204E 00YARSL3 =      0.83573E 00
I=       3
 YHRS3=        0.12077E 01YARS3=        0.73380E 00
I=       4
 YHRSR3=       0.85628E 00YARSR3=       0.79060E 00YHRSL3=       0.91779E 00YARSL3 =      0.82713E 00
I=       5
 YHRS3=        0.12059E 01YARS3=        0.73378E 00
I=       6
 YHRSR3=       0.85206E 00YARSR3=       0.79069E 00YHRSL3=       0.91351E 00YARSL3 =      0.82720E 00
I=       7
 YHRS3=        0.11570E 01YARS3=        0.73957E 00
I=       8
 YHRSR3=       0.79078E 00YARSR3=       0.83712E 00YHRSL3=       0.80799E 00YARSL3 =      0.84939E 00
I=       9
 YHRS3=        0.68920E 00YARS3=        0.12351E 01
I=       1
 YHRS4=        0.13986E 01YARS4=        0.75846E 00
I=       2
 YHRSR4=       0.87952E 00YARSR4=       0.79995E 00YHRSL4=       0.94204E 00      YARSL4=       0.83573E 00
I=       3
 YHRS4=        0.12077E 01YARS4=        0.73380E 00
I=       4
 YHRSR4=       0.85628E 00YARSR4=       0.79060E 00YHRSL4=       0.91779E 00      YARSL4=       0.82713E 00
I=       5
 YHRS4=        0.12059E 01YARS4=        0.73378E 00
I=       6
 YHRSR4=       0.85206E 00YARSR4=       0.79069E 00YHRSL4=       0.91351E 00      YARSL4=       0.82720E 00
I=       7
 YHRS4=        0.11570E 01YARS4=        0.73957E 00
I=       8
 YHRSR4=       0.79078E 00YARSR4=       0.83712E 00YHRSL4=       0.80799E 00      YARSL4=       0.84939E 00
I=       9
 YHRS4=        0.68920E 00YARS4=        0.12351E 01
I=       1
 YHRS5=        0.13986E 01YARS5=        0.75846E 00
I=       2
 YHRSR5=       0.97134E 00YARSR5=       0.91296E 00YHRSL5=       0.94204E 00YARSL5=       0.83573E 00
I=       3
 YHRS5=        0.12002E 01YARS5=        0.87514E 00
I=       4
 YHRSR5=       0.93217E 00YARSR5=       0.89949E 00YHRSL5=       0.91779E 00YARSL5=       0.82713E 00
I=       5
 YHRS5=        0.11921E 01YARS5=        0.87303E 00
I=       6
 YHRSR5=       0.92795E 00YARSR5=       0.89957E 00YHRSL5=       0.91351E 00YARSL5=       0.82720E 00
I=       7
 YHRS5=        0.11525E 01YARS5=        0.87854E 00
I=       8
```

```
 YHRSR5= 0.86453E 00YARSR5= 0.94579E 00YHRSL5= 0.80799E 00YARSL5= 0.84939E 00
I=       9
 YHRS5= 0.76745E 00YARS5= 0.13588E 01
I=       1
 YHRS6= 0.13986E 01YARS6= 0.75846E 00
I=       2
 YHRSR6= 0.97134E 00YARSR6= 0.91296E 00YHRSL6= 0.94204E 00YARSL6= 0.83573E 00
I=       3
 YHRS6= 0.12036E 01YARS6= 0.81090E 00
I=       4
 YHRSR6= 0.93217E 00YARSR6= 0.89949E 00YHRSL6= 0.91779E 00YARSL6= 0.82713E 00
I=       5
 YHRS6= 0.11984E 01YARS6= 0.80973E 00
I=       6
 YHRSR6= 0.92795E 00YARSR6= 0.89957E 00YHRSL6= 0.91351E 00YARSL6= 0.82720E 00
I=       7
 YHRS6= 0.11546E 01YARS6= 0.81537E 00
I=       8
 YHRSR6= 0.86453E 00YARSR6= 0.94579E 00YHRSL6= 0.80799E 00YARSL6= 0.84939E 00
I=       9
 YHRS6= 0.76745E 00YARS6= 0.13588E 01
I=       1
 YHRS7= 0.13144E 01YARS7= 0.66284E 00
I=       2
 YHRSL7= 0.11833E 01YARSL7= 0.86769E 00YHRSR7= 0.97134E 00YARSR7= 0.91296E 00
I=       3
 YHRS7= 0.11381E 01YARS7= 0.64167E 00
I=       4
 YHRSL7= 0.11586E 01YARSL7= 0.85913E 00YHRSR7= 0.93217E 00YARSR7= 0.89949E 00
I=       5
