b = dimensionless concentration, B/B_L
c = dimensionless concentration, C/A_i
D = molecular diffusivity
E_A^* = reaction factor, defined by Eq. (1)
E_{A0}^* = asymptotic value of E_A^* in penetration theory for short contact time, Eq. (40)
$E_{Aa,1}^*$ = asymptotic value of E_A^*, Eq. (32)
$E_{Aa,2}^*$ = asymptotic value of E_A^*, Eq. (34)
K = constant defined by Eq. (3a)
k_1, k_2 = rate constants of reactions, Eqs. (4) and (5) respectively
k_{L0} = physical mass transfer coefficient: $2\sqrt{\frac{D_A}{\pi t}}$ in penetration theory; $\frac{D_A}{\delta}$ in film theory
M = $k_1 B_L D_A / k_{L0}^2$ in general; $\pi q\theta/4$ in penetration theory; $k_1 B_L \delta^2 / D_A$ in film theory
N = mass transfer rate
p = k_2/k_1
q = B_L/A_i
r_B, r_C = defined by D_B/D_A and D_C/D_A, respectively
t = contact time in penetration theory
x = distance into the liquid phase
Z = dimensionless distance in film theory, x/δ
z = dimensionless distance in penetration theory, $x\sqrt{k_1 A_i / D_A}$
z_R = defined by Eq. (3a)

Greek Letters

α = ratio of the bulk liquid volume to film volume in film theory
γ' = constant defined by Eq. (30)
δ = film thickness in film theory
θ = dimensionless contact time, $k_1 A_i t$
ϵ = constant defined by Eq. (3a)
η = dimensionless distance defined by Eq. (3)

Subscripts

A,B,C = species A, B, and C, respectively
i = gas-liquid interface
L = bulk liquid in film theory

Superscript

$\prime$ = dummy variable in definite integral

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Manuscript received October 1, 1979; revision received March 13, and accepted April 10, 1980.

Separation of Proteins Via Multicolumn *p*H Parametric Pumping

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Fractionation of protein mixtures by multicolumn *p*H parametric pumping is investigated theoretically and experimentally. The parapumps considered consist of a series of columns packed alternately with cation and anion exchangers. Various methods of operation of the parapumps are discussed. Two separation problems are examined: enrichment and splitting. Experimental data were obtained for two-column systems and compared with the calculated results based on an equilibrium theory.

SCOPE

The name 'parametric pumping' was applied to the separation process in 1966 by the inventor of the batch pump, the late R. H. Wilhelm of Princeton University. Since the time of that invention, much experimental and theoretical work has been done on thermal and pressure cycling parametric pumping. Comprehensive reviews of the subject have been written by Sweed (1971), Wankat (1974*a*), Rice (1976) and Chen (1979*c*). By contrast, very little work has been done on the *p*H paramet-

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0001-1541-80-3313-0839-$01.25.

ric pump (Sabadell and Sweed, 1970; Shaffer and Hamrin, 1975).

The parapumping system considered consists of a series of columns packed alternately with cation and anion exchangers through which the protein solution to be separated flows. Periodic variation of pH in the system accompanied with synchronous changing of the direction of the flowing mixture makes the separation possible.

Chen and his co-workers (1977, 1979*a*) have studied the separation of haemoglobin and albumin by one-column pH parametric pumping. The experimental data have shown that the pH driven parapump is capable of yielding very high separation factors. In this paper, we will examine theoretically and experimentally multicolumn pH parametric pumps for the separation of proteins. Emphasis is placed on the configuration of columns and reservoirs necessary to achieve specific separation objectives.

CONCLUSIONS AND SIGNIFICANCE

Various modes of operation of two- and multicolumn pH parapumps are examined in terms of an equilibrium theory and compared for two specific objectives: enriching and splitting. A simple graphical method is presented to show how the separation builds from cycle to cycle and to give a better understanding of the origin of separation.

The separation capability of two-column parapumping is experimentally demonstrated for the system of haemoglobin-albumin-CM Sepharose-DEAE Sepharose. The data agree reasonably well with the theory.

The new processes developed can be used to selectively cause certain desired proteins to migrate toward one end of a chromatographic column, thereby effecting separation. Furthermore, the processes are capable of yielding high separation factors without the necessity of solid phase regeneration.

ONE-COLUMN SYSTEM

Proteins carry both positively and negatively charged groups and can be bound to anionic or cationic ion exchange media. The net charge on a protein is dependent on pH. At low pH, the net charge is positive, and the protein will be taken up by a cationic exchange; at high pH, it is negative, where it will be taken up by an anionic exchanger. The intermediate pH at which there is zero net charge is called the isoelectric point. These facts can serve as the basis for parametric pumping separation of a protein mixture.

Consider the separation of a protein A from a mixture or solution. Assume that 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 and will be taken up by a suitable cationic exchanger R^- at P_2 and released 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. A reversed effect will occur if an anion exchanger is selected.

The first system we will consider is shown in Figure 1 (Chen et al., 1979*b*). It consists of a column packed with an ion exchanger (cation or anion) and reservoirs attached to each end. The pump had 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 buffer ionic strengths for 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:

1. 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_1 is set to be

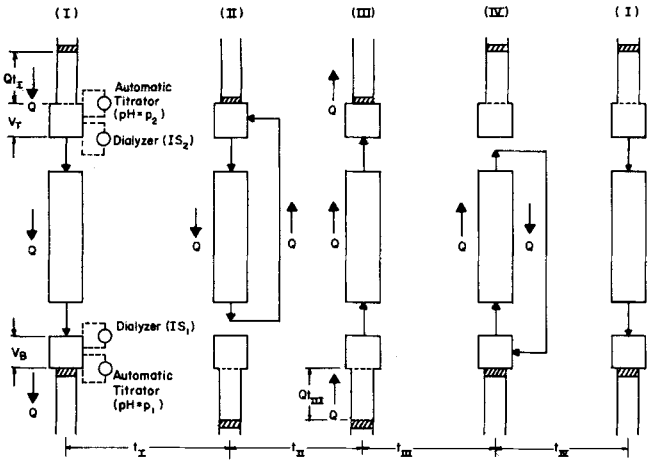

Figure 1. Schematic of one-column system.

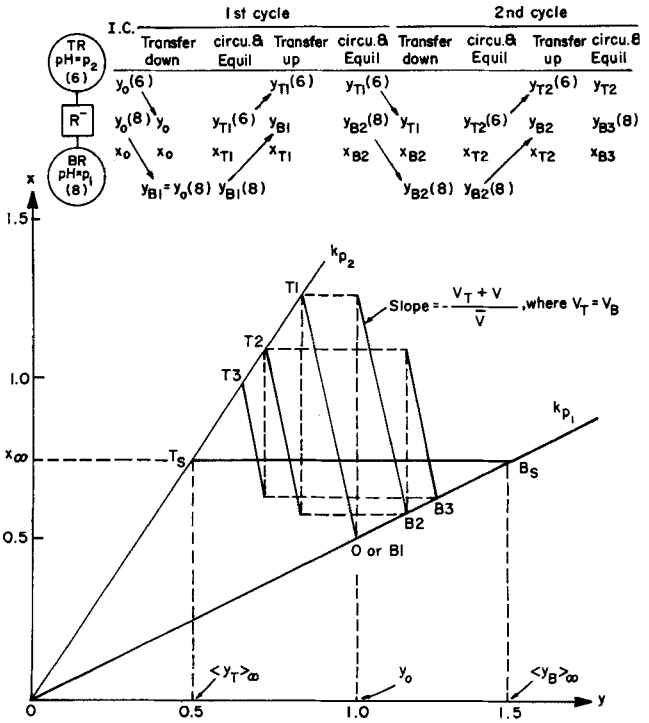

Figure 2. Graphical solution for one-column system.

equal to the void volume of the column V_e; that is, $Qt_{\rm I} = V_e$.

