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Continuous fractionation of protein mixtures by pH-parametric pumping

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CONTINUOUS FRACTIONATION OF PROTEIN MIXTURES BY pH-PARAMETRIC PUMPING

BY

YUK-WEI WONG

A THESIS PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

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This work is specially dedicated to my parents, who taught me the value of education.

Finally, I would like to acknowledge the substantial financial assistance I received while as a graduate student through the Department of Chemistry and Chemical Engineering.

This investigation was supported by a grant from National Science Foundation.

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ABSTRACT

Continuous pH-parametric pumping separations of a haemoglobin - albumin - CM Separose system were experimentally investigated. The parapump system has a feed, containing the protein mixture to be separated, introduced alternately to the top and bottom of the chromatographic column. The top and bottom products are withdrawn from the apparatus during the bottom and top feed respectively. It is shown that under certain conditions the pH-driven parametric pump has the capacity for removal of a protein component from one product fraction and large enrichment in the other fraction. Moreover, the continuous process can be operated with a large feed throughput.

SCOPE

Parametric pumping represents a new development in separation science. It has attracted considerable attention both because of its novelty and the possibility of continuous operation in small equipment with very high separation factors. Much experimental and theoretical work has been done on thermal and pressure parametric pumping. By contrast, very little work has been done on pH-parametric pumping. Included are Sabadell and Sweed (1970), Bradley (1973), Shaffer and Hamrin (1975), Busbice and Wankat (1975), and Chen et al. (1977).

The pH parapumping involves reciprocating flow of the protein mixture to be separated through an ion exchange bed and, simultaneously, synchronous cyclic variation of the pH. The change of pH displaces the interphase equilibrium and, in combination with the reciprocating flow, causes preferential movement of the sorbable components of the mixture towards one end of the bed, leading to a buildup of the separation from cycle to cycle.

Recent experimental results obtained by Chen et al. (1977), have shown that the parametric pump can separate a mixture of haemoglobin and albumin. The pump considered had a centre feed between an enriching column and a stripping column, and was operated batchwise during upflow and continuously during downflow.

In the present paper, the feed is alternately introduced at the top and bottom of the column, while the top and bottom products are withdrawn from the apparatus during the bottom and top feed respectively. Emphasis is placed on the operating conditions necessary to achieve high separations and high product rates. The system studied is haemoglobin-albumin on CM Sepharose. Contrary to the system with the center feed for which the haemoglobin initially present at the top of the stripping section above the point of high pH liquid penetration is immobilized there permanently, no haemoglobin immobilization problem occurs for the present arrangement.

CONCLUSIONS AND SIGNIFICANCE

The pH parametric pump has been extended to protein separations. The new continuous process developed can cause certain proteins in a mixture to migrate toward one end of a chromatographic column, thereby effecting separation. Experimental data are obtained for the system of haemoglobin -albumin on CM Sepharose.

The experiments show that after an initial transient the product concentrations reach a limiting condition and remain constant as the number of cycles continues to increase. Thus, as long as the system operates, two product streams are continually withdrawn from the apparatus. This offers significant advantages over the batch pump for which no benefit results by operating additional cycles after limiting concentrations are reached in the reservoirs attached to each end of the column. Furthermore, the process is capable of yielding high separation factors, with large feed throughput, in equipment of small size, without the necessity of solid-phase regeneration.

PROCESS DESCRIPTION

The parapump considered is shown in Figure 1. It consists of a column packed with an ion exchanger and reservoirs attached to each end. Initially, the column voids and the reservoirs are filled with the mixture to be separated. Reciprocating flow within the system causes the fluid to move up and down through the apparatus. As the flow direction changes, the column pH is also changed by changing the pH of the fluid entering the column. 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 pump has dead volumes V_T and V_B for the top and bottom reservoirs respectively. The flow system has four distinct stages in each cycle :

(I) Flow from the top reservoir through the column to the bottom reservoir for time, t_I.

(II) Feed at the bottom with the mixture of pH=P_2, and flow out of the top of the column as the top product for time, t_{II}.

(II) Flow from the bottom reservoir through the column to the top reservoir for time, t_{III}, and

(IV) Feed at the top with the mixture of pH=P_1, and flow out the bottom of the column as the bottom product for time, t_{IV}.

The flow rate within the column is always identical to the reservoir displacement rate, Q. The volumes of the bottom and top feeds (Qt_{II} and Qt_{IV}) are respectively equal to those of top and bottom products. For both the up and downflow, the reservoirs have the same displacement, i.e., $Qt_I = Qt_{III}$.

Proteins carry both negatively and positively charged groups, which can attach themselves to anionic or cationic exchangers. The net charge is dependent on the pH level. At low pH, the net charge is positive. At high pH, it is negative. At the isoelectric point (i.e. no net charge), the proteins are not bound to any type of ion exchangers.

Figure 2 shows the schematic description of the pH-parametric pumping principle. Suppose we are concerned with the separation of a two-protein system only. Let us assume that the two proteins, A and B, have isoelectric points, I_A and I_B, whereas $I_A > I_B$. Two constant pH fields, (i.e. high, P_1, and low, P_2, pH) are imposed periodically on the system, and $P_1 > I_A > P_2 > I_B$. The ion exchanger is assumed to be cationic with counter ions S^+. For the purpose of illustration, we will make the following assumptions:

(1) The displacement is equal to the void volume of the column (V_e), i.e., $Qt_I = Qt_{III} = V_e$.

(2) The volume of either the top or the bottom feed is identical to V_e, i.e., $Qt_{II} = Qt_{IV} = V_e$.

(3) The ion exchanger used (CM Sepharose) has high capacity, and the ionic exchange between the counter ions and the protein A, is essentially complete at the end of each stage. (Pharmacia Fine Chemicals, 1976; also see Results and Discussion.)

At the time zero, the void volume of the bed is filled with the high pH feed solution and the top reservoir is filled with a solution containing a feed of pH = P_2. Therefore the net charges for A and B in the column are negative, and in the top reservoir are positive and negative, respectively. During the first downflow stage, t_I, the low pH (P_2) fluid coming from the top reservoir enters the top of the column while the solution emerged from the other end enters the bottom reservoir. The pH of the column is then changed from P_1 to P_2. As a result, S^+ are exchanged for the A^+ originated from the top reservoir. During t_{II}, a feed with pH = P_2 is introduced through the bottom, and simultaneously a top product containing only pure B is removed from the column at the same rate. In addition, the A^+ initially present in the bottom feed are exchanged for S^+. After this adsorption process, an upflow is followed, and the high pH fluid enters the bottom of the column. The solution containing pure B flows out of the column to the top reservoir. Consequently, the pH in the column changes from P_2 to P_1, and desorption

of A occurs. S^+ shifts back to the bed, and the ion exchanger is then regenerated. During t_{IV}, a feed with pH = P_1 enters the top while a product rich in A is withdrawn from the bottom of the column. One whole cycle is thus completed. From Figure 2, one can see that all of the solute A entering from either the top or bottom always move toward the bottom product stream. Complete removal of A from the top product stream is achieved with one single complete cycle. Note that this result is based on the assumptions made above. In practice, it may not be possible to implement the operating conditions that satisfy the required assumptions. However, an optimum separation is attainable by repeating the process, illustrated in Figure 2, in succeeding cycles. In the limit of a large number of cycles, the system is capable of removing substantially all of the A from the top product stream and transfer it to the bottom stream. The separation factor will therefore become very large.

EXPERIMENTAL

The experimental apparatus is shown schematically in Figure 3. The column (0.016m inside diameter and 0.4 m length) was packed with ion exchangers, and maintained at a constant temperature of 288°K. Reciprocating flow within the system was introduced by a P-3 peristaltic pump manufactured by Pharmacia Fine Chemicals. The pump was connected to a dual timer to have the flow direction reversed automatically at the end of each downflow or upflow. Four automatic valves, wired to two timers, were used so that the low and high pH feeds were alternately directed to the bottom and top of the column. At the same time, the top and bottom products were withdrawn from the column respectively during the bottom and top feeds.

The pH levels in the reservoirs were maintained constant by titrating with hydrochloric acid and sodium hydroxide solutions. The strength of the 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 reservoirs, magnetic stirrers were used.

A haemoglobin and albumin mixture was selected to examine experimentally the feasibility of this parametric pumping separation scheme. Worthington human haemoglobin and human serum albumin were used. The isoelectric points for haemoglobin and albumin are 6.7 and 4.7, respectively. For all

runs, $P_1 = 8$ and $P_2 = 6$ so that only the isoelectric point of haemoglobin lies between the two pH levels. This will lead to the result that haemoglobin would be removed from the top product stream,and concentrated in the bottom product stream.

For the solid phase,CM Sepharose (Registrated Trademark) ion exchange media manufactured by Pharmacia Fine Chemicals was chosen. CM Sepharose is a macroporous, bead-formed ion exchanger derived from the cross-linked agarose gel Sepharose CL-6B. The ion exchange capacity of this material is high, and in addition the exchanger has an extremely stable bed volume.

Samples taken from the product streams at the end of each cycle were analyzed by using a spectrophotometer (Bausch & Lomb Spectronic System 400-3). The haemoglobin concentration was determined directly from the absorbance at a wavelength of 403 μm. The Bio-Rad Protein assay was used to obtain the total protein concentration. Hence, subtraction of the haemoglobin concentration from the total gave the concentration of the albumin.

RESULTS AND DISCUSSION

All parametric pump separation experiments were carried out in the apparatus depicted in Figure 3. Table 1 summarizes all the experimental parameters, and the results are plotted in Figures 4 to 10. The experimental results confirm the parapump theory described above. Haemoglobin does migrate downward and accumulate at the bottom end.

Figure 4 shows the weight fraction of solute based on the total weight of proteins (W) as a function of the number of cycles (n). W is given in Kg haemoglobin or albumin/ Kg total protein. The haemoglobin weight fraction decreases in the top product stream, and increases in the bottom product stream, with increasing number of cycles. The reverse holds true for albumin, as observed from the same figure. As n becomes large, the weight fractions in both streams level off to steady-state values.

The effect of Qt_{II} on the separation factor (S.F.) is demonstrated in Figure 5. The separation factor is defined as the quotient of the bottom and top weight fractions, ($\langle W_{BP} \rangle_n / \langle W_{TP} \rangle_n$). One may see that for runs 1, 2, and 3, an increase in Qt_{II} produces an increase in the steady state S.F. for haemoglobin and has an opposite effect on albumin. However, if Qt_{II} becomes too excessive, as in run 4, haemoglobin from the bottom feed passes through the column and out in the top product. The separation becomes worse. Note that

Qt_{IV} should have a similar effect on separation as Qt_{II}.