 YHRS7= 0.11363E 01YARS7= 0.64165E 00
I=       6
 YHRSL7= 0.11446E 01YARSL7= 0.85949E 00YHRSR7= 0.92795E 00YARSR7= 0.89957E 00
I=       7
 YHRS7= 0.10894E 01YARS7= 0.64762E 00
I=       8
 YHRSL7= 0.94832E 00YARSL7= 0.90702E 00YHRSR7= 0.86453E 00YARSR7= 0.94579E 00
I=       9
 YHRS7= 0.76745E 00YARS7= 0.13588E 01
I=       1
 YHRS8= 0.13144E 01YARS8= 0.66284E 00
I=       2
 YHRSL8= 0.11833E 01YARSL8= 0.86769E 00YHRSR8= 0.97134E 00YARSR8= 0.91296E 00
I=       3
 YHRS8= 0.11381E 01YARS8= 0.64167E 00
I=       4
 YHRSL8= 0.11586E 01YARSL8= 0.85913E 00YHRSR8= 0.93217E 00YARSR8= 0.89949E 00
I=       5
 YHRS8= 0.11363E 01YARS8= 0.64165E 00
I=       6
 YHRSL8= 0.11446E 01YARSL8= 0.85949E 00YHRSR8= 0.92795E 00YARSR8= 0.89957E 00
I=       7
 YHRS8= 0.10894E 01YARS8= 0.64762E 00
I=       8
 YHRSL8= 0.94832E 00YARSL8= 0.90702E 00YHRSR8= 0.86453E 00YARSR8= 0.94579E 00
I=       9
 YHRS8= 0.76745E 00YARS8= 0.13588E 01
I=       1
```

```
   YHRS1=          0.13144E 01YARS1=          0.66284E 00
I=       2
   YHRSL1=      0.99519E 00YARSL1=      0.81122E 00YHRSR1=      0.97134E 00YARSR1=      0.91296E 00
I=       3
   YHRS1=          0.96184E 00YARS1=          0.62344E 00
I=       4
   YHRSL1=      0.96411E 00YARSL1=      0.80103E 00YHRSR1=      0.93217E 00YARSR1=      0.89949E 00
I=       5
   YHRS1=          0.95904E 00YARS1=          0.62327E 00
I=       6
   YHRSL1=      0.95326E 00YARSL1=      0.80132E 00YHRSR1=      0.92795E 00YARSR1=      0.89957E 00
I=       7
   YHRS1=          0.92137E 00YARS1=          0.62894E 00
I=       8
   YHRSL1=      0.80007E 00YARSL1=      0.84123E 00YHRSR1=      0.86453E 00YARSR1=      0.94579E 00
I=       9
   YHRS1=          0.65719E 00YARS1=          0.13042E 01
***J=    5
I=       1
   YHRS2=          0.13144E 01YARS2=          0.66284E 00
I=       2
   YHRSL2=      0.99519E 00YARSL2=      0.81122E 00YHRSR2=      0.97134E 00   YARSR2=      0.91296E 00
I=       3
   YHRS2=          0.10420E 01YARS2=          0.63172E 00
I=       4
   YHRSL2=      0.96411E 00YARSL2=      0.80103E 00YHRSR2=      0.93217E 00   YARSR2=      0.89949E 00
I=       5
   YHRS2=          0.10396E 01YARS2=          0.63163E 00
I=       6
   YHRSL2=      0.95326E 00YARSL2=      0.80132E 00YHRSR2=      0.92795E 00   YARSR2=      0.89957E 00
I=       7
   YHRS2=          0.99776E 00YARS2=          0.63743E 00
I=       8
   YHRSL2=      0.80007E 00YARSL2=      0.84123E 00YHRSR2=      0.86453E 00   YARSR2=      0.94579E 00
I=       9
   YHRS2=          0.65719E 00YARS2=          0.13042E 01
I=       1
   YHRS3=          0.14869E 01YARS3=          0.71063E 00
I=       2
   YHRSR3=      0.90469E 00YARSR3=      0.76593E 00YHRSL3=      0.99519E 00YARSL3 =     0.81122E 00
I=       3
   YHRS3=          0.12764E 01YARS3=          0.68701E 00
I=       4
   YHRSR3=      0.87540E 00YARSR3=      0.75502E 00YHRSL3=      0.96411E 00YARSL3 =     0.80103E 00
I=       5
   YHRS3=          0.12714E 01YARS3=          0.68702E 00
I=       6
   YHRSR3=      0.86477E 00YARSR3=      0.75534E 00YHRSL3=      0.95326E 00YARSL3 =     0.80132E 00