2. Circulation between the top reservoir and the column 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.

3. 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 $Qt_{\rm III} = V_\epsilon$.

4. Circulation between the column and the bottom reservoir allows 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 $t_{\rm II}$ or $t_{\rm IV}$, 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 2 is a graphical solution for one-column system. The assumptions made here are:

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

$$x = ky \tag{1}$$

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

The graphical method described below is based on a simple discrete transfer equilibrium model (Pigford et al., 1969; Jenczewski and Meyer, 1970; Wankat, 1974*b*; Grevillot and Tondeur, 1976; 1977). It should be pointed out that the treatment in Figure 2 differs from that of Grevillot and Tondeur (1976) by inclusion of the reservoir dead volume in the expression for the slope of the operating line. In addition, they are concerned with the direct mode of thermal parametric pumping, and we deal with the pH driven parametric pumping.

From Figure 2, 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, everywhere at equilibrium with the solid. The initial pH in the column is high (or $P_1 = 8$). 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), 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 for 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 equilibration at P_2. The column pH 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 *T*1. 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 lines is $-(V_T + V)/V$ and is obtained by the mass balance constraint; that is

$$(V_T + V)\, y_{T0} + \bar{V}X_{B1} = (V_T + V)\, y_{T1} + \bar{V}X_{T1} \tag{2}$$

or

$$(V_T + V)\, y_{T(n-1)} + \bar{V}X_{Bn} = (V_T + V)\, y_{Tn} + \bar{V}X_{Tn} \tag{3}$$

where $n = 1, 2, \cdots$

Also $y_{T0} = y_0$, $X_{B1} = X_0$, and $V = V_\epsilon$

3. Transfer up. The solution in 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 reestablished. The new equilibrium point $(y_{B2}; x_{B2})$, represented by the point *B*2, is located at the intersection of the equilibrium line k_{p_1} and of the operating line passing through $(y_{B1}; x_{T1})$ and having a slope of $-(V_B + V)/\bar{V}$. It is obtained from the following mass balance

$$(V_B + V)\, y_{B(n-1)} + \bar{V}X_{T(n-1)} = (V_B + V)\, y_{Bn} + \bar{V}X_{Bn} \tag{4}$$

where $n = 2, 3, \cdots$, 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 2). 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; that is, $<y_T>_\infty$ and $<y_B>_\infty$, respectively. At steady state, the solid phase has a constant composition which is in equilibrium with both $<y_T>_\infty$ and $<y_B>_\infty$, that is

$$X_\infty = k_{p_1} <y_B>_\infty = k_{p_2} <y_T>_\infty \tag{5}$$

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

TWO-COLUMN SYSTEMS

We will consider four modes of two-column systems shown in Figures 3, 5, 7 and 9. For all systems, 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, whereas Bwill be taken up at P_3 and released at P_2 or P_1. A reversed effect will occur if an anion exchanger is selected. The steady state concentrations in the reservoirs are graphically shown in Figures 4, 6, 8 and 10. Also, Table 1 shows the direction of flow of A and B for different modes and summarizes the notations of reservoirs, and steady state concentrations. 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. The four modes of pump operations are described as follows.

Mode 1 (Chen et al., 1979b)

The system has three reservoirs as shown in Figure 3. 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:

1. 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 $t_{\rm I}$.

2. Circulate the fluid between the *TR* and the R^+ column and between the *MR* and the R^- column, for time $t_{\rm II}$.

3. Pump the fluid from the bottom reservoir through the R^- column, *MR* and R^+ column to the top reservoir, for time $t_{\rm III}$.

4. Circulate the fluid between the *MR* and the R^+ column and between *BR* and the R^- column.

The graphical construction for the concentration profile (Figure 4) 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^-

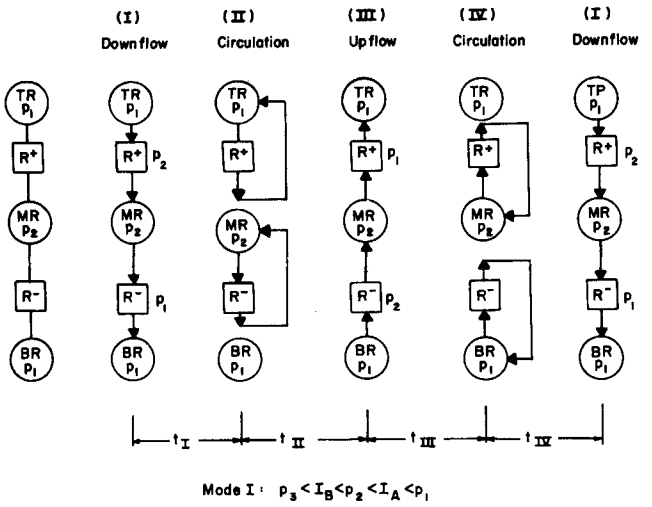

Figure 3. Schematic of two-column system, Mode 1.

columns converge to two limits, X_{R^+} and X_{R^-}, respectively. Thus, a two-step staircase is formed (upper diagram). 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; that is

$$X_{R^+} = k^+_{p_1} <y_T>_\infty = k^+_{p_2} <y_M>_\infty$$

and

$$X_{R^-} = k^-_{p_1} <y_B>_\infty = k^-_{p_2} <y_M>_\infty \qquad (6)$$

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, that is, the bottom reservoir. By comparing Figures 2 and 4 one can see that the separation factor ($<y_B>_\infty/<y_T>_\infty$) for the two-column system is much higher than that for the one-column system. Also, from the lower diagram (Figure 4), no separation occurs for protein B, that is, $[<y_B>_\infty/<y_T>_\infty]=1$. 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.

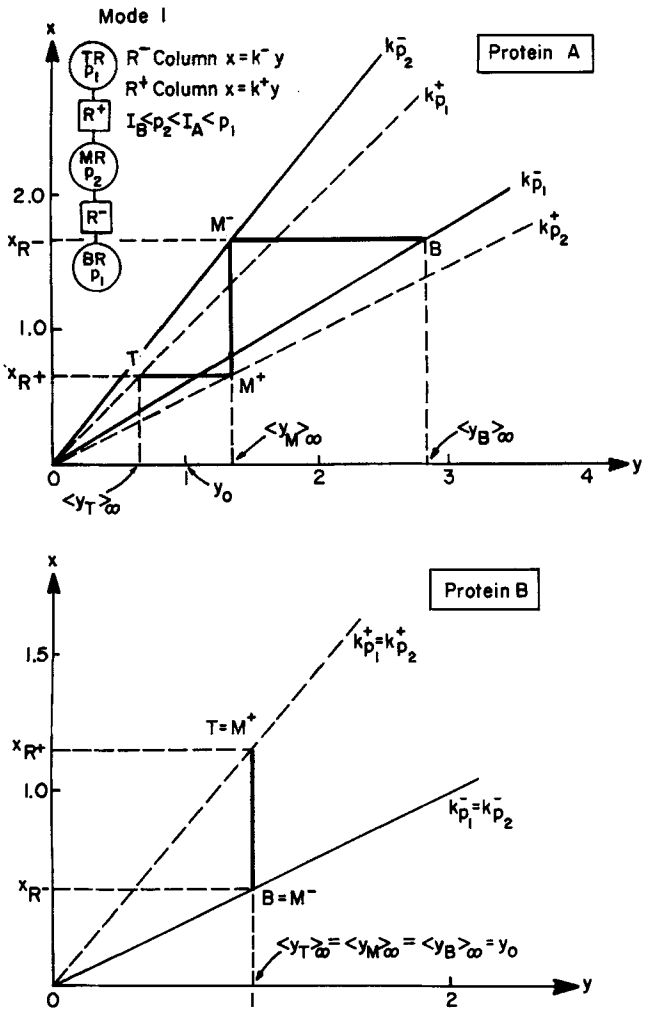

Figure 4. Graphical solution for two-column system, Mode 1.