The dependence of the separation on reservoir displacement rate, Q, is shown in Figure 6. From the figure, it appears that one can improve the separation by decreasing Q. However, in general practice, one would want to achieve a desired steady state separation as quickly as possible. If we plot S.F. versus time, as shown in Figure 7, we find that the highest Q is in fact more preferable. Smaller Q would only be used when the greater ones do not give the desired extent of separation. Also, if Q becomes too small (run 5) axial diffusion may be important and poor separation may result.

The effect of reservoir displacement on concentration transients are shown in Figure 8. The ordinate is the average product concentration divided by the feed concentration for haemoglobin and is dimensionless. These values are always greater than one for the bottom product, while those for the top product are always less than one. Comparing runs 1 and 12 ($Q = 8.33 \times 10^{-3}$ cm^3/s), as well as runs 6 and 11 ($Q = 16.67 \times 10^{-3}$ cm^3/s), one can see that separation is virtually non-existent, when the displacement (Qt_I) is much less or much greater than the column void volume ($V_e = 22.5$ cm^3). The separation will gradually increase as Qt_I approaches V_e (see curves 1, 6).

The effect of the buffer ionic concentration on the separation is demonstrated in Figure 9. An increase in the

ionic concentration (the sodiom ion concentration) of the high pH solution results in an increase in the desorption of haemoglobin from the exchanger. It thus enhances the haemoglobin concentration in the bottom product, while the top concentration is essentially constant.

Figure 10 illustrates the change of the separation factor, S.F., with n for two different feed concentrations. The separation factors for both cases are close to each other. Thus, the protein concentration in the feed has no significant influence on the separation. As long as the feeds are not too concentrated, the components in the mixture will not interact with one another and compete for adsorption sites on the exchangers. Under this condition, it is possible to obtain a complete separation of the two proteins by passing the product streams from one column into several other columns, placed in series.

Figure 11 shows the results of a simple experiment carried out in a column packed with a cationic exchanger (CM Sepharose). Initially, the exchanger was in equilibrium with a high pH ($P_1 = 8$) buffer. At $t = 0$, a low pH feed ($P_2 = 6$) containing 0.02 weight percent of haemoglobin (upper diagram) was introduced to the top of the column, and product samples were collected from the bottom of the column in equal time intervals. Since haemoglobin at pH = 6 carries a positive charge, and the exchanger had large capacity under the specific buffer concentration used, most of the haemoglobin supplied by the feed was adsorbed in the exchanger. The product concentrat-

ion was essentially equal to zero during the feeding period, r_1. At $t = r_1$, the high pH buffer ($P_1 = 8$) entered the column as elutant. Haemoglobin became negatively charged and was eluted from the ion exchanger. Hence, the exit stream concentration rose sharply to a high value and dropped to zero as soon as the haemoglobin was completely removed from the exchanger. A similar experiment was done for albumin (lower diagram). For albumin, the net charge was negative at pH = 6 or 8. The exit concentration approached a steady value (approximately equal to the feed concentration) for a time period of r_2, and then returned to zero. This implied that no appreciable ionic exchange took place between albumin and the exchanger.

The results shown in Figure 11 illustrates the importance of selecting the appropriate pH levels for the parapumping separation. Consider a solution of n proteins ordered according to their isoelectric point, I_i. Choose two pH values, P_1 and P_2, such that:

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

The first m components will bear a negative charge, whereas the others will carry a negative charge at P_1 and a positive charge at P_2. Therefore, the latter group will be bound to a suitable cationic exchanger at P_2 and released at P_1, while the first m components will be unaffected. Thus, a parametric pump operating with levels of P_1 and P_2 should be capable of

removing the components m+1, ..., n from one product stream and enriching the other product stream with these components.

Many versions of parametric pumps are conceivable. The pump examined here appears to be the most promising for separating two or multi-compoment protein mixtures.

NOTATION

$(BOT/TOP)_{Hb}$ = ratio of the average haemoglobin concentration of the bottom product to that of top product

F_{Alb} = ratio of the average albumin concentration of the product to that of the feed

F_{Hb} = ratio of the average haemoglobin concentration of the product to that of the feed

F_{total} = ratio of the total protein concentration of the product to that of the feed

I_i = isoelectric point for component i

n = number of cycles of pump

P_1 = high pH

P_2 = low pH

Q = reservoir displacement rate, cm^3/s

Qt_I = upflow displacement, cm^3

Qt_{II} = volume of top product, cm^3

Qt_{III} = downflow displacement, cm^3

Qt_{IV} = volume of bottom product, cm^3

$(R_{sB})_{403}$ = absorbance reading for the bottom product at a wavelength of 403 um,*

$(R_{sB})_{595}$ = absorbance reading for the bottom product at a wavelength of 595 um,*

$(R_{sT})_{403}$ = absorbance reading for the top product at a wavelength of 403 um,*

$(R_{sT})_{595}$ = absorbance reading for the top product at a wavelength of 595 um,*

$(R_{LF})_{403}$ = absorbance reading for the low pH feed at a wavelength of 403 um,*

$(R_{LF})_{595}$ = absorbance reading for the low pH feed at a wavelength of 595 um,*

$(R_{HF})_{403}$ = absorbance reading for the high pH feed at a wavelength of 403 um,*

$(R_{HF})_{595}$ = absorbance reading for the high pH feed at a wavelength of 595 um,*

t_I = duration of downflow, s

t_{II} = duration of bottom feed, s

t_{III} = duration of upflow, s

t_{IV} = duration of top feed, s

V_B = bottom reservoir dead volume, cm^3

V_e = void volume, cm^3

V_T = top reservoir dead volume, cm^3

W = weight fraction of solute based on total weight of proteins, (Kg/Kg)

W_{Alb} = weight fraction of albumin based on total weight of proteins, (Kg/Kg)

W_{Hb} = weight fraction of haemoglobin based on total weight of protein, (Kg/Kg)

$\langle W_P \rangle_n$ = average W in the product at n^{th} cycle, (Kg/Kg)

$\langle W_{BP} \rangle_n$ = average W in the bottom product at n^{th} cycle, (Kg/Kg)

$\langle W_{TP} \rangle_n$ = average W in the top product at n^{th} cycle, (Kg/Kg)

$\langle y_0 \rangle$ = concentration of solute in the feed, kg moles/cm^3

$\langle y_{BP} \rangle_n$ = average concentration of solute in the bottom product at n^{th} cycle, Kg moles/cm^3

$\langle y_{TP} \rangle_n$ = average concentration of solute in the top product at n^{th} cycle, Kg moles/cm^3

The absorbance reading for the corresponding buffer solutions without any solute protein is assigned to be 0.000

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Table 1: Experimental Parameters

Run	Feed (Weight %) Haemoglobin	Feed (Weight %) Albumin	Displacement Rate, Q cm^3/s	Feed Volume (cm^3) Bottom Qt_{II}	Feed Volume (cm^3) Top Qt_{IV}	Reservoir Displacement $Qt_I = Qt_{III}$ (cm^3)	Ionic Conc. Molarity, M Bottom Buffer	Ionic Conc. Molarity, M Bottom NaCl	Ionic Conc. Molarity, M Top Buffer
1	0.02	0.02	8.33×10^{-3}	10	10	22.5	0.2	0.1	0.05
2	0.02	0.02	8.33×10^{-3}	5	10	22.5	0.2	0.1	0.05
3	0.02	0.02	8.33×10^{-3}	15	10	22.5	0.2	0.1	0.05
4	0.02	0.02	8.33×10^{-3}	20	10	22.5	0.2	0.1	0.05
5	0.02	0.02	3.33×10^{-3}	10	10	22.5	0.2	0.1	0.05
6	0.02	0.02	16.67×10^{-3}	10	10	22.5	0.2	0.1	0.05
7	0.02	0.02	25.00×10^{-3}	10	10	22.5	0.2	0.1	0.05
8	0.02	0.02	8.33×10^{-3}	15	10	22.5	0.05	0.65	0.05
9	0.02	0.02	8.33×10^{-3}	15	10	22.5	0.25	-	0.025
10	0.02	0.02	8.33×10^{-3}	15	10	22.5	0.05	0.25	0.05
11	0.02	0.02	16.67×10^{-3}	10	10	35	0.2	0.1	0.05
12	0.02	0.02	8.33×10^{-3}	10	10	10	0.2	0.1	0.05
13	0.01	0.01	8.33×10^{-3}	15	10	22.5	0.25	-	0.025

For all runs: column length = 0.15m; $V_T = V_B = 10\ cm^3$; $P_1 = 8$ and $P_2 = 6$