I=       7
   YHRS3=          0.11871E 01YARS3=          0.69937E 00
I=       8
   YHRSR3=      0.76542E 00YARSR3=      0.82252E 00YHRSL3=      0.80007E 00YARSL3 =     0.84123E 00
I=       9
   YHRS3=          0.65719E 00YARS3=          0.13042E 01
I=       1
   YHRS4=          0.14869E 01YARS4=          0.71063E 00
I=       2
   YHRSR4=      0.90469E 00YARSR4=      0.76593E 00YHRSL4=      0.99519E 00   YARSL4=      0.81122E 00
```

```
I=  5
 YHRS4=      0.12764E 01YARS4=      0.68701E 00
I=  4
 YHRSR4=     0.87540E 00YARSR4=     0.75502E 00YHRSL4=     0.96411E 00    YARSL4=     0.80103E 00
I=  5
 YHRS4=      0.12714E 01YARS4=      0.68702E 00
I=  6
 YHRSR4=     0.86477E 00YARSR4=     0.75534E 00YHRSL4=     0.95326E 00    YARSL4=     0.80132E 00
I=  7
 YHRS4=      0.11871E 01YARS4=      0.69937E 00
I=  8
 YHRSR4=     0.76542E 00YARSR4=     0.82252E 00YHRSL4=     0.80007E 00    YARSL4=     0.84123E 00
I=  9
 YHRS4=      0.65719E 00YARS4=      0.13042E 01
I=  1
 YHRS5=      0.14869E 01YARS5=      0.71063E 00
I=  2
 YHRSR5=     0.10034E 01YARSR5=     0.87137E 00YHRSL5=     0.99519E 00YARSL5=     0.81122E 00
I=  3
 YHRS5=      0.12613E 01YARS5=      0.82314E 00
I=  4
 YHRSR5=     0.95665E 00YARSR5=     0.85657E 00YHRSL5=     0.96411E 00YARSL5=     0.80103E 00
I=  5
 YHRS5=      0.12491E 01YARS5=      0.82071E 00
I=  6
 YHRSR5=     0.94595E 00YARSR5=     0.85688E 00YHRSL5=     0.95326E 00YARSL5=     0.80132E 00
I=  7
 YHRS5=      0.11798E 01YARS5=      0.83249E 00
I=  8
 YHRSR5=     0.84270E 00YARSR5=     0.92446E 00YHRSL5=     0.80007E 00YARSL5=     0.84123E 00
I=  9
 YHRS5=      0.73505E 00YARS5=      0.14211E 01
I=  1
 YHRS6=      0.14869E 01YARS6=      0.71063E 00
I=  2
 YHRSR6=     0.10034E 01YARSR6=     0.87136E 00YHRSL6=     0.99519E 00YARSL6=     0.81122E 00
I=  3
 YHRS6=      0.12682E 01YARS6=      0.76126E 00
I=  4
 YHRSR6=     0.95665E 00YARSR6=     0.85657E 00YHRSL6=     0.96411E 00YARSL6=     0.80103E 00
I=  5
 YHRS6=      0.12592E 01YARS6=      0.75994E 00
I=  6
 YHRSR6=     0.94595E 00YARSR6=     0.85688E 00YHRSL6=     0.95326E 00YARSL6=     0.80132E 00
I=  7
 YHRS6=      0.11831E 01YARS6=      0.77198E 00
I=  8
 YHRSR6=     0.84270E 00YARSR6=     0.92446E 00YHRSL6=     0.80007E 00YARSL6=     0.84123E 00
I=  9
 YHRS6=      0.73505E 00YARS6=      0.14211E 01
I=  1
 YHRS7=      0.13964E 01YARS7=      0.62141E 00
I=  2
 YHRSL7=     0.12484E 01YARSL7=     0.84083E 00YHRSR7=     0.10034E 01YARSR7=     0.87136E 00
I=  3
 YHRS7=      0.12019E 01YARS7=      0.60108E 00
I=  4
 YHRSL7=     0.12163E 01YARSL7=     0.83069E 00YHRSR7=     0.95665E 00YARSR7=     0.85657E 00
```

```
I=          5
  YHRS7=          0.11970E 01YARS7=          0.60109E 00
I=          6
  YHRSL7=       0.11886E 01YARSL7=       0.83161E 00YHRSR7=       0.94595E 00YARSR7=       0.85688E 00
I=          7
  YHRS7=          0.11163E 01YARS7=          0.61311E 00
I=          8
  YHRSL7=       0.93270E 00YARSL7=       0.90234E 00YHRSR7=       0.84270E 00YARSR7=       0.92446E 00