Mode 1 just described is for an enrichment problem. The objective is to obtain a product in which the concentration of a component is larger than the corresponding concentration in the feed. The other problem of interest is a split problem and will be discussed below.

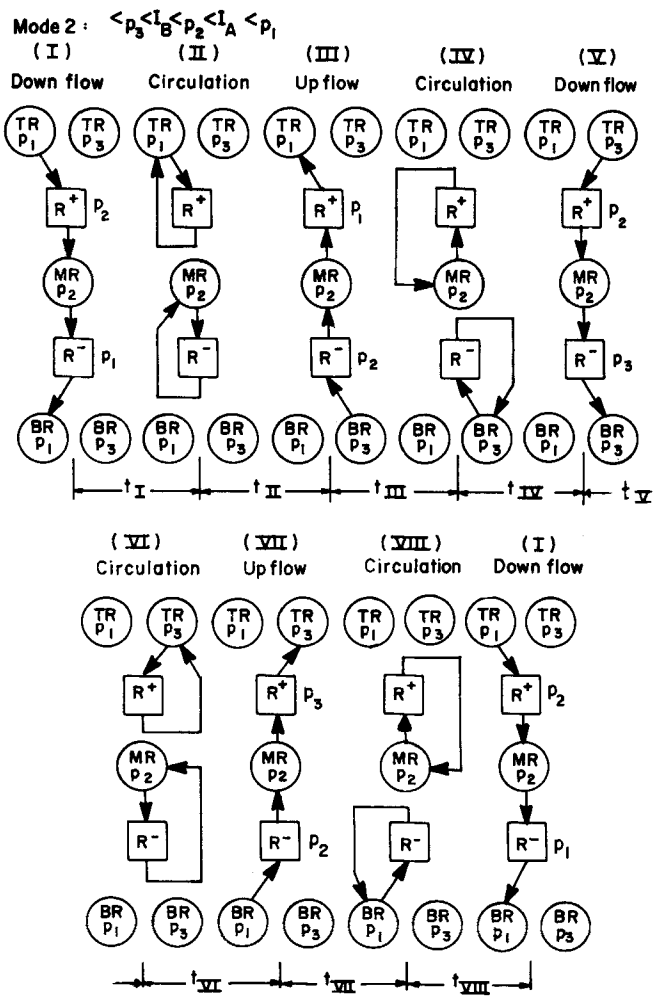

Figure 5. Schematic of two-column system, Mode 2.

Mode 2

The system contains five reservoirs, two top, one middle and two bottom reservoirs, respectively, with pH = P_1, P_3, P_2, P_1 and P_3 (Figure 5). The flow system has eight distinct steps in each cycle:

1. Pump the fluid from the $TR(P_1)$ through the R^+ column, MR and R^- column to the BR (P_1), for time t_I.
2. Circulate the fluid between the TR (P_1) and R^+ column and between the MR and the R^- column.
3. Pump the fluid from the BR (P_3) through the R^- column, MR, and the R^+ column to the TR (P_1), for time t_{III}.
4. Circulate the fluid between the BR (P_3) and the R^- column and between the MR and the R^+ column, for time t_{IV}.

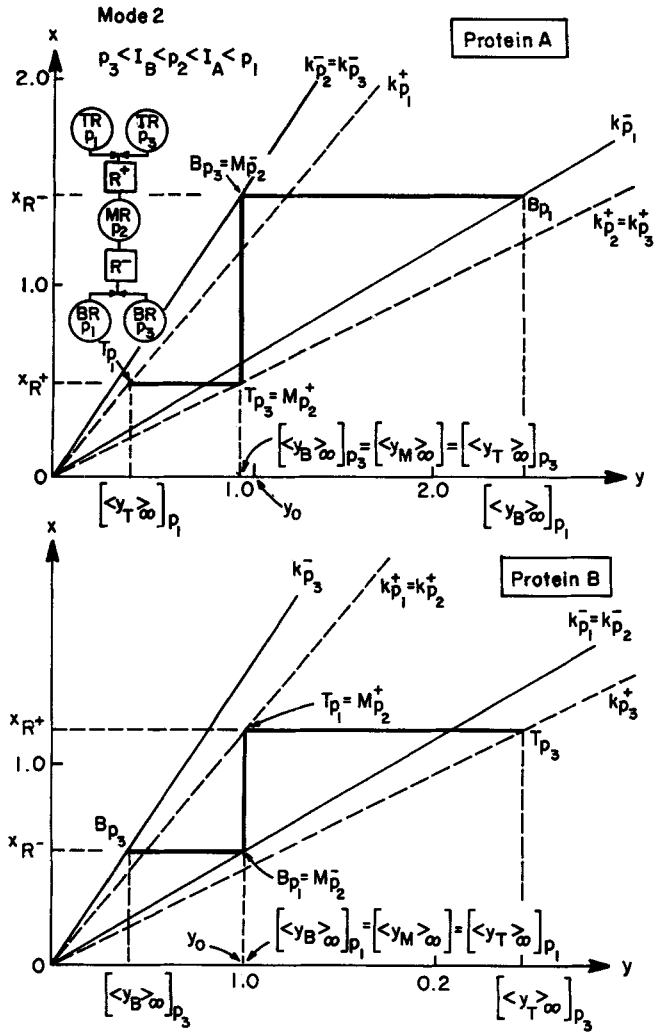

Figure 6. Graphical solution for two-column system, Mode 2.

5. 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.
6. Circulate the fluid between the TR (P_3) and the R^+ column and between the MR and the R^- column, for time t_{VI}.
7. 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}.
8. 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 Figure 6. At steady state ($n\to\infty$)

$$X_{R^+} = k^+_{p_1}[\langle y_T\rangle_\infty]_{P1} = k^+_{p_3}[\langle y_T\rangle_\infty]_{P3} = k^+_{p_2}[\langle y_M\rangle_\infty]$$

and

$$X_{R^-} = k^-_{p_1}[\langle y_B\rangle_\infty]_{P_1} = k^-_{p_3}[\langle y_B\rangle_\infty]_{P_3} = k^-_{p_2}[\langle y_M\rangle_\infty] \tag{7}$$

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 $BR(P_1)$ ($[\langle y_B\rangle_\infty]_{P_1}$) is much higher than that in the TR (P_1) ($[\langle y_T\rangle_\infty]_{P_1}$), while the concentration of B in the top reservoir TR (P_3) ($[\langle y_T\rangle_\infty]_{P_3}$) is much greater than that in the BR (P_3) ($[\langle y_B\rangle_\infty]_{P_3}$). 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, that is, $P_2<I_A<P_1$ and $P_3<I_B<P_2$. Thus, in the R^- column, 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 other words, A and B migrate in opposite directions and concentrate, respectively, in BR (P_1) and TR (P_3).

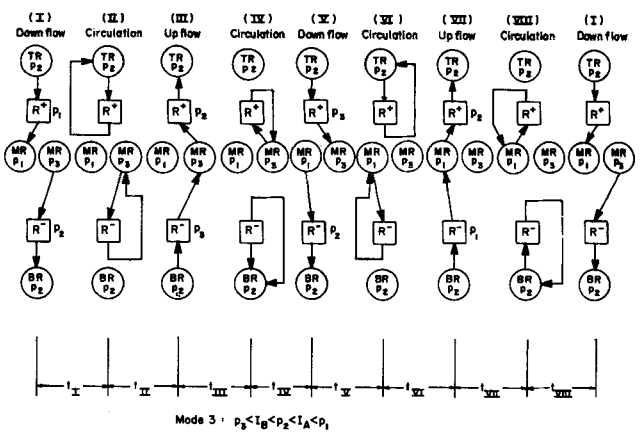

Figure 7. Schematic of two-column system, Mode 3.

Mode 3

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 7) for one cycle are:

1. 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.
2. Circulate the fluid between the TR and the R^+ column and between the MR (P_3) and the R^- column, for time t_{II}.
3. 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}.
4. Circulate the fluid between the BR and the R^- column and between the MR (P_3) and the R^+ column, for time t_{IV}.
5. 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.