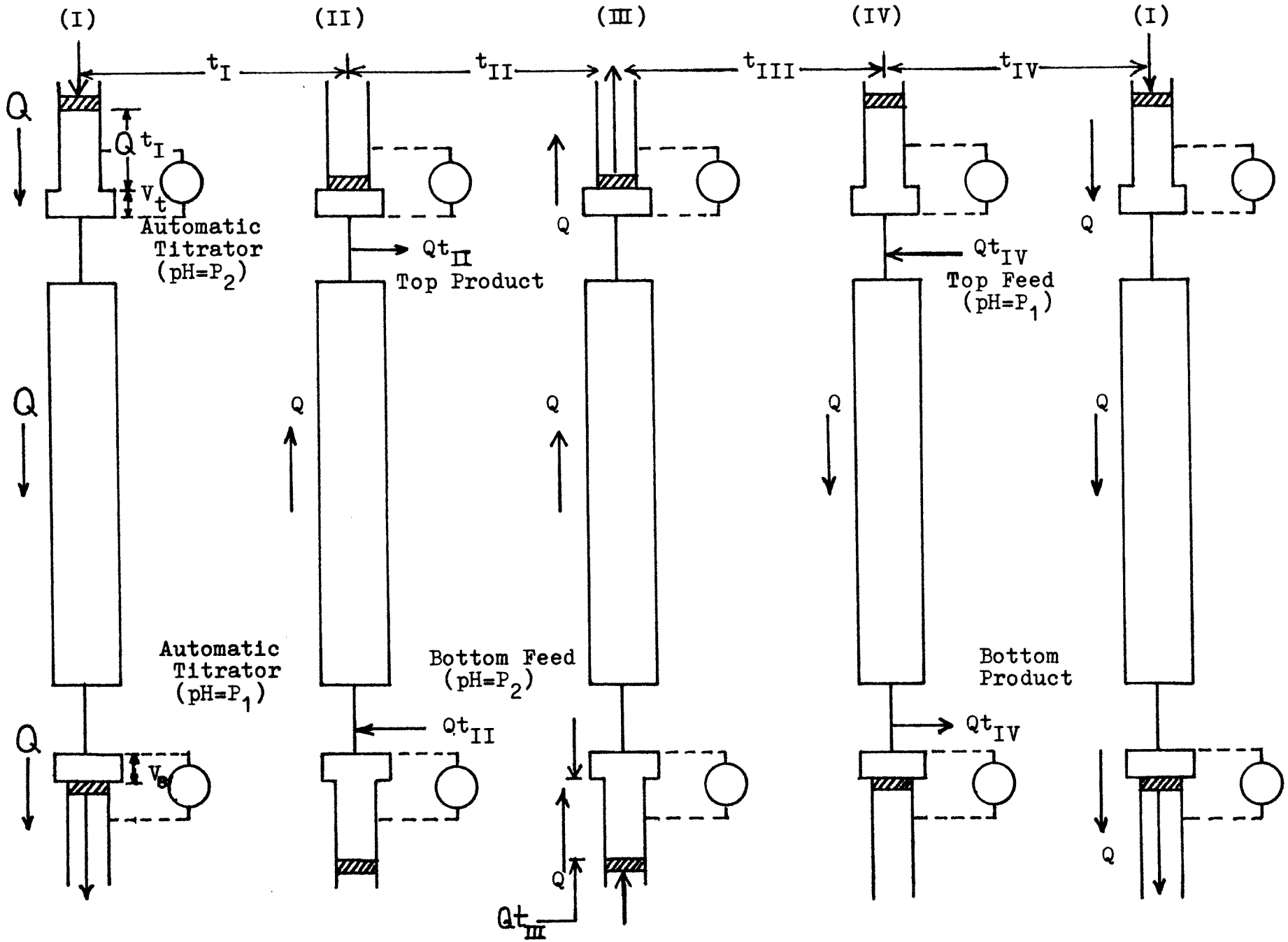
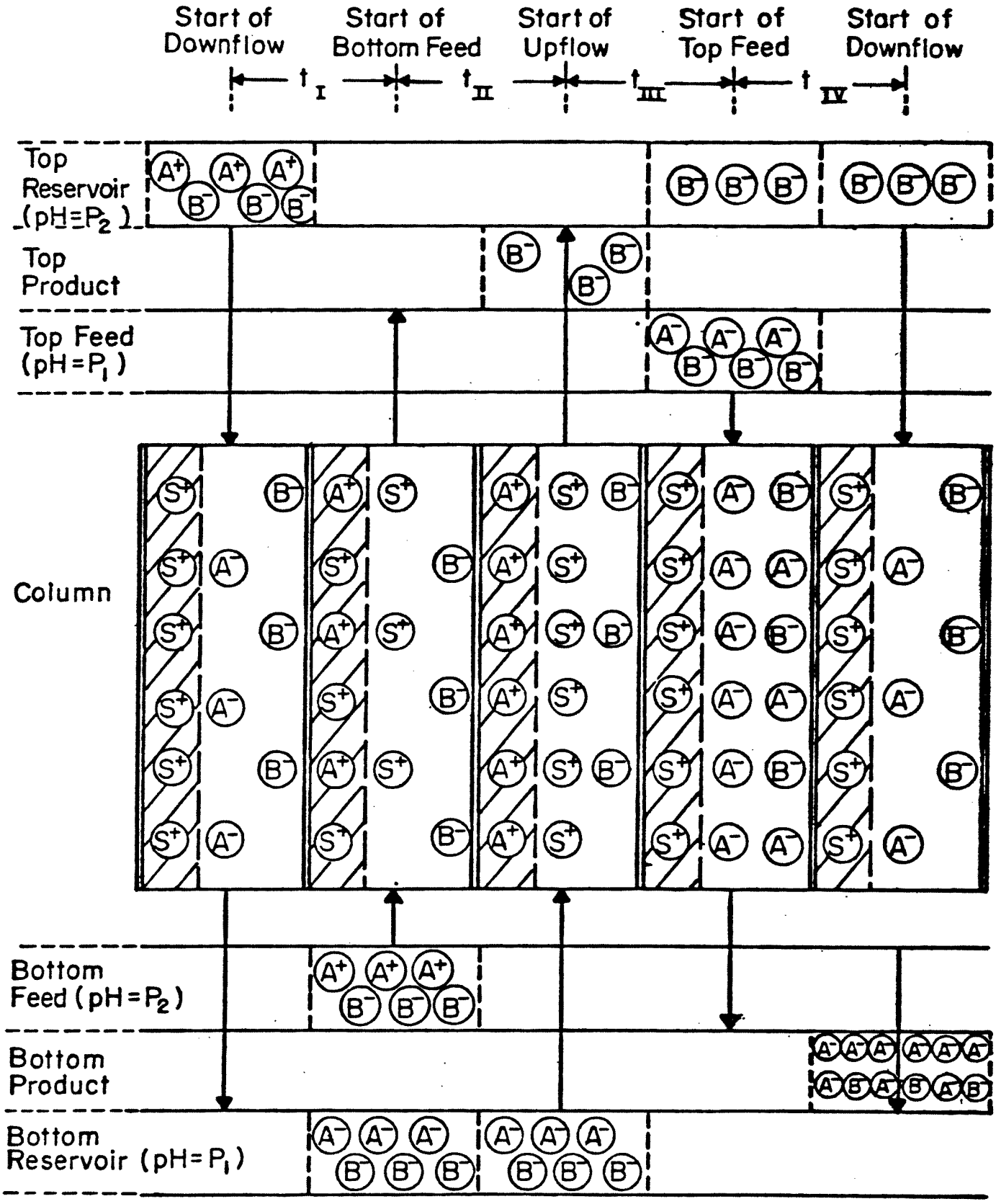


Figure 1. Column diagram for continuous pH parametric pumping.



A, B = substances with isoelectric points, I_A and I_B respectively,
$P_2 < I_A < P_1$; $I_B < P_2$; P_1 = high pH ; P_2 = low pH