I=          9
  YHRS7=          0.73505E 00YARS7=          0.14211E 01
I=          1
  YHRS8=          0.13964E 01YARS8=          0.62141E 00
I=          2
  YHRSL8=       0.12484E 01YARSL8=       0.84083E 00YHRSR8=       0.10034E 01YARSR8=       0.87136E 00
I=          3
  YHRS8=          0.12019E 01YARS8=          0.60108E 00
I=          4
  YHRSL8=       0.12163E 01YARSL8=       0.83069E 00YHRSR8=       0.95665E 00YARSR8=       0.85657E 00
I=          5
  YHRS8=          0.11970E 01YARS8=          0.60109E 00
I=          6
  YHRSL8=       0.11886E 01YARSL8=       0.83161E 00YHRSR8=       0.94595E 00YARSR8=       0.85688E 00
I=          7
  YHRS8=          0.11163E 01YARS8=          0.61311E 00
I=          8
  YHRSL8=       0.93270E 00YARSL8=       0.90234E 00YHRSR8=       0.84270E 00YARSR8=       0.92446E 00
I=          9
  YHRS8=          0.73505E 00YARS8=          0.14211E 01
I=          1
  YHRS1=          0.13964E 01YARS1=          0.62141E 00
I=          2
  YHRSL1=       0.10506E 01YARSL1=       0.78298E 00YHRSR1=       0.10034E 01YARSR1=       0.87136E 00
I=          3
  YHRS1=          0.10157E 01YARS1=          0.58449E 00
I=          4
  YHRSL1=       0.10126E 01YARSL1=       0.77162E 00YHRSR1=       0.95665E 00YARSR1=       0.85657E 00
I=          5
  YHRS1=          0.10100E 01YARS1=          0.58433E 00
I=          6
  YHRSL1=       0.99107E 00YARSL1=       0.77238E 00YHRSR1=       0.94595E 00YARSR1=       0.85688E 00
I=          7
  YHRS1=          0.94500E 00YARS1=          0.59573E 00
I=          8
  YHRSL1=       0.79000E 00YARSL1=       0.83223E 00YHRSR1=       0.84270E 00YARSR1=       0.92446E 00
I=          9
  YHRS1=          0.63084E 00YARS1=          0.13631E 01
***J=       6
I=          1
  YHRS2=          0.13964E 01YARS2=          0.62141E 00
I=          2
  YHRSL2=       0.10506E 01YARSL2=       0.78298E 00YHRSR2=       0.10034E 01      YARSR2=       0.87136E 00
I=          3
  YHRS2=          0.11003E 01YARS2=          0.59203E 00
I=          4
  YHRSL2=       0.10126E 01YARSL2=       0.77162E 00YHRSR2=       0.95665E 00      YARSR2=       0.85657E 00
I=          5
  YHRS2=          0.10950E 01YARS2=          0.59195E 00
I=          6
```

APPENDIX D

TABLES:

pH-PARAMETRIC PUMPING

Conditions for Computational the Results with variable parameters:

Operating Variable	Value
Volume of Fluid Phase per Column	30.0 cm^3
Volume of Solid Phase per Column	20.0 cm^3
Initial Solute Concentration (Normalized)	1.0 kg mole/cm^3
Dead Volume of Top Reservoir	30.0 cm^3
Dead Volume of Middle Reservoir	30.0 cm^3
Dead Volume of Bottom Reservoir	30.0 cm^3
Number of Cycles	30

TABLE D-1

SEPARATION OF PROTEIN A Via TWO-COLUMNS

$\beta = 2.14$

$k_{P_1}^- = 0.6$, $k_{P_1}^+ = 1.2$, $k_{P_2}^- = k_{P_3}^- = 1.5$, $k_{P_2}^+ = k_{P_3}^+ = 0.7$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	1.0783	0.7639	1.4007
2	1.1489	0.7213	1.5928
3	1.2100	0.6882	1.7583
4	1.2615	0.6621	1.9053
5	1.3043	0.6416	2.0330
6	1.3392	0.6253	2.1417
7	1.3675	0.6125	2.2327
8	1.3902	0.6024	2.3079