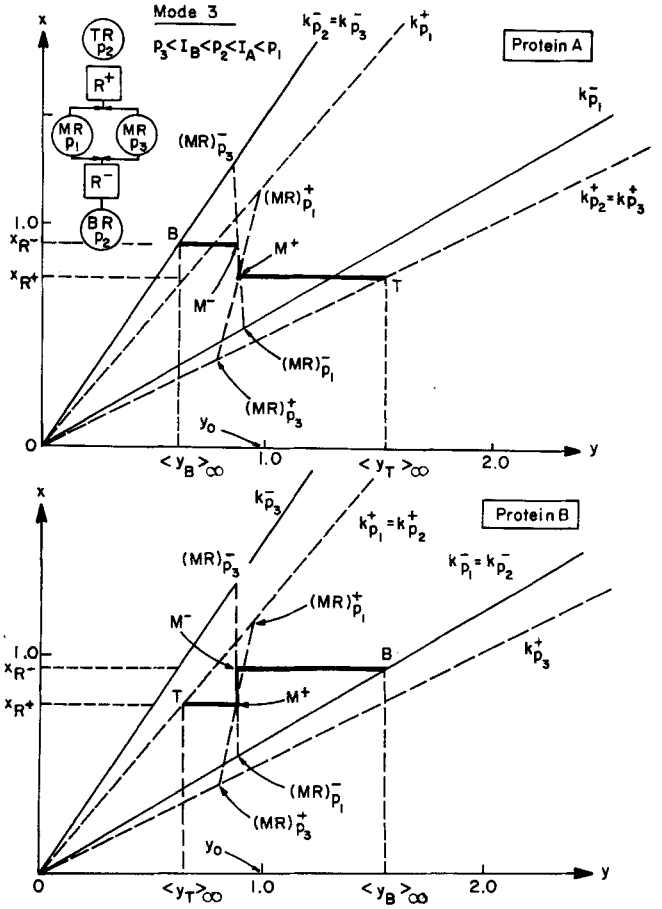

Figure 8. Graphical solution of two-column system, Mode 3.

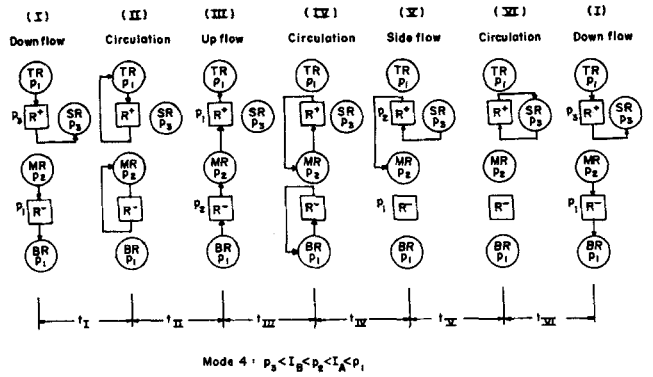

Figure 9. Schematic of two-column system, Mode 4.

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

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

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

Figure 8 shows 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; that is

$$X_{R^+} = k^+_{p_2} <y_T>_\infty = \bar{k}^+ <y_{M^+}>_\infty$$
$$X_{R^-} = k^-_{p_2} <y_B>_\infty = \bar{k}^- <y_{M^-}>_\infty \tag{8}$$

and

$$<y_{M^+}>_\infty = 0.5(y_{MR^+_{P_1}} + y_{MR^+_{P_3}}) = <y_{M^-}>_\infty = 0.5(y_{MR^-_{P_1}} + y_{MR^-_{P_3}}) \tag{9}$$

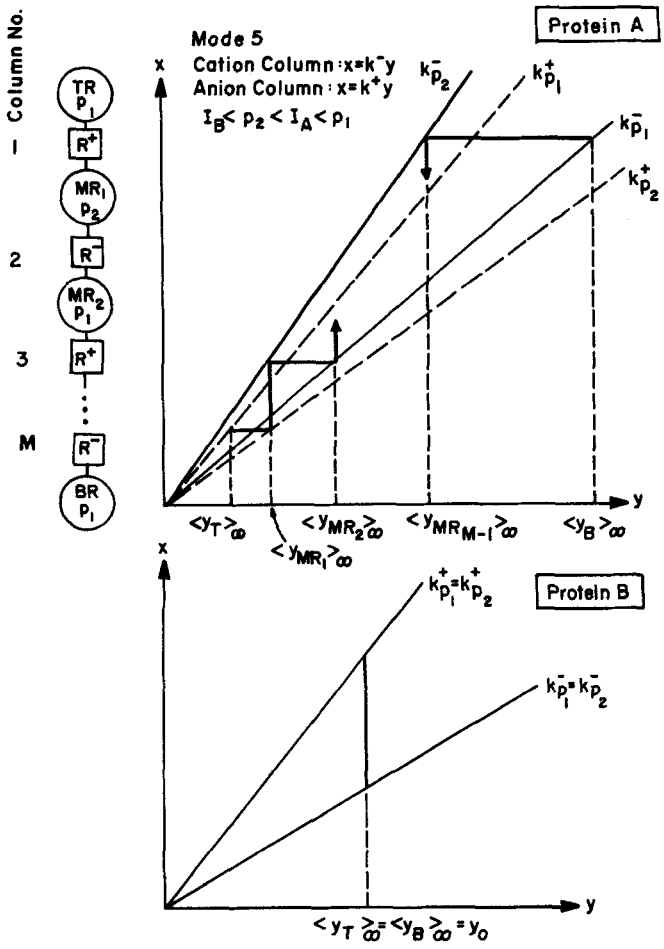

Figure 11. Graphical solution for M-column system, Mode 5.

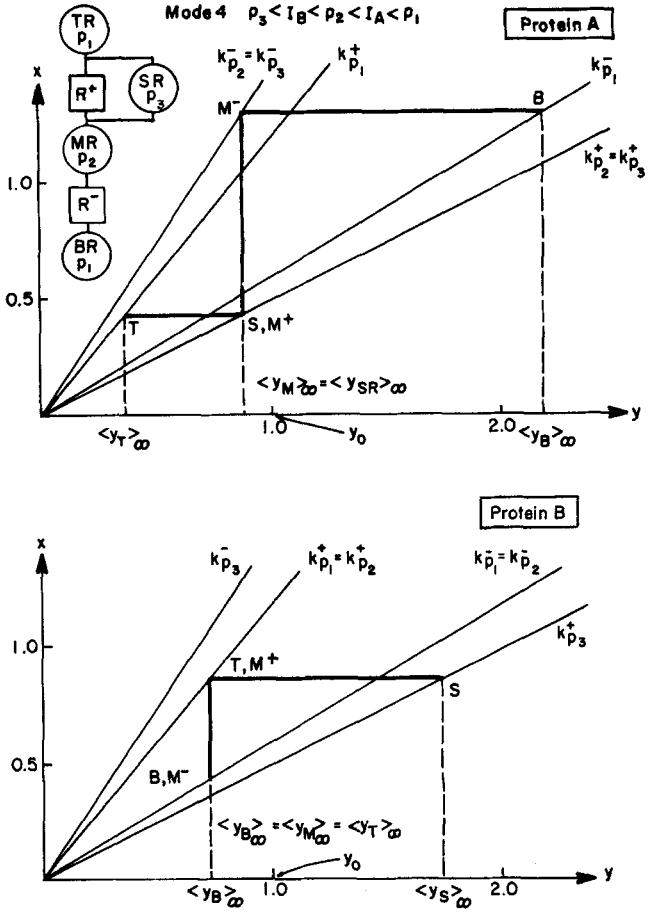

Figure 10. Graphical solution for two-column system, Mode 4.

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.