S^+ = Counter ion

= Cationic exchanger

Figure 2. Schematic description of pH-parametric pumping principle

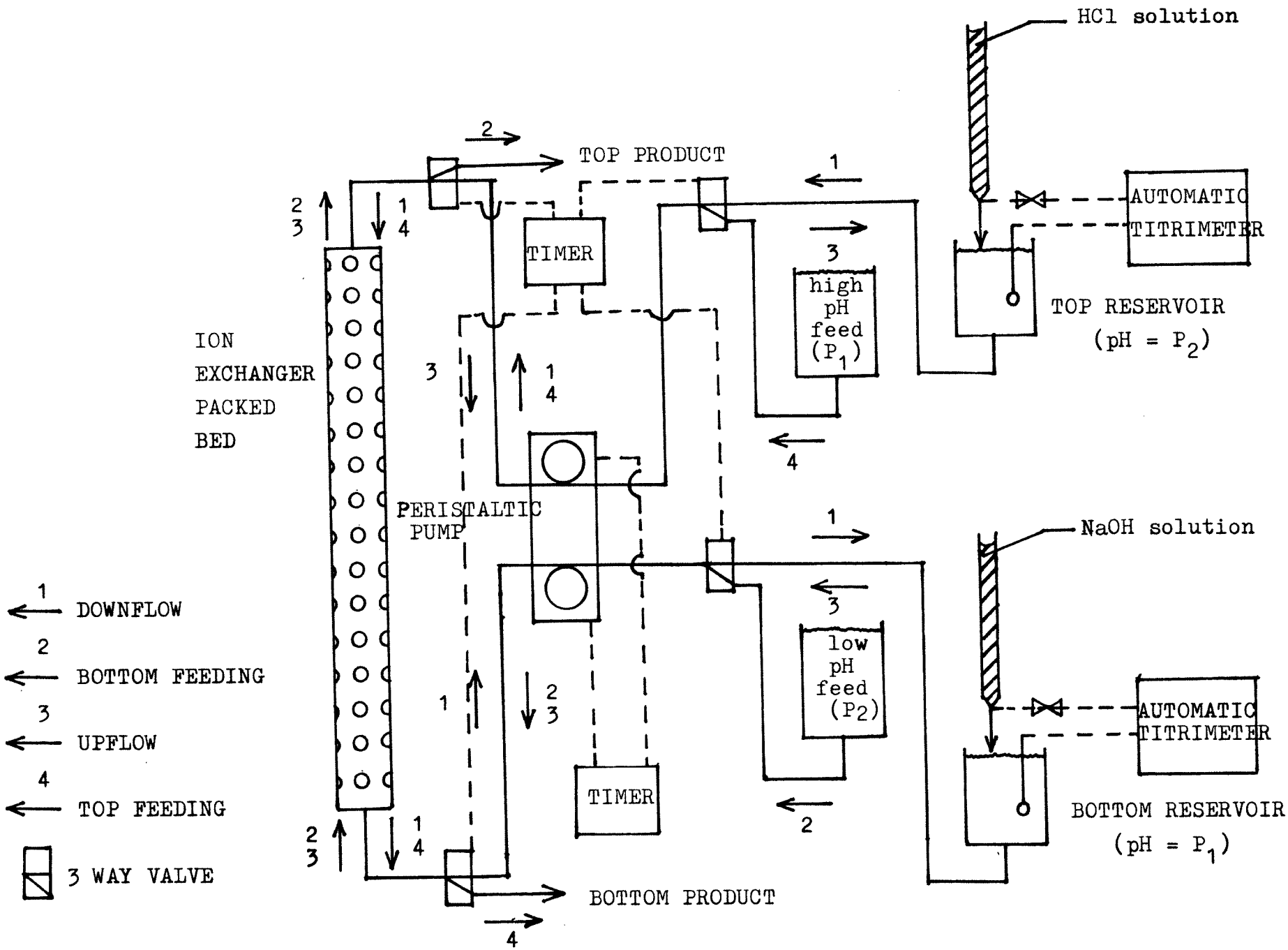


Fig. 3. Experimental apparatus for continuous pH parametric pumping

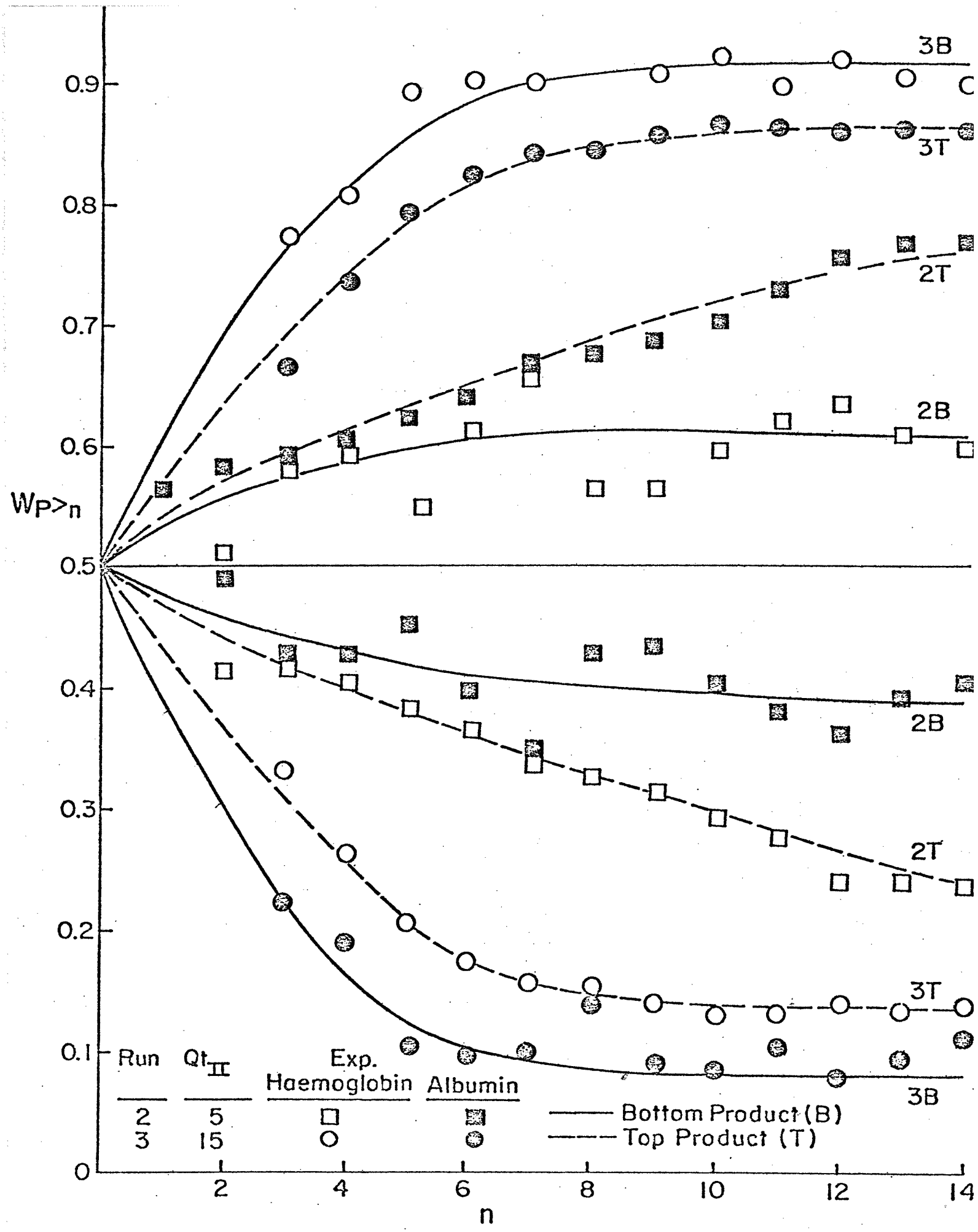


Figure 4. Concentration transients for the top and bottom products.

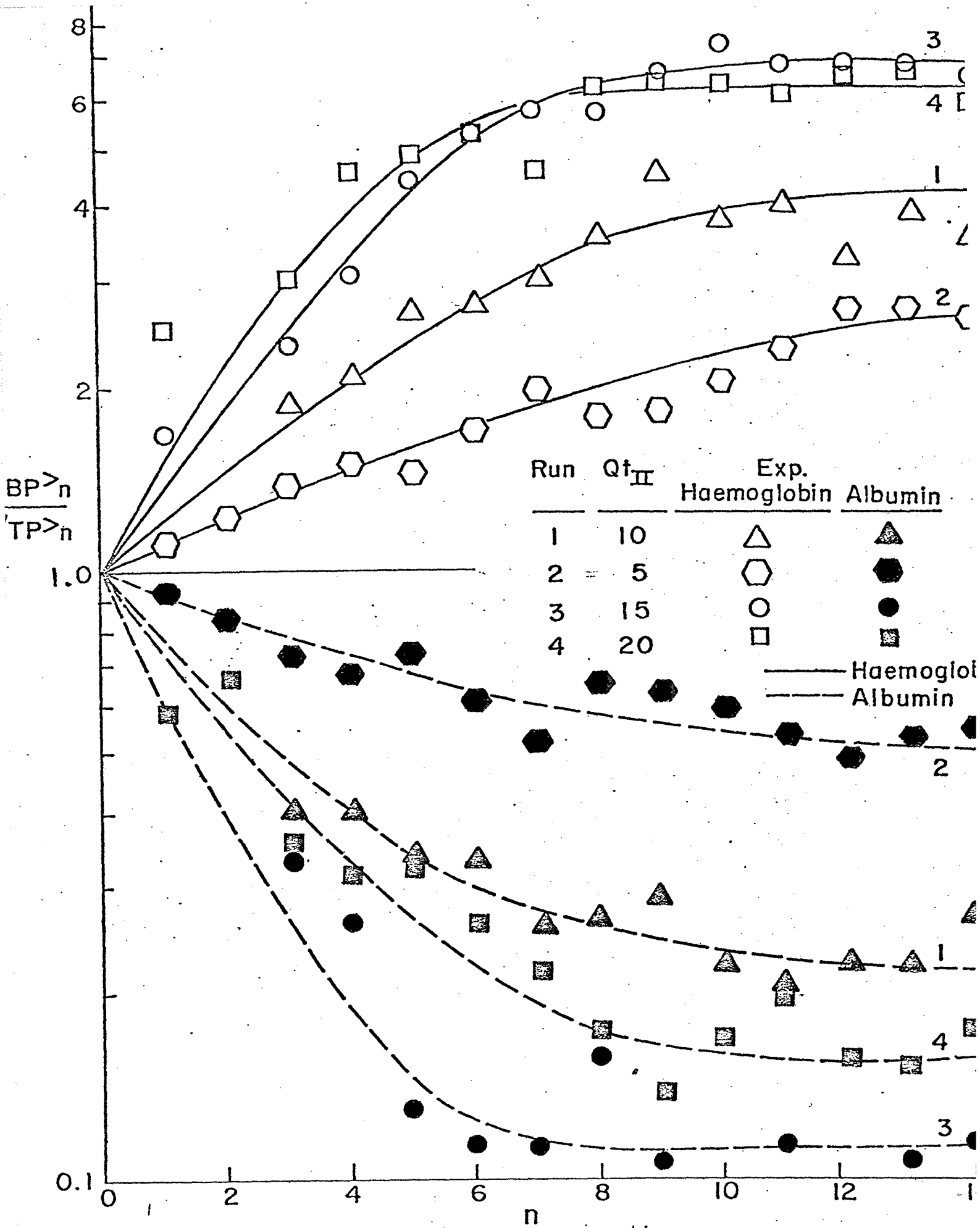


Figure 5. Effect of Qt_{II} on separation

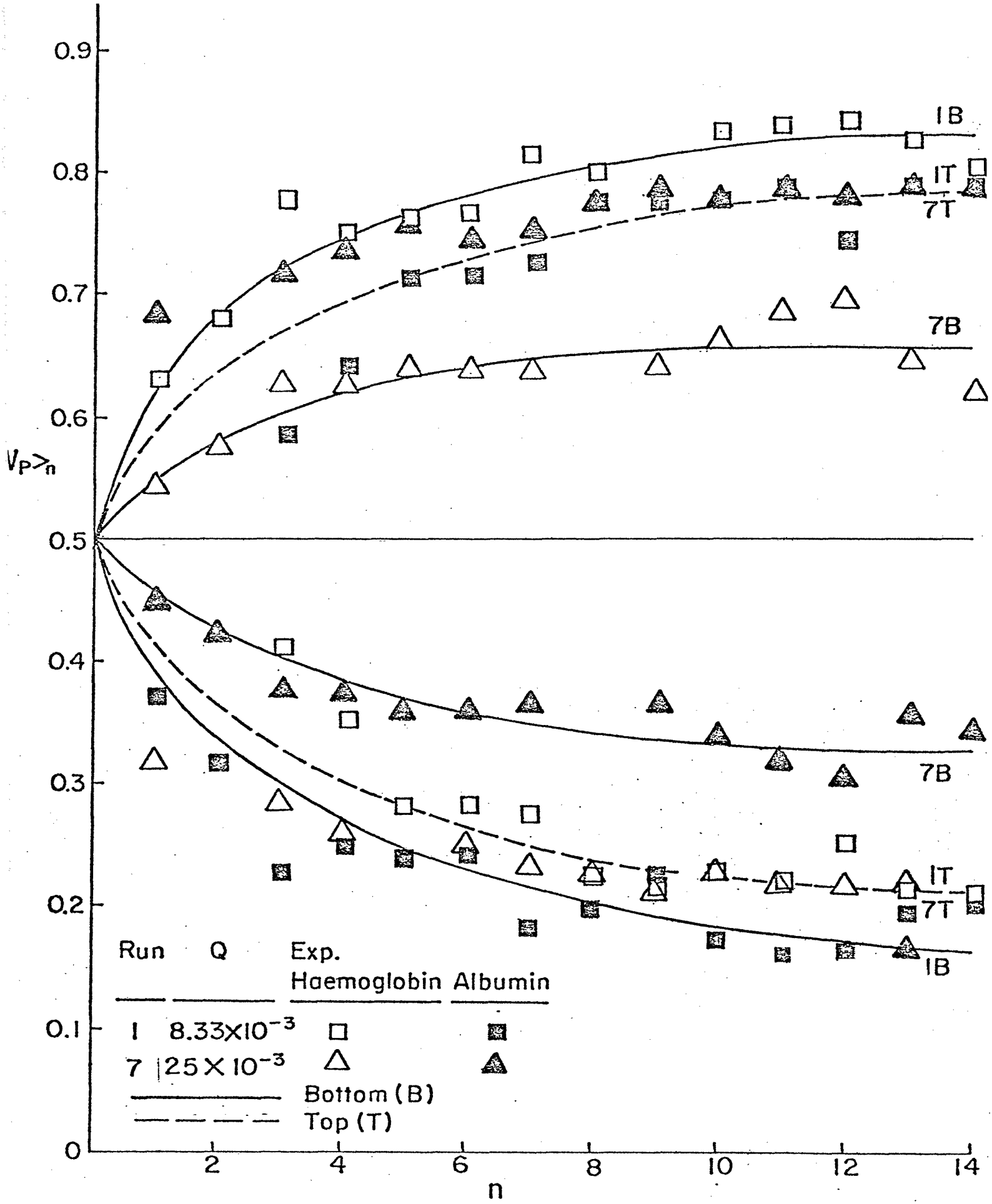


Figure 6. Dependence of separation on the reservoir displacement rate.