9	1.4038	0.5945	2.3691
10	1.4227	0.5882	2.4186
11	1.4340	0.5834	2.4580
12	1.4429	0.5796	2.4894
13	1.4498	0.5767	2.5139
14	1.4553	0.5744	2.5334
15	1.4595	0.5727	2.5485
16	1.4627	0.5713	2.5602
17	1.4653	0.5703	2.5694
18	1.4672	0.5695	2.5763
19	1.4687	0.5689	2.5817
20	1.4699	0.5684	2.5860
25	1.4726	0.5673	2.5959
30	1.4734	0.5670	2.5987

TABLE D-2

SEPARATION OF PROTEIN A Via TWO-COLUMNS

$\beta = 3.00$

$k^-_{P_1} = 0.6$, $k^+_{P_1} = 1.2$, $k^-_{P_2} = k^-_{P_3} = 1.5$, $k^+_{P_2} = k^+_{P_3} = 0.5$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Producy $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	1.1539	0.8691	1.3278
2	1.2827	0.7834	1.6375
3	1.3873	0.7247	1.9142
4	1.4706	0.6832	2.1526
5	1.5363	0.6529	2.3530
6	1.5878	0.6304	2.5187
7	1.6279	0.6135	2.6535
8	1.6590	0.6007	2.7619
9	1.6831	0.5909	2.8485
10	1.7017	0.5834	2.9170
11	1.7161	0.5777	2.9708
12	1.7272	0.5732	3.0131
13	1.7358	0.5699	3.0461
14	1.7424	0.5673	3.0717
15	1.7474	0.5653	3.0914
16	1.7513	0.5637	3.1067
17	1.7543	0.5625	3.1185
18	1.7566	0.5616	3.1277
19	1.7584	0.5609	3.1347
20	1.7598	0.5604	3.1403
25	1.7630	0.5591	3.1532
30	1.7638	0.5588	3.1564

TABLE D-3

SEPARATION OF PROTEIN A Via TWO-COLUMNS

$\rho = 3.20$

$k^-_{P_1} = 1.0$, $k^+_{P_1} = 1.2$, $k^-_{P_2} = k^-_{P_3} = 1.6$, $k^+_{P_2} = k^+_{P_3} = 0.5$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	1.1539	0.8691	1.3278
2	1.2907	0.7834	1.6477
3	1.4192	0.7242	1.9598
4	1.5441	0.6812	2.2667
5	1.6673	0.6488	2.5699
6	1.7895	0.6234	2.8706
7	1.9103	0.6029	3.1686
8	2.0290	0.5859	3.4629
9	2.1448	0.5716	3.7523
10	2.2571	0.5592	4.0362
11	2.3653	0.5483	4.3136
12	2.4688	0.5386	4.5836
13	2.5673	0.5298	4.8455
14	2.6606	0.5218	5.0992
15	2.7486	0.5143	5.3444
16	2.8312	0.5073	5.5807
17	2.9086	0.5007	5.8087
18	2.9807	0.4945	6.0278
19	3.0479	0.4885	6.2389
20	3.1101	0.4828	6.4415
25	3.3545	0.4571	7.3380
30	3.3546	0.4570	7.3400

TABLE D-4

SEPARATION OF PROTEIN A Via TWO-COLUMNS

$\beta = 3.50$

$k^-_{P_1} = 0.8$, $k^+_{P_1} = 1.2$, $k^-_{P_2} = k^-_{P_3} = 1.4$, $k^+_{P_2} = k^+_{P_3} = 0.4$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	1.1250	0.8214	1.3697
2	1.2421	0.7691	1.6151
3	1.3528	0.7105	1.9040
4	1.4576	0.6534	2.2310
5	1.5568	0.6009	2.5909
6	1.6500	0.5542	2.9773
7	1.7375	0.5133	3.3848
8	1.8190	0.4779	3.8066
9	1.8948	0.4472	4.2367
10	1.9650	0.4208	4.6692
11	2.0297	0.3981	5.0986
12	2.0893	0.3785	5.5203
13	2.1440	0.3615	5.9302
14	2.1940	0.3469	6.3246
15	2.2398	0.3342	6.7016
16	2.2815	0.3232	7.0587
17	2.3195	0.3137	7.3952
18	2.3541	0.3053	7.7103
19	2.3855	0.2981	8.0034
20	2.4140	0.2917	8.2753
25	2.5209	0.2699	9.3398
30	2.5853	0.2583	10.0093

TABLE D-5

SEPARATION OF PROTEIN A Via TWO-COLUMNS

$\beta = 4.00$

$k^-_{P_1} = 0.8$, $k^+_{P_1} = 1.0$, $k^-_{P_2} = k^-_{P_3} = 1.6$, $k^+_{P_2} = k^+_{P_3} = 0.4$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	1.1860	0.8214	1.4440
2	1.3548	0.7634	1.7746
3	1.5100	0.6978	2.1640
4	1.6534	0.6339	2.6085
5	1.7855	0.5755	3.1023
6	1.9070	0.5240	3.6390
7	2.0182	0.4793	4.2104
8	2.1196	0.4409	4.8073
9	2.2116	0.4080	5.4203