The results shown in Figure 8 are very similar to those for Mode 2. A and B move in opposite directions, but for this case, protein A migrates upward to the top reservoir (pH $= P_2$), while B moves downward to the bottom reservoir (pH $= P_2$). Also, by comparing Figures 6 and 8, one can see that separations by Mode 2 are better than those by Mode 3; that is

$$\text{for protein A: } \left\{\frac{[<y_B>_\infty]_{P_1}}{[<y_T>_\infty]_{P_1}}\right\}_{\text{Mode 2}} > \left[\frac{<y_T>_\infty}{<y_B>_\infty}\right]_{\text{Mode 3}}$$
$$\text{for protein B: } \left\{\frac{[<y_T>_\infty]_{P_3}}{[<y_B>_\infty]_{P_3}}\right\}_{\text{Mode 2}} > \left[\frac{<y_B>_\infty}{<y_T>_\infty}\right]_{\text{Mode 3}} \tag{10}$$

Mode 4

This mode includes four reservoirs, TR, MR, SR (side reservoir) and BR, respectively, with pH $= P_1$, P_2, P_3 and P_1. One cycle of operation is described (Figure 9) as follows:

1. Pump the fluid from TR through the R^+ column to the SR, and at the same time pump the fluid from the MR through the R^- column to the BR, for time t_I.

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

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

4. Circulate between the BR and R^- column and between the MR and R^+ column, for time t_{IV}.

5. Pump the fluid from the SR through the R^+ column to the MR, for time t_V.

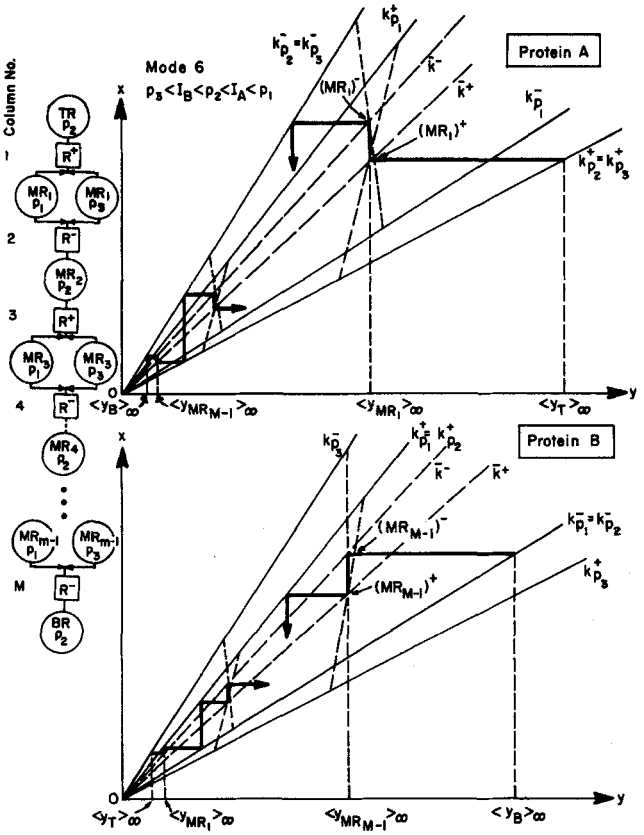

Figure 12. Graphical solution for M-column system, Mode 6.

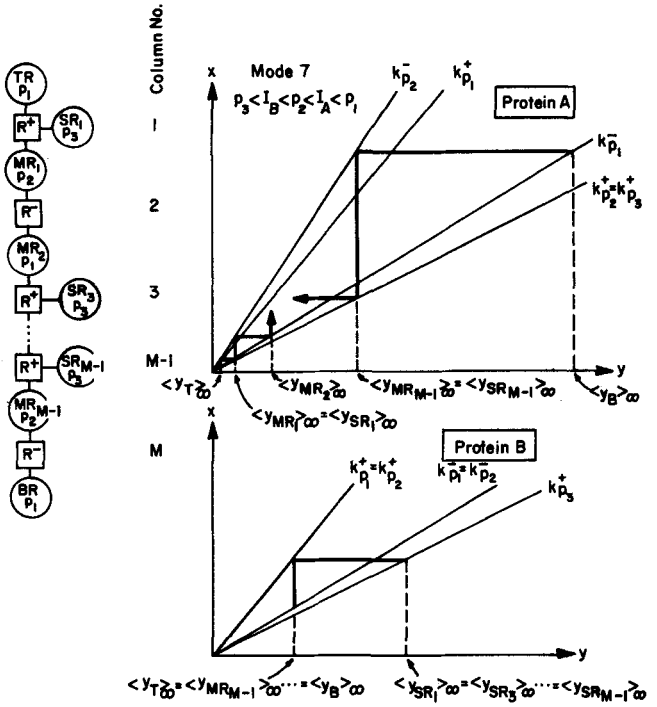

Figure 13. Graphical solution for M-column system, Mode 7.

6. Circulate between the SR and R^+ column for time t_{VI}.

The steady state concentrations are shown in Figure 10. Proteins A and B are enriched in the BR and SR, respectively. Note that by this mode protein B has no separation in the R^- column, and thus only a one-step staircase is formed.

M COLUMN SYSTEMS

The two-column system described above can be extended to the multicolumn systems (Figures 11, 12 and 13). The systems consist of a series of M columns, where M = even numbers. The column with odd numbers is packed with anion exchangers, and the remaining columns are with cation exchangers. The graphical construction for concentration profile can be made in the same way as described for the one- and two-column parapump systems.

Mode 5 (Figure 11)

This system is a generalization of Mode 1. The pH in both top and bottom reservoirs is maintained at P_1. The pH values for the middle reservoirs, MR_1, MR_2, ---, MR_{M-1} are, respectively, P_2, P_1, P_2, ---, P_2. For protein A, an M-step staircase is formed, and a very high separation factor ($<y_B>_\infty/<y_T>_\infty$) can be obtained when M becomes large. Thus, this parapump system operating with pH levels of P_1 and P_2 and $P_2<I_A<P_1$ is capable of removing protein A from the top reservoir and concentrating it in the bottom reservoir. However, protein B remains unaffected.

Mode 6

This mode (Figure 12) is a combination of Modes 2 and 3. The top and bottom reservoirs and the middle reservoirs with even numbers are maintained at pH = P_2. The remaining reservoirs are arranged in pairs. Each pair of reservoirs is kept at pH = P_1 and P_3, respectively. As shown in Figure 12, this mode gives an M-step staircase for both protein A and B on an x-y diagram. Furthermore, protein A migrates upward and concentrates in the top reservoir, while protein B moves downward. Thus, A and B split from each other. As M become larger, $S.F.$ ($<y_T>_\infty/<y_B>_\infty$) for A approaches infinity, while $S.F.$ for B approaches zero.

Mode 7 (Figure 13)

This mode is a generalization of Mode 4. There are top and bottom reservoirs and $M-1$ middle reservoir and ($M/2$) side reservoirs. The side reservoirs are maintained at pH = P_3, and the remaining reservoirs have the same pH values as those for Mode 5. As shown in Figure 13, for protein A, an M-step staircase is formed, and $<y_B>_\infty/<y_{SR_1}>_\infty$ increases as M increases. For B, $<y_B>_\infty/<y_{SR_1}>_\infty$ is less than unity (but independent of M). This is to say that Mode 5 is capable of concentrating A and B, respectively, in the BR and SR.

Figure 14 shows the dependence of the steady state separation factor ($S.F.$) on M. For all three modes (Modes 5, 6 and 7), $S.F.$ for protein A increases without limit as M increases. In the case of protein B, $S.F.$ decreases as M increases for a parapump operating with Mode 6 and remains constant for Mode 7. However, no separation will take place for B when Mode 5 is used.