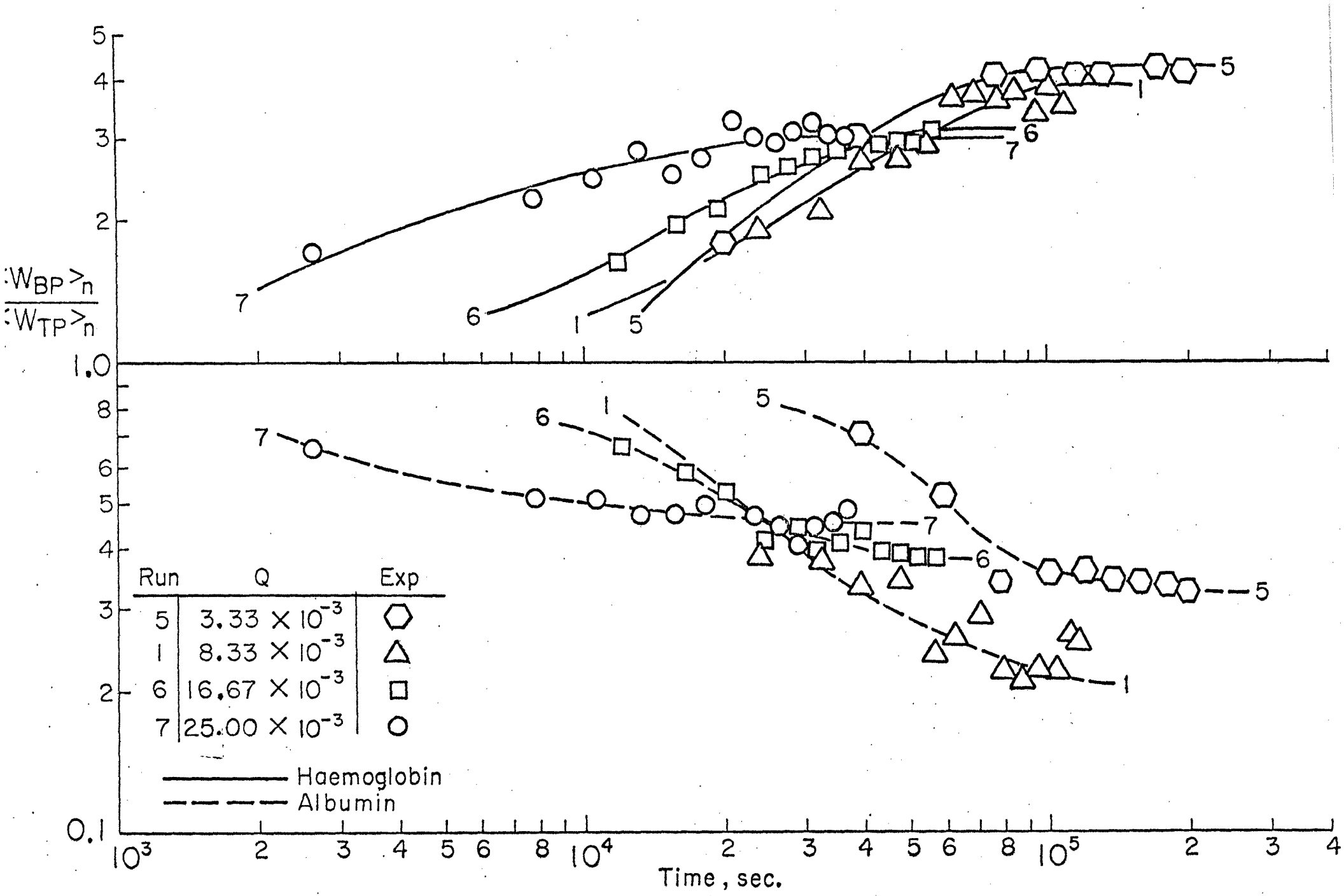


Figure 7. Separation factors versus time.

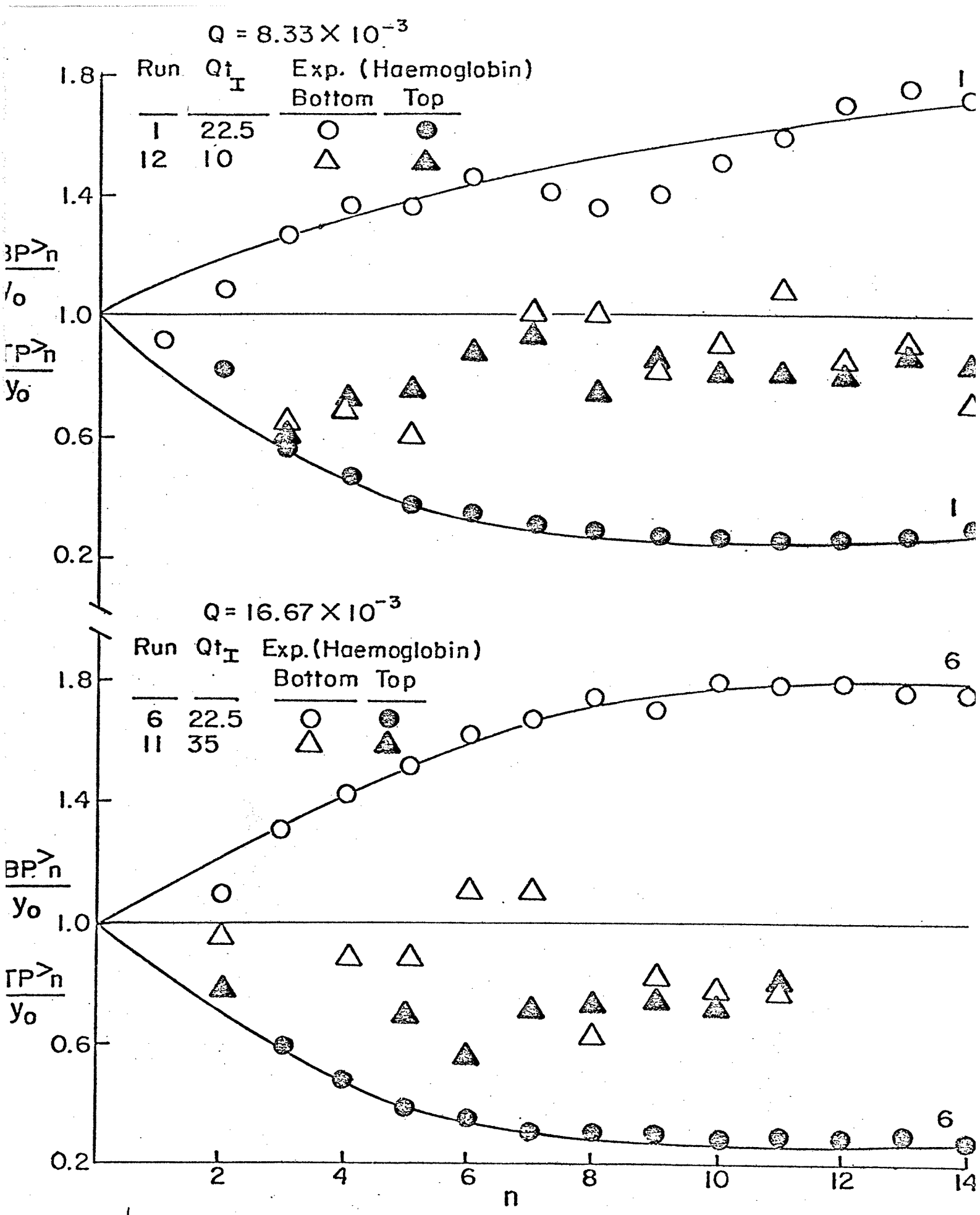


Figure 8. Effect of reservoir displacement on separation.

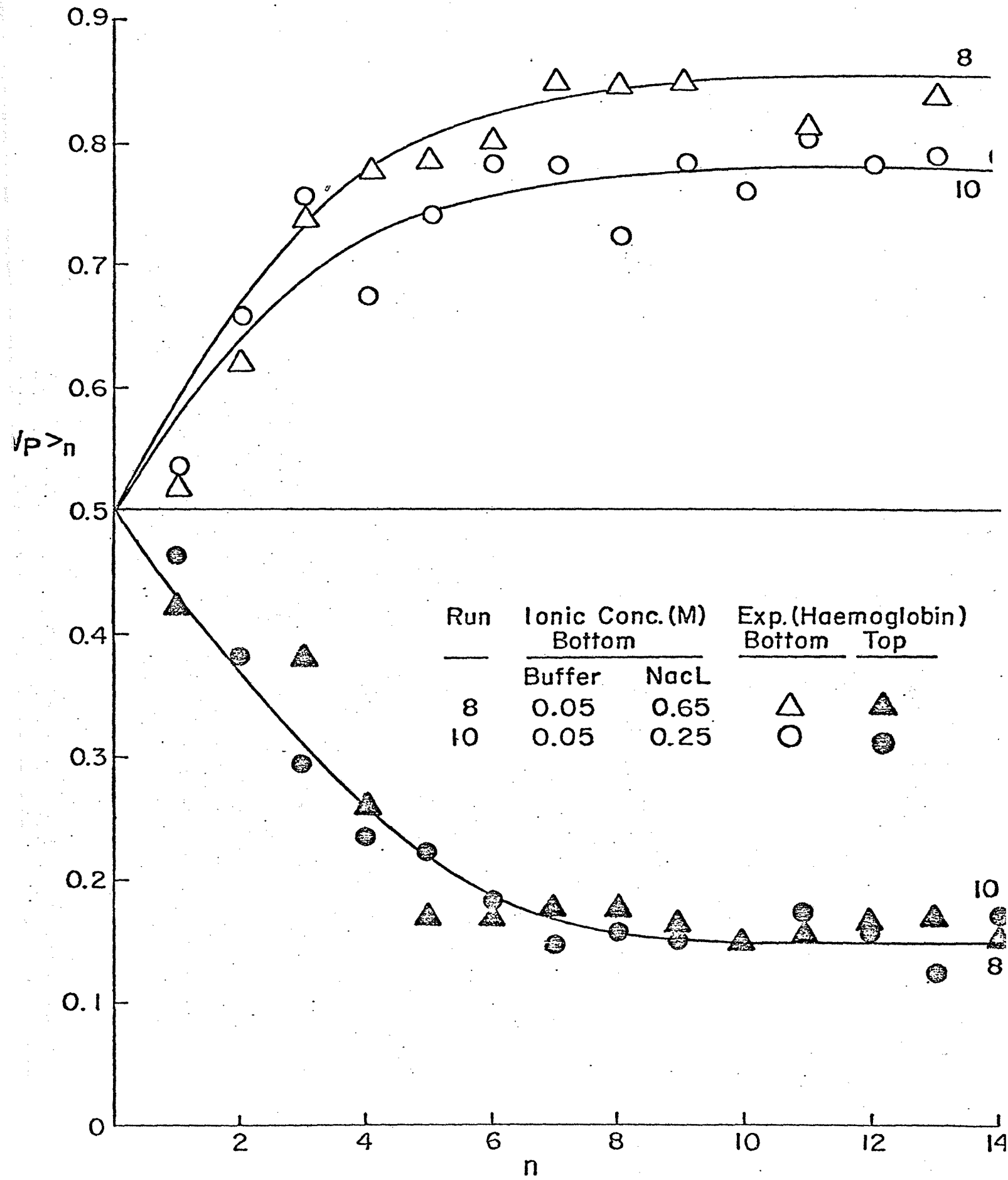


Figure 9. Dependence of separation on ionic strength.