10	2.2949	0.3799	6.0405
11	2.3700	0.3559	6.6588
12	2.4375	0.3354	7.2668
13	2.4981	0.3179	7.8579
14	2.5523	0.3029	8.4259
15	2.6007	0.2901	8.9661
16	2.6439	0.2790	9.4753
17	2.6823	0.2695	9.9514
18	2.7165	0.2614	10.3929
19	2.7468	0.2543	10.7997
20	2.7737	0.2483	11.1721
25	2.8683	0.2282	12.5703
30	2.9191	0.2183	13.3738

TABLE D-6

SEPARATION OF PROTEIN A Via TWO- COLUMNS

$\beta = 5.67$

$k^-_{P_1} = 0.6$, $k^+_{P_1} = 1.0$, $k^-_{P_2} = k^-_{P_3} = 1.7$, $k^+_{P_2} = k^+_{P_3} = 0.3$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	1.2746	0.8214	1.5518
2	1.5133	0.7556	2.0027
3	1.7248	0.6806	2.5343
4	1.9136	0.6081	3.1471
5	2.0821	0.5427	3.8368
6	2.2321	0.4857	4.5956
7	2.3653	0.4369	5.4133
8	2.4831	0.3956	6.2768
9	2.5869	0.3607	7.1715
10	2.6780	0.3314	8.0818
11	2.7577	0.3067	8.9924
12	2.8272	0.2859	9.8888
13	2.8878	0.2684	10.7585
14	2.9404	0.2537	11.5905
15	2.9860	0.2413	12.3757
16	3.0255	0.2308	13.1082
17	3.0596	0.2220	13.7838
18	3.0890	0.2145	14.4016
19	3.1144	0.2082	14.9609
20	3.1363	0.2028	15.4635
25	3.2073	0.1861	17.2380
30	3.2403	0.1787	18.1367

TABLE D-7

SEPARATION OF PROTEIN B Via TWO-COLUMNS

$\beta = 0.18$

$k^-_{P_1} = k^-_{P_2} = 0.3$, $k^+_{P_1} = k^+_{P_2} = 1.7$, $k^-_{P_3} = 2.0$, $k^+_{P_3} = 0.3$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	1.0803	1.3361	0.8086
2	1.1085	1.4436	0.7679
3	1.1103	1.5155	0.7326
4	1.0994	1.5662	0.7020
5	1.0832	1.6037	0.6754
6	1.0656	1.6327	0.6527
7	1.0484	1.6558	0.6332
8	1.0326	1.6748	0.6166
9	1.0184	1.6906	0.6024
10	1.0060	1.7039	0.5904
11	0.9952	1.7152	0.5802
12	0.9859	1.7248	0.5716
13	0.9779	1.7330	0.5643
14	0.9710	1.7400	0.5580
15	0.9651	1.7459	0.5528
16	0.9601	1.7510	0.5483
17	0.9559	1.7554	0.5445
18	0.9522	1.7591	0.5413
19	0.9491	1.7622	0.5386
20	0.9465	1.7649	0.5363
25	0.9381	1.7735	0.5290
30	0.9343	1.7773	0.5257

TABLE D-8

SEPARATION OF PROTEIN B Via TWO-COLUMNS

$\beta = 0.33$

$k^-_{P_1} = k^-_{P_2} = 0.5$, $k^+_{P_1} = k^+_{P_2} = 1.5$, $k^-_{P_3} = 1.6$, $k^+_{P_3} = 0.4$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	0.8709	1.0788	0.8073
2	0.7807	1.1689	0.6679
3	0.7163	1.2492	0.5734
4	0.6693	1.3194	0.5073
5	0.6342	1.3798	0.4597
6	0.6075	1.4312	0.4245
7	0.5869	1.4747	0.3980
8	0.5706	1.5113	0.3776
9	0.5577	1.5420	0.3617
10	0.5472	1.5676	0.3491
11	0.5388	1.5891	0.3391
12	0.5319	1.6070	0.3310
13	0.5263	1.6219	0.3245
14	0.5216	1.6343	0.3192
15	0.5178	1.6447	0.3148
16	0.5146	1.6533	0.3113
17	0.5120	1.6605	0.3083
18	0.5098	1.6665	0.3059
19	0.5080	1.6714	0.3039
20	0.5064	1.6756	0.3022
25	0.5019	1.6879	0.2974
30	0.5001	1.6929	0.2954

TABLE D-9

SEPARATION OF PROTEIN B Via TWO-COLUMNS

$\beta = 0.40$

$k^-_{P_1} = k^-_{P_2} = 0.6$, $k^+_{P_1} = k^+_{P_2} = 1.5$, $k^-_{P_3} = 1.5$, $k^+_{P_3} = 0.5$

Number of Cycles, n	Top Product $\langle y_T \rangle_n$	Bottom Product $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$
1	0.8597	1.1209	0.7670
2	0.7698	1.2302	0.6258
3	0.7092	1.3233	0.5359
4	0.6665	1.4002	0.4760
5	0.6355	1.4625	0.4345
6	0.6123	1.5123	0.4049
7	0.5948	1.5519	0.3833
8	0.5815	1.5831	0.3673
9	0.5712	1.6076	0.3553