EXPERIMENTAL

Experimental studies were made for two-column parapumps. A schematic figure of the experimental apparatus used for Mode 1 is shown in Figure 15. The equipment maintained at 4.5°C (40°F) consisted of two chromatographic columns. One column was packed with DEAE-Sepharose (anion exchanger) and the other with CM-Sepharose (cation exchanger). There were three reservoirs (TR, MR and BR) which were connected by two columns. Reciprocating flow within the columns was introduced by two P-3 peristaltic pumps. The pumps were wired to a dual timer to have flow direction reversed automatically at the end of each half cycle. The pH levels in the reservoirs were maintained constant by titrating with hydrochloric acid and sodium hydroxide solutions. The strengths of acid and base were so chosen that the effects on the product and reservoir concentrations were minimal. To ensure perfect mixing with the titrant in the reservoir, magnetic stirrers were used. Three hollow fiber dialyzers were used to keep the ionic strength of reservoirs constant. The dialyzers use natural turbulance similar to that found in heat exchangers to mix the buffer solution. For $pH = P_1$ and P_2, the buffer was a mixture of tris (hydroxy methyl) aminomethane-maleate, sodium hydroxide and sodium chloride and for pH = P_3, that

TABLE 1. COMPARISON OF VARIOUS MODES OF OPERATION

$P_3 < I_B < P_2 < I_A < P_1$

Modes	1 (Figure 4)	2 (Figure 6)	3 (Figure 8)	4 (Figure 10)
pH levels	P_1, P_2	P_1, P_2, P_3	P_1, P_2, P_3	P_1, P_2, P_3
Reservoirs				
Top	$TR(P_1)$	$TR(P_1)$; $TR(P_3)$	$TR(P_2)$	$TR(P_1)$
Middle	$MR(P_2)$	$MR(P_2)$	$MR(P_1)$; $MR(P_3)$	$MR(P_2)$
Bottom	$BR(P_1)$	$BR(P_1)$; $BR(P_3)$	$BR(P_2)$	$BR(P_1)$
Side				$SR(P_3)$
Direction of flow of solutes				
A	$TR(P_1)\rightarrow MR(P_2)\rightarrow BR(P_1)$;	$TR(P_1)\rightarrow MR(P_2)\rightarrow BR(P_1)$;	$BR(P_2)\rightarrow MR(P_1)\rightarrow TR(P_2)$;	$TR(P_1)\rightarrow MR(P_2)\rightarrow BR(P_1)$
B	—	$BR(P_3)\rightarrow MR(P_2)\rightarrow TR(P_3)$;	$TR(P_2)\rightarrow MR(P_3)\rightarrow BR(P_2)$;	$TR(P_2)\&MR(P_2)\rightarrow SR(P_3)$
Steady state concentrations				
Top	$<y_T>_\infty$	$[<y_T>_\infty]_{P_1}$;$[<y_T>_\infty]_{P_3}$	$<y_T>_\infty$	$<y_T>_\infty$
Middle	$<y_M>_\infty$	$<y_M>_\infty$	$[<y_M>_\infty]_{P_1}$;$[<y_M>_\infty]_{P_3}$	$<y_M>_\infty$
Bottom	$<y_B>_\infty$	$[<y_B>_\infty]_{P_1}[<y_B>_\infty]_{P_3}$	$<y_B>_\infty$	$<y_B>_\infty$
Side				$<y_S>_\infty$

was a mixture of acetic acid, sodium acetate and sodium chloride. Note that modifications of the apparatus shown in Figure 15 were also made to carry out the separation experiments by Modes 2 and 4.

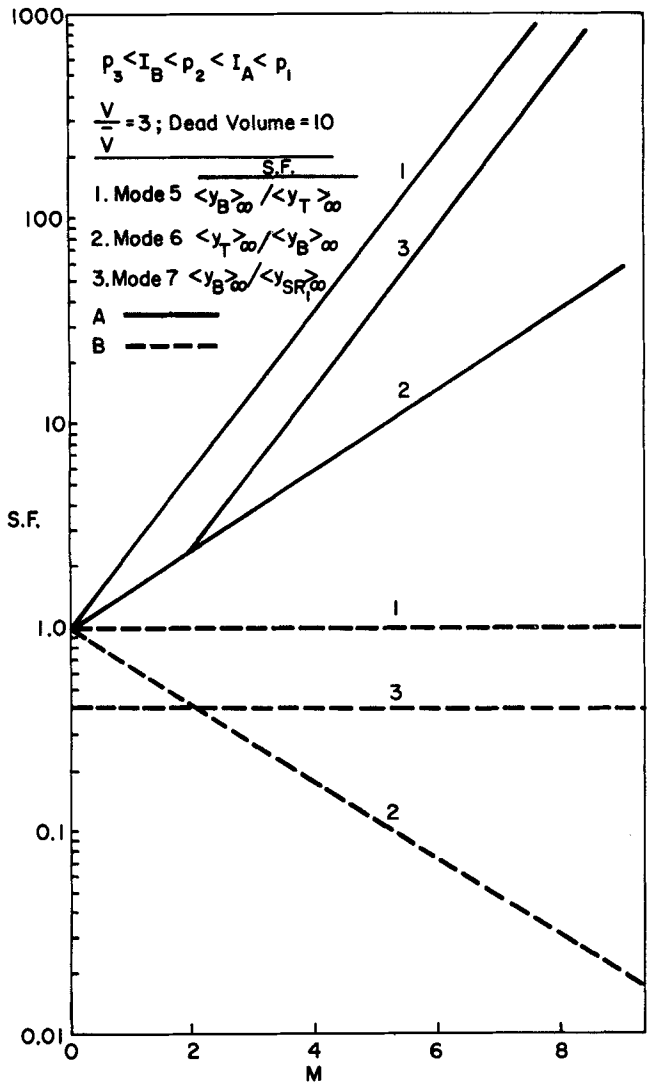

Figure 14. Steady state separation factors vs. number of columns (for protein A: $k^-_{\nu_1} = 0.6$; $k^-_{\nu_2} = k^-_{\nu_3} = 1.5$; $k^+_{\nu_1} = 1.2$; $k^+_{\nu_2} = k^+_{\nu_3} = 0.5$. For protein B: $k^-_{\nu_1} = k^-_{\nu_2} = 0.6$; $k^-_{\nu_3} = 1.5$; $k^+_{\nu_1} = k^+_{\nu_2} = 1.5$; $k^+_{\nu_3} = 0.5$).

A two-component protein mixture was selected as a model system for examining the feasibility of the proposed parapumping separation scheme:

Component	Protein	Molecular weight	Isoelectric weight
A	Haemoglobin	63 000	6.7
B	Albumin	69 000	4.7

Worthington human haemoglobin and human serum albumin were used. The pH levels were so chosen that the isoelectric points of A and B lie, respectively, between P_1 and P_2 and P_2 and P_3. For the solid phase,

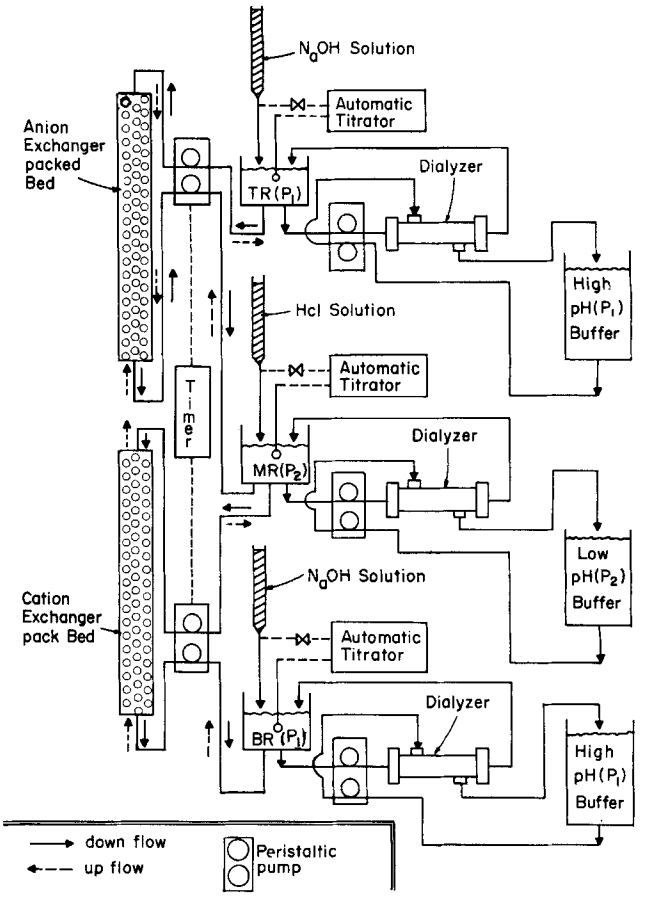

Figure 15. Schematic diagram of a two-column experimental apparatus.