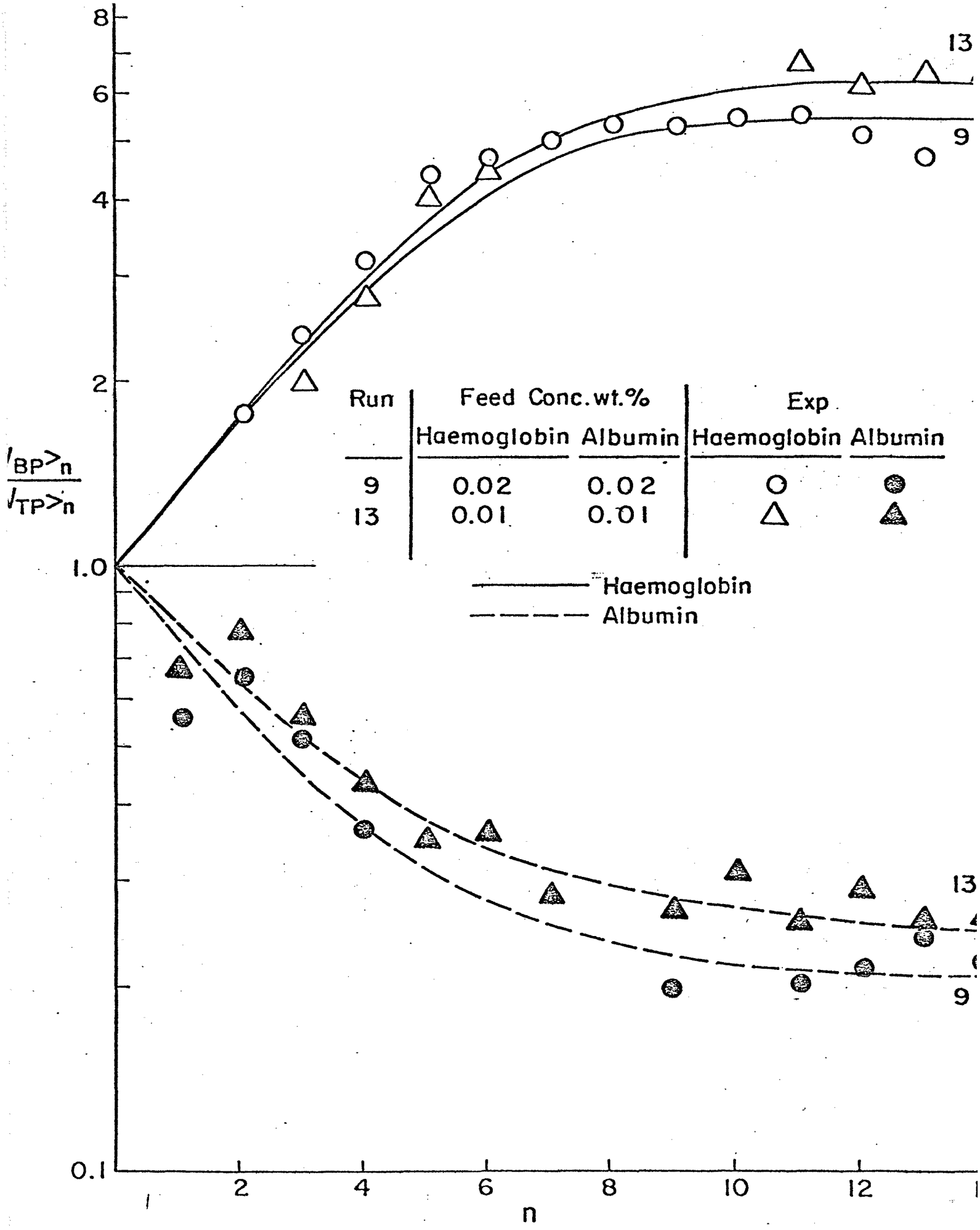
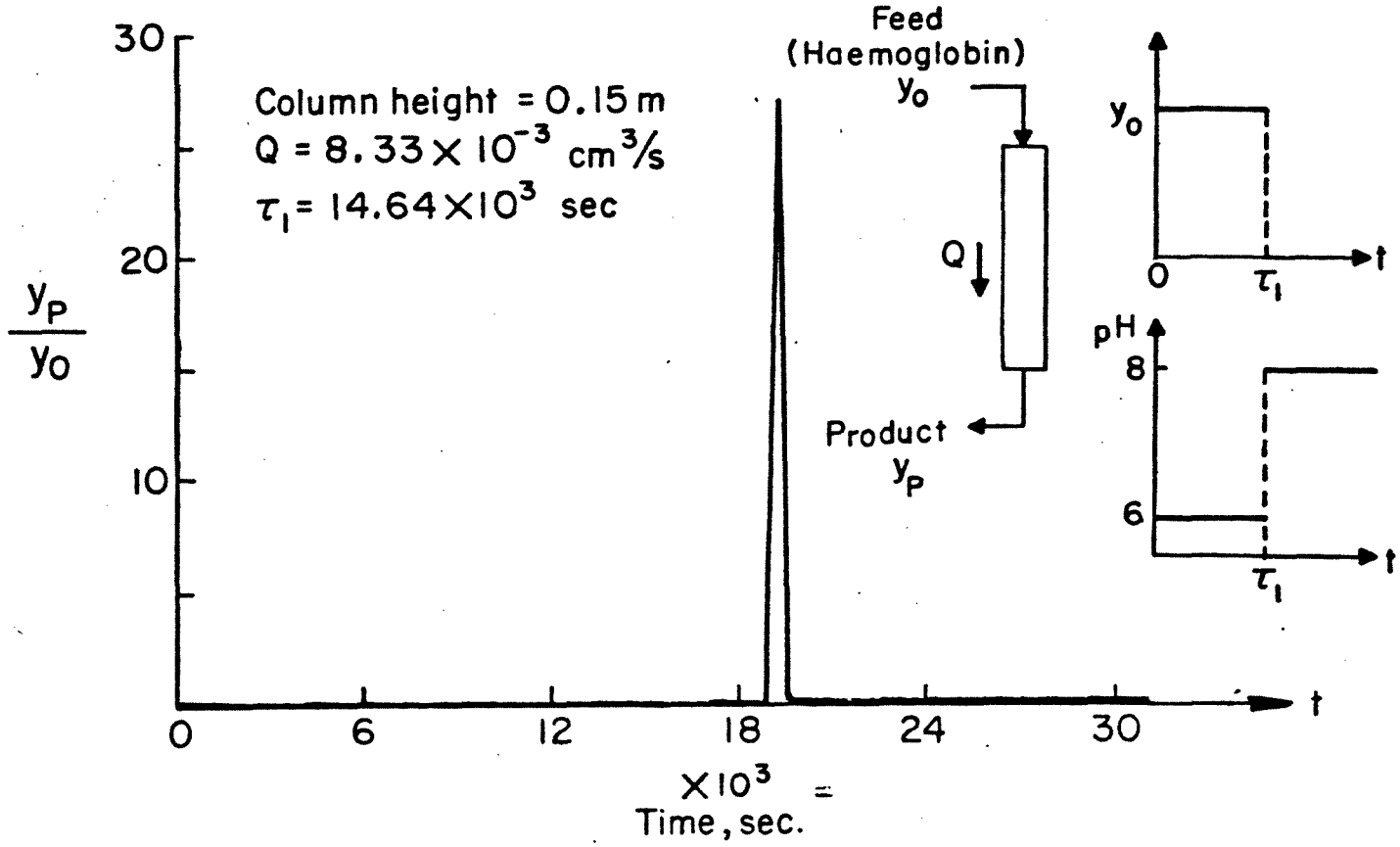


Figure 10. Effect of feed concentration on separation.



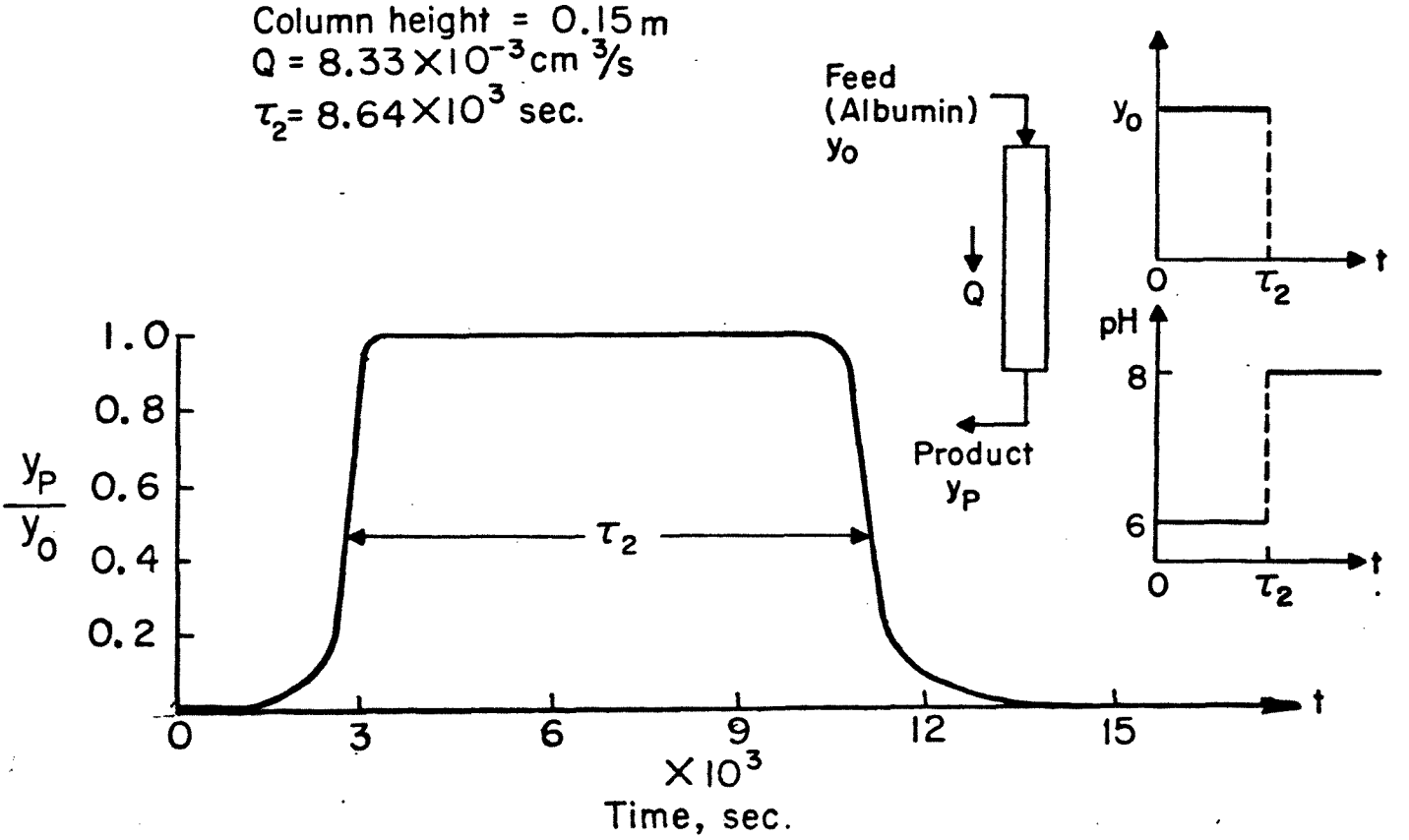


Figure 11. Elution curves for haemoglobin and albumin

APPENDIX

SAMPLE CALCULATION

The absorbance readings of the samples at a wavelength of of 403 um from each half cycle can be used to determine the percentage by weight of haemoglobin in the mixture as albumin shows no absorbance at this particular wavelength.