10	0.5633	1.6268	0.3462
11	0.5571	1.6418	0.3393
12	0.5524	1.6535	0.3340
13	0.5487	1.6626	0.3300
14	0.5459	1.6696	0.3270
15	0.5437	1.6751	0.3246
16	0.5420	1.6793	0.3228
17	0.5407	1.6826	0.3213
18	0.5397	1.6851	0.3203
19	0.5389	1.6871	0.3194
20	0.5383	1.6886	0.3188
25	0.5368	1.6923	0.3172
30	0.5365	1.6932	0.3168

TABLE D-10

SEPARATION OF PROTEIN MIXTURE A AND B Via TWO-COLUMNS

Protein A : $k_{P_1}^- = 0.6$, $k_{P_1}^+ = 1.2$, $k_{P_2}^- = k_{P_3}^- = 1.5$, $k_{P_2}^+ = k_{P_3}^+ = 0.5$

Protein B : $k_{P_1}^- = 0.5$, $k_{P_1}^+ = k_{P_2}^+ = 1.5$, $k_{P_2}^- = 0.5$, $k_{P_3}^- = 1.6$, $k_{P_3}^+ = 0.4$

Number of Cycles, n	Protein A $\langle y_T \rangle_n$	Protein A $\langle y_B \rangle_n$	Protein B $\langle y_T \rangle_n$	Protein B $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. A)	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. B)
1	1.1539	0.8691	0.8709	1.0788	1.3278	0.8037
5	1.5363	0.6529	0.6342	1.3798	2.3530	0.4597
10	1.7017	0.5834	0.5472	1.5676	2.9170	0.3491
15	1.7474	0.5653	0.5178	1.6447	3.0914	0.3148
20	1.7598	0.5604	0.5064	1.6756	3.1403	0.3022
25	1.7630	0.5591	0.5019	1.6879	3.1532	0.2974
30	1.7638	0.5588	0.5001	1.6929	3.1564	0.2954

TABLE D-11

SEPARATION OF PROTEIN MIXTURE C AND D Via MULTI-COLUMN

M = 4

Protein C : $k^-_{P_3} = 0.6$, $k^+_{P_3} = 1.2$, $k^-_{P_4} = k^-_{P_5} = 1.5$, $k^+_{P_4} = k^+_{P_5} = 0.5$

Protein D : $k^-_{P_3} = k^-_{P_4} = 0.5$, $k^+_{P_3} = k^+_{P_4} = 1.5$, $k^-_{P_5} = 1.6$, $k^+_{P_5} = 0.4$

Number of Cycles, n	Protein C $\langle y_T \rangle_n$	Protein C $\langle y_B \rangle_n$	Protein D $\langle y_B \rangle_n$	Protein D $\langle y_T \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. C)	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot.D)
1	1.1539	0.8691	1.1209	0.8597	1.3278	0.7670
5	1.6673	0.6488	1.4637	0.6076	2.5699	0.4152
10	2.2571	0.5592	1.6334	0.4483	4.0362	0.2745
15	2.7486	0.5143	1.6862	0.3468	5.3444	0.2057
20	3.1101	0.4828	1.7008	0.2827	6.4415	0.1662
25	3.3545	0.4571	1.7022	0.2428	7.3380	0.1427
30	3.3548	0.4570	1.7025	0.2180	7.4100	0.1299

TABLE D-12

SEPARATION OF PROTEIN MIXTURE B, C AND D Via MULTI-COLUMN

M = 6

Protein B : $k^-_{P_2} = 0.6$, $k^+_{P_2} = 1.2$, $k^-_{P_3} = k^-_{P_5} = 1.5$, $k^+_{P_3} = k^+_{P_5} = 0.5$

Protein C & D: $k^-_{P_2} = k^-_{P_3} = 0.5$, $k^+_{P_2} = k^+_{P_3} = 1.5$, $k^-_{P_5} = 1.6$, $k^+_{P_5} = 0.4$

Number of Cycles, n	Protein B $\langle y_T \rangle_n$	Protein B $\langle y_B \rangle_n$	Protein C&D $\langle y_B \rangle_n$	Protein C&D $\langle y_T \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. B)	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. C&D)
1	1.1539	0.8691	1.1209	0.8597	1.3278	0.7670
5	1.6735	0.6488	1.4637	0.6073	2.5794	0.4149
10	2.3763	0.5592	1.6334	0.4420	4.2498	0.2706
15	3.1951	0.5139	1.6864	0.3259	6.2169	0.1933
20	4.0603	0.4818	1.7012	0.2431	8.4270	0.1429
25	4.8908	0.4552	1.7029	0.1848	10.7455	0.1085
30	5.6319	0.4316	1.6994	0.1443	13.0489	0.0849

TABLE D-13

SEPARATION OF PROTEIN MIXTURE A, B, C AND D Via MULTI-COLUMN

M = 8