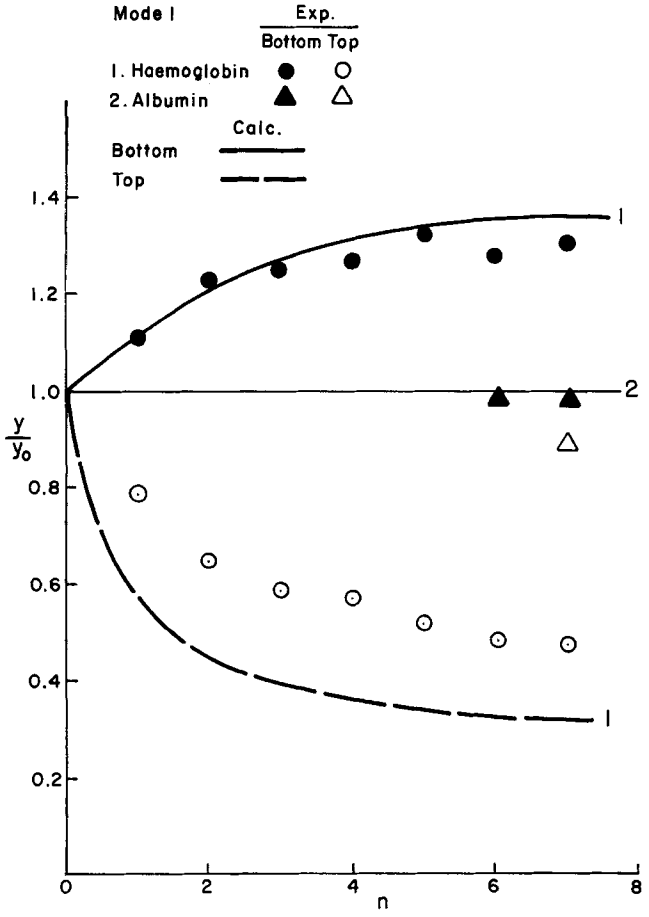

Figure 16. Experimental results, Mode 1.

CM-Sepharose and DEAE-Sepharose were chosen. The CM- and DEAE-Sepharose are macroporous, bead formed ion exchangers derived from cross linked agarose gel Sepharose CL-6B. The ion exchange capacity of the materials is high, and in addition the exchangers have an extremely stable bed volume (Pharmacia Fine Chemicals, 1976). Note that separations of haemoglobin-albumin on CM-Sepharose (and SP Sephadex) by a one-column parapumping have been reported previously (Chen et al., 1977).

RESULTS AND DISCUSSION

The experimental results are presented in Figures 16 to 18 for Modes 1, 2 and 4. Table 2 summarizes the experimental and model parameters. These plots show the top and bottom reservoir concentrations (also the side reservoir concentrations for Mode 4) vs. number of cycles. Curves computed from the theory described above are also given. The calculated results agree reasonably well with the observed values.

Figures 16 and 17 show that by the use of Modes 1 or 4, the haemoglobin concentration in the bottom reservoir builds up from cycle to cycle and approaches a steady value as n becomes large. However, albumin migrates toward the side reservoir when Mode 4 is used, whereas no separation occurs when Mode 1 is applied. The concentration profiles for Mode 2 are presented in Figure 18. As the theory predicts, both haemoglobin and albumin migrate in the opposite direction, and concentrate in the bottom (with P_1) and top (with P_3) reservoirs, respectively.

The equilibrium constants k used for computations (Table 2) were determined experimentally. Note that k is a function of ionic strength and pH. In subsequent papers, we will discuss the

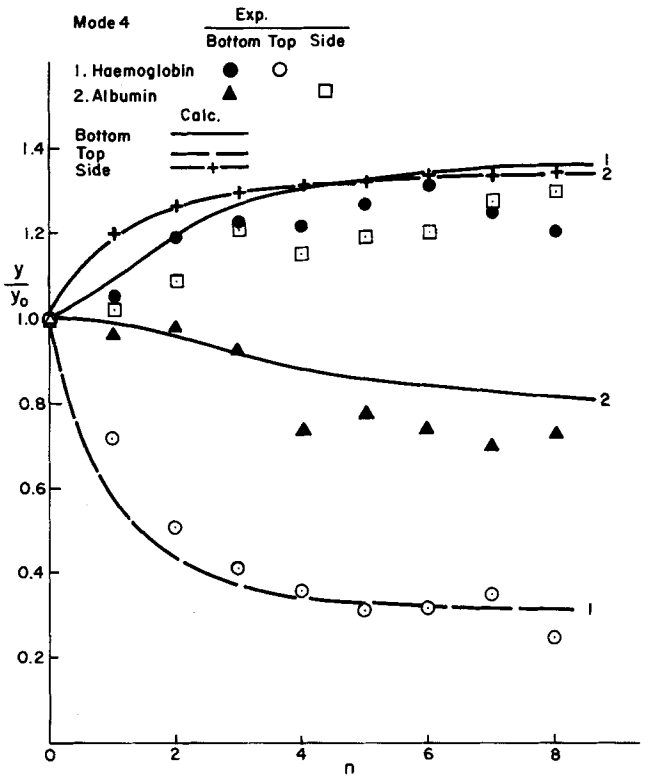

Figure 17. Experimental results, Mode 4.

procedure for determining the k value. Also, various modes of pump operation will be optimized and compared.

The results discussed thus far with reference to the separation

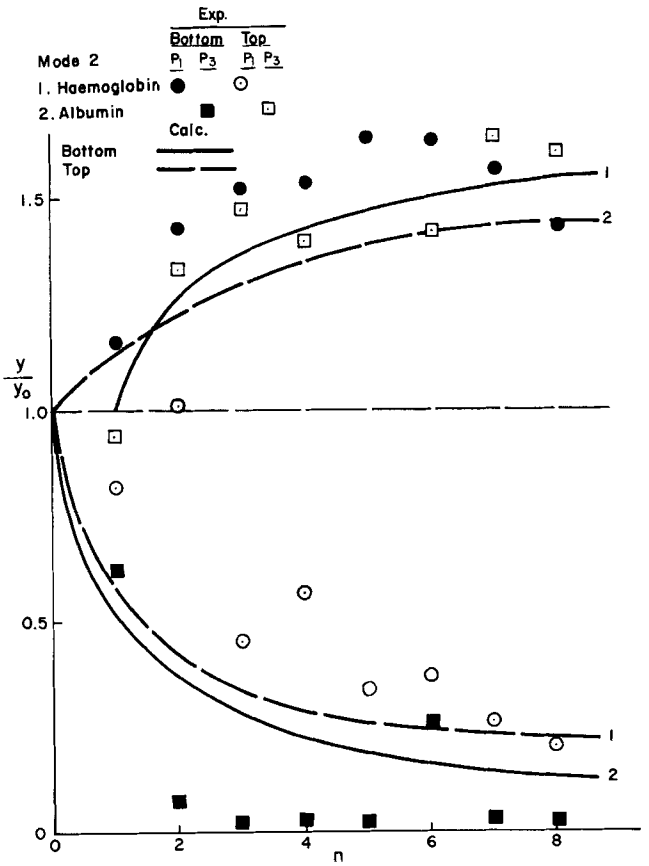

Figure 18. Experimental results, Mode 2.