The feed solutions used in all runs except Run 13 (see Table 1.) were made from dissolving 0.02 gm of human haemoglobin and 0.02 gm of human serum albumin in 100 ml of the corresponding buffer solutions, giving a concentration of 0.02% by weight of haemoglobin and 0.02% by weight of albumin. The percentage by weight of haemoglobin in the sample is then calculated as follows:

$$\frac{(R_{sB})_{403}}{(R_{HF})_{403}} \times 0.02\% \qquad \text{or,}$$

$$\frac{(R_{sT})_{403}}{(R_{LF})_{403}} \times 0.02\%$$

i.e. $F_{Hb} \times 0.02\%$

Since the absorbance reading at a wavelength of 595 um arises from both haemoglobin and albumin, the percentage by weight of the total proteins in the sample is determined as follows:

$$\frac{(R_s)_{595}}{(R_F)_{595}} \times 0.04\% = F_{total} \times 0.04\%$$

From which the weight percent of albumin in the sample can be computed as the following:

$$F_{total} \text{ X } 0.04\% = F_{Alb} \text{ X } 0.02\% + F_{Hb} \text{ X } 0.02\%$$

i.e.

$$F_{Alb} \text{ X } 0.02\% = \frac{(R_s)_{595}}{(R_F)_{595}} \text{ X } 0.04\% - \frac{(R_s)_{403}}{(R_F)_{403}} \text{ X } 0.02\%$$

Thus,

$$F_{Alb} = F_{total} \text{ X } 2 - F_{Hb}$$

The weight fraction of haemoglobin in the protein mixture, W_{Hb}, is given by :

$$W_{Hb} = \frac{F_{Hb} \text{ X } 0.02\%}{(F_{Hb} + F_{Alb}) \text{ X } 0.02\%}$$

$$= \frac{F_{Hb}}{(F_{Hb} + F_{Alb})}$$

Similarly, the weight fraction of albumin in the protein mixture, W_{Alb}, is given by :

$$W_{Alb} = \frac{F_{Alb} \text{ X } 0.02\%}{(F_{Hb} + F_{Alb}) \text{ X } 0.02\%}$$

$$= \frac{F_{Alb}}{(F_{Hb} + F_{Alb})}$$

TABLE 2 **Bottom Sample Data from Run 1**

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	1.009	0.566	0.929	0.541	0.632	0.368
2	1.166	0.608	1.073	0.504	0.680	0.320
3	1.358	0.624	1.250	0.370	0.772	0.228
4	1.488	0.704	1.370	0.458	0.749	0.251
5	1.478	0.689	1.360	0.430	0.760	0.240
6	1.571	0.731	1.446	0.452	0.762	0.238
7	1.515	0.661	1.395	0.321	0.813	0.187
8	1.477	0.656	1.367	0.344	0.798	0.202
9	1.489	0.686	1.394	0.388	0.782	0.218
10	1.601	0.696	1.499	0.309	0.829	0.171
11	1.688	0.723	1.580	0.298	0.841	0.159
12	1.830	0.785	1.713	0.325	0.841	0.159
13	1.870	0.815	1.750	0.366	0.827	0.173
14	1.831	0.827	1.714	0.426	0.810	0.190
15	1.827	0.806	1.711	0.383	0.817	0.183

$(R_{HF})_{403}$= 1.086

$(R_{HF})595$= 0.770

TABLE 3 Top Sample Data from Run 1

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.665	0.265	0.517	0.203	0.718	0.282
2	1.029	0.419	0.801	0.337	0.704	0.296
3	0.735	0.505	0.572	0.798	0.418	0.583
4	0.581	0.465	0.452	0.810	0.358	0.642
5	0.473	0.472	0.368	0.912	0.288	0.713
6	0.427	0.434	0.332	0.846	0.282	0.718
7	0.396	0.448	0.308	0.820	0.273	0.727
8	0.371	0.465	0.288	0.974	0.228	0.772
9	0.326	0.428	0.260	0.902	0.224	0.776
10	0.315	0.376	0.251	0.869	0.224	0.776
11	0.312	0.426	0.249	0.907	0.215	0.785
12	0.312	0.418	0.249	0.885	0.261	0.739
13	0.324	0.443	0.258	0.944	0.215	0.785
14	0.347	0.436	0.277	0.907	0.234	0.766
15	0.323	0.468	0.258	1.012	0.203	0.797

$(R_{LF})_{403} = 1.284$

$(R_{LF})_{595} = 0.737$

TABLE 4 Bottom Sample Data from Run 2

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.913	0.672	0.856	0.924	0.481	0.519
2	1.032	0.719	0.968	0.937	0.508	0.492
3	1.079	0.658	1.012	0.731	0.581	0.419
4	1.148	0.688	1.077	0.746	0.591	0.409
5	1.088	0.702	1.021	0.839	0.549	0.451
6	1.202	0.694	1.128	0.710	0.614	0.386
7	1.372	0.740	1.287	0.673	0.657	0.343
8	1.094	0.683	1.026	0.783	0.567	0.432
9	1.142	0.717	1.071	0.828	0.564	0.436
10	1.218	0.727	1.143	0.783	0.594	0.406
11	1.368	0.781	1.283	0.786	0.620	0.380
12	1.297	0.716	1.208	0.687	0.637	0.363
13	1.343	0.778	1.250	0.811	0.607	0.393
14	1.335	0.793	1.243	0.858	0.592	0.408
15	1.336	0.790	1.245	0.850	0.593	0.407

$(R_{HF})_{403} = 1.066$

$(R_{HF})_{595} = 0.755$

TABLE 5 Top Sample Data from Run 2

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.601	0.419	0.481	0.629	0.433	0.567
2	0.785	0.574	0.628	0.893	0.413	0.587
3	0.782	0.562	0.626	0.863	0.420	0.580
4	0.657	0.502	0.526	0.804	0.395	0.605
5	0.682	0.545	0.546	0.898	0.378	0.622
6	0.611	0.514	0.489	0.873	0.359	0.641
7	0.534	0.480	0.427	0.845	0.336	0.664
8	0.531	0.496	0.425	0.889	0.323	0.677
9	0.516	0.500	0.413	0.912	0.312	0.688
10	0.480	0.490	0.384	0.914	0.296	0.704
11	0.429	0.473	0.343	0.910	0.274	0.726
12	0.389	0.482	0.309	0.968	0.242	0.758
13	0.364	0.468	0.291	0.949	0.235	0.765
14	0.361	0.461	0.289	0.932	0.237	0.763
15	0.360	0.459	0.288	0.930	0.236	0.764

$(R_{LF})_{403} = 1.250$

$(R_{LF})_{595} = 0.755$

TABLE 6 Bottom Sample Data from Run 3

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.609	0.595	0.702	0.789	0.471	0.529
2	0.907	0.754	1.045	0.845	0.553	0.447
3	1.406	0.825	1.600	0.468	0.774	0.226
4	1.593	0.913	1.789	0.424	0.808	0.192
5	1.833	0.947	2.059	0.237	0.897	0.103
6	1.988	0.983	2.233	0.231	0.906	0.094
7	1.951	0.968	2.191	0.235	0.903	0.097
8	1.998	1.036	2.245	0.351	0.865	0.135
9	2.022	0.994	2.272	0.219	0.912	0.088
10	2.167	1.054	2.435	0.207	0.922	0.078
11	2.050	1.033	2.304	0.285	0.900	0.100
12	2.177	1.056	2.446	0.201	0.924	0.076
13	2.192	1.082	2.463	0.249	0.908	0.092
14	2.160	1.089	2.427	0.303	0.889	0.111
15	2.304	1.099	2.589	0.300	0.902	0.098

$(R_{HF})_{403} = 0.890$

$(R_{HF})_{595} = 0.798$

TABLE 7 Top Sample Data from Run 3

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.452	0.497	0.361	0.933	0.279	0.721
2	1.042	0.684	0.833	0.948	0.468	0.532
3	0.662	0.611	0.529	1.062	0.332	0.668
4	0.471	0.547	0.376	1.048	0.264	0.736
5	0.348	0.522	0.278	1.082	0.204	0.796
6	0.293	0.517	0.234	1.112	0.174	0.826
7	0.266	0.524	0.212	1.153	0.155	0.845
8	0.259	0.509	0.207	1.119	0.156	0.844
9	0.244	0.538	0.195	1.206	0.139	0.861
10	0.215	0.517	0.172	1.174	0.128	0.872
11	0.228	0.527	0.182	1.190	0.133	0.867
12	0.235	0.520	0.188	1.166	0.139	0.861
13	0.234	0.529	0.187	1.191	0.136	0.864
14	0.228	0.496	0.182	1.110	0,141	0.859
15	0.227	0.501	0.181	1.111	0.139	0.861

$(R_{LF})_{403} = 1.250$

$(R_{LF})_{595} = 0.768$

TABLE 8 Bottom Sample Data from Run 4

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.940	0.680	0.877	0.732	0.545	0.455
2	1.371	0.854	1.279	0.743	0.633	0.367
3	1.690	0.903	1.576	0.561	0.737	0.263
4	1.750	0.931	1.632	0.572	0.740	0.260
5	1.761	0.945	1.643	0.594	0.734	0.266
6	1.944	0.981	1.813	0.509	0.781	0.219
7	1.963	0.935	1.831	0.383	0.827	0.173
8	2.118	0.978	1.976	0.339	0.854	0.146
9	2.178	0.974	2.032	0.273	0.882	0.118
10	2.308	1.065	2.153	0.367	0.854	0.146
11	2.184	1.030	2.037	0.401	0.836	0.164
12	2.194	0.996	2.047	0.310	0.868	0.132
13	2.459	1.110	2.294	0.333	0.873	0.127
14	2.379	1.098	2.219	0.380	0.854	0.146
15	2.360	1.108	2.201	0.421	0.839	0.161