Protein A : $k_{P_1}^- = 0.6$, $k_{P_1}^+ = 1.2$, $k_{P_2}^- = k_{P_5}^- = 1.5$, $k_{P_2}^+ = k_{P_5}^+ = 0.5$

Protein B,C & D : $k_{P_1}^- = k_{P_2}^- = 0.5$, $k_{P_1}^+ = k_{P_2}^+ = 1.5$, $k_{P_5}^- = 1.6$, $k_{P_5}^+ = 0.4$

Number of Cycles, n	Protein A $\langle y_T \rangle_n$	Protein A $\langle y_B \rangle_n$	Protein B,C&D $\langle y_B \rangle_n$	Protein B,C&D $\langle y_T \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. A)	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. B,C&D)
1	1.1540	0.8691	1.1209	0.8597	1.3278	0.7670
5	1.3964	0.6308	1.3631	0.6214	2.2136	0.4559
10	1.8542	0.5488	1.5422	0.4790	3.3789	0.3106
15	2.4158	0.5041	1.6106	0.3790	4.7928	0.2353
20	3.0853	0.4716	1.6331	0.3005	6.5416	0.1840
25	3.8341	0.4442	1.6370	0.2383	8.6309	0.1456
30	4.6144	0.4196	1.6332	0.1890	10.9977	0.1158

TABLE D-14

SEPARATION OF PROTEIN MIXTURE A,B AND C,D Via MULTI-COLUMN

M = 8

Protein A & B : $k_{P_1}^- = 0.5$, $k_{P_1}^+ = 1.2$, $k_{P_3}^- = k_{P_5}^- = 1.6$, $k_{P_3}^+ = k_{P_5}^+ = 0.3$

Protein C & D : $k_{P_1}^- = k_{P_3}^- = 0.6$, $k_{P_1}^+ = k_{P_3}^+ = 1.4$, $k_{P_5}^- = 0.4$, $k_{P_5}^+ = 1.6$

Number of Cycles, n	Protein A&B $\langle y_T \rangle_n$	Protein A&B $\langle y_B \rangle_n$	Protein C&D $\langle y_T \rangle_n$	Protein C&D $\langle y_B \rangle_n$	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. A&B)	$\frac{\langle y_T \rangle_n}{\langle y_B \rangle_n}$ (Prot. C&D)
1	1.1540	0.8691	0.8597	1.1209	1.3278	0.7670
5	1.6736	0.6488	0.6073	1.4637	2.5796	0.4149
10	2.3884	0.5592	0.4419	1.6334	4.2719	0.2705
15	3.2997	0.5139	0.3246	1.6864	6.4204	0.1926
20	4.4356	0.4818	0.2390	1.7012	9.2059	0.1405
25	5.7646	0.4551	0.1767	1.7029	12.6658	0.1038
30	7.2160	0.4316	0.1314	1.6995	16.7207	0.0773

APPENDIX D

GLOSSARY

batch	a parametric pump with no feed input or product withdrawals
continuous	operation with feed during all parts of the cycle
cycling zone adsorption	a cyclic separation processes with unidirectional flow of fluid and one or more beds in series. The bed temperatures or the inlet fluid temperatures to each bed are varied periodically and are out of phase with the adjoining bed
parametric pumping	a cyclic separation with periodic reversal of fluid flow direction utilizing fluid stored in reservoirs. The bed or fluid temperature (or other cyclic variable) are varied periodically to force the separation
parapump, PP	abbreviations for parametric pumping
recuperative mode	parametric pumping operation where the fluid flowing into the column is heated or cooled and the column itself is adiabatic. In general applies to operation where cyclic variable is changed in fluid flowing into the column
semi-batch	operation of open parametric pump with product withdrawal at one end of column only. Concentration in reservoir at other end increases
separation factor	(Solute concentration in concentrated product)/(Solute concentration in dilute product)
semi-continuous	operation with feed during part of a cycle but not all of the cycle

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