TABLE 2. EXPERIMENTAL AND MODEL PARAMETERS

$Q = 1.67\times10^{-8}$ m³/s ; Feed: Haemoglobin = 0.02 wt %
Albumin = 0.02 wt %

$P_1 = 8.5$; $P_2 = 6.2$

IS_1 = [Tris.-NaOH 0.15M; -Maleate; NaCl 0.05M]; IS_2 = [Tris.-NaOH 0.15M; -Maleate; NaCl 0.05M]

Haemoglobin: $k^-_{p1} = 1.4$; $k^-_{p2} = 2.0$
$k^+_{p1} = 3.0$; $k^+_{p2} = 1.0$
Albumin: $k^-_{p1} = 1.5$; $k^-_{p2} = 1.5$
$k^+_{p1} = 2.5$; $k^+_{p2} = 2.5$

	Mode 1	Mode 2	Mode 4
$(V+V_T^*)/\bar{V}$	1.5	1.88	1.5
P_3	4.2	3.8	4
IS_3 [Acetic Acid- Na-Acetate; NaCl]	—	0.15M	0.2M
	—	0.05M	0.2M
$m^3 \times 10^6$	$Qt_I=Qt_{III}=15$ $Qt_{II}=Qt_{IV}=30$	$Qt_I=Qt_{III}=Qt_V=Qt_{VII}=15$ $Qt_{II}=Qt_{IV}=Qt_{VI}=Qt_{VIII}=30$	$Qt_I=Qt_{III}=Qt_V=15$ $Qt_{II}=Qt_{IV}=Qt_{VI}=30$
	Mode 1	Mode 2	Mode 4
Haemoglobin			
k^-_{p3}	—	4.0	—
k^+_{p3}	—	0.3	1.0
Albumin			
k^-_{p3}	—	4.5	—
k^+_{p3}	—	2.0	1.5

*$V_T = V_B = V_{MR} = V_{SR}$

of two-protein mixtures may be extended to multicomponent separations. Consider a solution of n proteins ordered according to their isoelectric point I_i.
Choose three pH values P_1, P_2 and P_3, such that

$$P_3<I_1<I_2---<I_m<P_2<I_{m+1}---<I_{n-1}<I_n<P_1$$

All of the components will bear a positive charge at P_3. The first m components will carry a negative charge at P_1 or P_2, whereas the remainder will carry a negative charge at P_1 and a positive charge at P_2. Therefore, a parapump operating with pH levels of P_1, P_2 and P_3 is capable of enriching components $m+1$, --- n in the top or bottom reservoirs, or split the components 1, 2, ---, m from $m+1$, $m+2$, ---, n, depending on the mode used:

Mode	Components to be concentrated: Top reservoir	Bottom reservoir	Side reservoir
5 (enrichment)		$m+1,---,n$	
6 (splitting)	$m+1,---,n$	$1,2,---,m$	
7 (splitting)		$m+1,---,n$	$1,2,---,m$

TABLE 3. COMPARISON BETWEEN TWO-COLUMN AND TWO SINGLE-COLUMN SYSTEMS

$k_{p1}=0.5$; $k_{p2}=1.5$; $(V/\bar{V})=3$; $V_T=V_B=0$

	Two-column system (Mode 1)		*Two single-column system			
			Case 1		Case 2	
	Column I R^+	Column II R^-	Column I R^-	Column II R^-	Column I R^-	Column II R^-
Initial condition, y_0 $(n=0)$	1.0	1.0	1.0	1.49	1.0	0.49
Steady state condition $(n=\infty)$						
Top reservoir $<y_T>_\infty$	0.23	0.75	0.49	(1.49)0.49; =0.73	0.49	(0.49)0.49 =0.24
Bottom reservoir $<y_B>_\infty$	0.75	2.22	1.49	(1.49)1.49; =2.22	1.49	(1.49)0.49 =0.73

*Both columns I and II can be either R^- or R^+.

The multicolumn parapumps presented here are batchwise and are able to extend to continuous processes. The continuous pump receives as feed a protein mixture which it separates into two or more product streams. After an initial transient, the product concentration reaches a limiting value and remains constant as the number of cycles continues to increase. Thus, as long as the system operates, product streams can be continually withdrawn from the apparatus, thereby tending to minimize both processing time and degradation. Also, the continuous process can be operated with high separation factors.

Table 3 shows a comparison on separation between the two-column (Mode 1) and a two single-column system. Both systems have two columns, I and II. For Mode 1, the columns I and II are, respectively, packed with anion (R^+) and cation (R^-) exchangers, and initially both columns are filled with a mixture of the concentration y_0 (=1.0). The separation factor ($S.F.$) defined as $[<y_B>_\infty]_{II}/[<y_T>_\infty]_I$ is equal to 9.66. In the case of the two single-column system, both columns I and II have the same ion exchanger (either R^+ or R^-). For the purpose of illustration we assume that the columns are packed with the R^-. The system is operated in sequence so that the final reservoir concentration ($[<y_B>_\infty]_I$ or $[<y_T>_\infty]_I$) of the column I is used as the initial concentration for the column II. Two cases are considered. For case 1, $[y_0]_{II} = [<y_B>_\infty]_I$, and for case 2, $[y_0]_{II} = [<y_T>_\infty]_I$. Thus, the separation factors for both cases are

$$\text{Case 1: } S.F. = ([<y_B>_\infty]_{II}/[<y_T>_\infty]_I) = 4.53$$
$$\text{Case 2: } S.F. = ([<y_B>_\infty]_I/[<y_T>_\infty]_{II}) = 6.21$$

It is obvious that the two-column system has much higher separating capability than the two single-column unit. Therefore, the M-column pumps examined in this paper should be very promising devices for separating multicomponent mixtures.

ACKNOWLEDGMENT

The National Science Foundation (CPE 7910540) provided financial support for this research.

NOTATION

I_i = isoelectric point of i
IS_1 = ionic strength in the bottom reservoir
IS_2 = ionic strength in the top reservoir
k = x/y, equilibrium constant, function of pH and ionic strength
k_{P1} = equilibrium constant at pH = P_1
k_{P2} = equilibrium constant at pH = P_2
k_{P3} = equilibrium constant at pH = P_3
n = number of cycles of pump operation
M = number of columns
P_1 = high pH level
P_2 = middle pH level
P_3 = low pH level
Q = reservoir displacement rate, m^3/s
V = volume of fluid phase, m^3
$\bar{V}$ = volume of solid phase, cm^3
V_B = bottom reservoir dead volume, m^3
V_{MR} = middle reservoir dead volume, m^3
V_{SR} = side reservoir dead volume, m^3
V_T = top reservoir dead volume, m^3
V_ϵ = column void volume, m^3
x = concentration of solute in the solid phase, kg mole/m^3
y = concentration of solute in the fluid phase, kg mole/m^3
y_0 = concentration of solute in the feed, kg mole/m^3
y_B = concentration of solute in the bottom reservoir, kg mole/m^3
y_{MR} = concentration of solute in the middle reservoir, kg mole/m^3
y_{SR} = concentration of solute in the side reservoir, kg mole/m^3
y_T = concentration of solute in the top reservoir, kg mole/m^3
$<y_B>_\infty$ = steady state concentration of solute in the bottom reservoir, kg mole/m^3
$<y_{MR}>_\infty$ or $<y_M>_\infty$ = steady state concentration in the middle reservoir, kg mole/m^3
$<y_{SR}>_\infty$ or $<y_S>_\infty$ = steady state concentration in the side reservoir, kg mole/m^3
$<y_T>_\infty$ = steady state concentration of solute in the top reservoir, kg mole/m^3
t = duration, s

Superscripts

$+$ = anion exchanger
$-$ = cation exchanger

Subscripts

R^+ = anion exchanger
R^- = cation exchanger

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Manuscript received August 20, 1979; revision received March 3 and accepted March 13, 1980.