$(R_{HF})_{403} = 1.072$
$(R_{HF})_{595} = 0.845$

TABLE 9 Top Sample Data from Run 4

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.333	0.496	0.265	0.952	0.218	0.782
2	0.923	0.670	0.735	0.909	0.447	0.553
3	0.428	0.580	0.341	1.082	0.240	0.760
4	0.264	0.526	0.210	1.081	0.163	0.837
5	0.228	0.488	0.182	1.016	0.152	0.848
6	0.213	0.469	0.170	0.981	0.148	0.852
7	0.288	0.537	0.229	1.030	0.182	0.818
8	0.224	0.513	0.178	1.081	0.141	0.859
9	0.223	0.526	0.178	1.113	0.138	0.862
10	0.213	0.502	0.170	1.062	0.138	0.862
11	0.223	0.510	0.178	1.074	0.142	0.858
12	0.220	0.536	0.175	1.141	0.133	0.867
13	0.214	0.521	0.171	1.107	0.134	0.866
14	0.243	0.532	0.194	1.112	0.149	0.851
15	0.217	0.528	0.173	1.123	0.133	0.867

$(R_{LF})_{403} = 1.255$

$(R_{LF})_{595} = 0.815$

TABLE 10 Bottom Sample Data from Run 5

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.685	0.525	0.637	0.728	0.467	0.533
2	0.838	0.555	0.778	0.665	0.539	0.461
3	0.964	0.571	0.895	0.590	0.603	0.397
4	1.219	0.599	1.131	0.427	0.726	0.274
5	1.161	0.581	1.079	0.432	0.714	0.286
6	1.370	0.692	1.273	0.527	0.707	0.293
7	1.353	0.670	1.257	0.486	0.721	0.279
8	1.282	0.656	1.239	0.467	0.726	0.274
9	1.242	0.632	1.201	0.443	0.731	0.269
10	1.364	0.687	1.319	0.468	0.738	0.262
11	1.392	0.697	1.346	0.468	0.742	0.258

$(R_{HF})_{403} = 1.076$

$(R_{HF})_{595} = 0.769$

TABLE 11 Top Sample Data from Run 5

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.375	0.404	0.294	0.820	0.264	0.736
2	0.460	0.385	0.371	0.691	0.349	0.651
3	0.251	0.309	0.202	0.650	0.237	0.763
4	0.224	0.347	0.180	0.777	0.188	0.812
5	0.122	0.203	0.098	0.462	0.175	0.825
6	0.270	0.434	0.218	0.980	0.182	0.818
7	0.276	0.449	0.222	1.017	0.179	0.821
8	0.286	0.460	0.230	1.039	0.181	0.819
9	0.291	0.471	0.230	1.069	0.177	0.823
10	0.290	0.456	0.230	1.028	0.183	0.817
11	0.342	0.449	0.270	0.969	0.218	0.782

$(R_{LF})_{403} = 1.241$

$(R_{LF})_{595} = 0.725$

TABLE 12 Bottom Sample Data from Run 6

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.837	0.727	0.808	1.123	0.418	0.582
2	1.117	0.768	1.078	0.962	0.528	0.472
3	1.333	0.853	1.286	0.980	0.568	0.432
4	1.471	0.872	1.419	0.897	0.613	0.387
5	1.586	0.893	1.530	0.842	0.645	0.355
6	1.672	0.879	1.613	0.722	0.691	0.309
7	1.713	0.930	1.653	0.817	0.669	0.331
8	1.784	0.921	1.722	0.724	0.704	0.296
9	1.932	0.922	1.671	0.778	0.682	0.318
10	1.803	0.971	1.740	0.839	0.675	0.325
11	1.776	0.938	1.739	0.753	0.698	0.302
12	1.775	0.933	1.738	0.740	0.701	0.299
13	1.783	0.938	1.746	0.745	0.701	0.299
14	1.776	0.932	1.739	0.736	0.703	0.297

$(R_{HF})_{403} = 1.036$

$(R_{HF})_{595} = 0.753$

TABLE 13 Top Sample Data from Run 6

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.604	0.440	0.478	0.792	0.376	0.624
2	1.159	0.590	0.917	0.786	0.538	0.462
3	0.747	0.573	0.591	1.063	0.357	0.643
4	0.594	0.495	0.470	0.959	0.329	0.671
5	0.491	0.437	0.388	0.873	0.307	0.692
6	0.436	0.435	0.345	0.910	0.275	0.725
7	0.394	0.415	0.311	0.887	0.260	0.740
8	0.373	0.437	0.295	0.873	0.253	0.747
9	0.363	0.411	0.287	0.899	0.242	0.758
10	0.343	0.392	0.271	0.860	0.240	0.760
11	0.340	0.410	0.281	0.902	0.238	0.762
12	0.334	0.410	0.276	0.902	0.234	0.766
13	0.333	0.406	0.275	0.896	0.242	0.758
14	0.322	0.420	0.266	0.946	0.219	0.781
15	0.320	0.419	0.264	0.928	0.232	0.768

$(R_{LF})_{403} = 1.263$

$(R_{LF})_{595} = 0.693$

TABLE 14 Bottom Sample Data from Run 7

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	1.040	0.718	0.965	0.812	0.543	0.457
2	1.148	0.749	1.065	0.789	0.574	0.426
3	1.365	0.817	1.266	0.756	0.626	0.374
4	1.485	0.932	1.378	0.822	0.626	0.374
5	1.591	0.932	1.476	0.831	0.640	0.360
6	1.600	0.938	1.484	0.838	0.639	0.361
7	1.655	0.977	1.535	0.883	0.633	0.367
8	1.681	0.862	1.559	0.675	0.731	0.269
9	1.486	0.869	1.378	0.773	0.641	0.359
10	1.651	0.939	1.532	0.792	0.659	0.341
11	1.599	0.871	1.483	0.673	0.688	0.312
12	1.578	0.853	1.464	0.674	0.694	0.306
13	1.569	0.908	1.455	0.793	0.647	0.353
14	1.463	0.888	1.357	0.841	0.617	0.383
15	1.439	1.000	1.335	1.140	0.539	0.461
16	1.514	0.900	1.404	0.824	0.630	0.370
17	1.388	0.866	1.288	0.856	0.601	0.399

$(R_{HF})_{403} = 1.078$ $(R_{HF})_{595} = 0.808$

TABLE 15 Top Sample Data from Run 7

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.500	0.596	0.396	0.848	0.318	0.682
2	0.132	0.467	0.104	0.784	0.117	0.883
3	0.592	0.751	0.468	1.169	0.286	0.714
4	0.496	0.683	0.392	1.072	0.268	0.732
5	0.424	0.659	0.335	1.069	0.239	0.762
6	0.451	0.651	0.357	1.026	0.258	0.742
7	0.420	0.658	0.332	1.069	0.237	0.763
8	0.386	0.643	0.305	1.058	0.224	0.776
9	0.395	0.675	0.313	1.131	0.216	0.783
10	0.399	0.640	0.316	1.039	0.233	0.767
11	0.359	0.617	0.284	0.981	0.225	0.775
12	0.399	0.668	0.316	1.110	0.222	0.778
13	0.401	0.649	0.317	1.061	0.230	0.770
14	0.363	0.638	0.287	1.063	0.213	0.787
15	0.481	0.669	0.381	1.048	0.267	0.733
16	0.396	0.658	0.313	1.088	0.223	0.777
17	0.369	0.675	0.292	1.152	0.202	0.798

$(R_{LF})_{403} = 1.264$ $(R_{LF})_{595} = 0.894$

TABLE 16 Bottom Sample Data from Run 8

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.738	0.534	0.675	0.633	0.516	0.484
2	1.154	0.698	1.055	0.655	0.617	0.383
3	1.472	0.745	1.346	0.480	0.737	0.263
4	1.820	0.880	1.664	0.493	0.771	0.229
5	1.767	0.838	1.615	0.439	0.786	0.214
6	1.807	0.844	1.652	0.417	0.798	0.202
7	1.963	0.866	1.794	0.329	0.845	0.155
8	1.948	0.863	1.781	0.334	0.842	0.158
9	1.934	0.857	1.768	0.332	0.842	0.158
10	1.840	0.852	1.682	0.406	0.806	0.194
11	1.823	0.856	1.666	0.432	0.794	0.206
12	1.890	0.897	1.728	0.471	0.786	0.214
13	2.312	1.032	2.113	0.416	0.835	0.165
14	1.872	0.856	1.711	0.387	0.815	0.185
15	1.960	0.945	1.792	0.524	0.859	0.141
16	2.428	1.054	2.219	0.364	0.859	0.141

$(R_{HF})_{403}$=1.094 $(R_{HF})_{595}$ = 0.816

TABLE 17 Top Sample Data from Run 8

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.505	0.535	0.462	0.810	0.421	0.579
2	0.930	0.659	0.852	0.715	0.543	0.456
3	0.541	0.549	0.495	0.811	0.379	0.621
4	0.323	0.482	0.296	0.850	0.258	0.742
5	0.188	0.430	0.172	0.851	0.168	0.832
6	0.200	0.458	0.183	0.906	0.168	0.832
7	0.257	0.482	0.203	0.943	0.177	0.823
8	0.232	0.439	0.184	0.860	0.176	0.824
9	0.214	0.445	0.169	0.889	0.160	0.840
10	0.208	0.470	0.165	0.953	0.148	0.852
11	0.223	0.481	0.176	0.968	0.154	0.846
12	0.210	0.440	0.166	0.880	0.159	0.841
13	0.248	0.485	0.196	0.957	0.170	0.830
14	0.209	0.469	0.165	0.950	0.148	0.852
15	0.168	0.461	0.133	0.963	0.121	0.879
16	0.229	0.470	0.181	0.937	0.162	0.838

$(R_{LF})_{403}$ = 1.092 $(R_{LF})_{595}$ = 0.841

TABLE 18 Bottom Sample Data from Run 9

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	1.005	0.711	0.937	0.758	0.553	0.447
2	0.967	0.681	0.901	0.722	0.555	0.445
3	1.262	0.796	1.176	0.721	0.620	0.380
4	1.487	0.806	1.386	0.535	0.721	0.279
5	1.881	0.830	1.753	0.226	0.886	0.114
6	1.821	0.822	1.697	0.262	0.866	0.134
7	1.880	0.851	1.752	0.276	0.864	0.136
8	2.329	0.992	2.171	0.194	0.918	0.082
9	1.838	0.862	1.713	0.344	0.833	0.167
10	1.875	0.844	1.747	0.265	0.868	0.132
11	1.866	0.878	1.739	0.354	0.831	0.169
12	1.890	0.895	1.761	0.372	0.826	0.174
13	1.956	0.953	1.823	0.449	0.802	0.198
14	2.016	0.962	1.879	0.414	0.819	0.181
15	2.044	0.955	1.905	0.372	0.837	0.163

$(R_{HF})_{403} = 1.073$

$(R_{HF})_{595} = 0.839$

TABLE 19 Top Sample Data from Run 9

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.091	0.138	0.072	0.305	0.191	0.809
2	0.711	0.649	0.563	1.210	0.318	0.682
3	0.518	0.575	0.410	1.161	0.261	0.739
4	0.404	0.514	0.320	1.084	0.228	0.772
5	0.345	0.492	0.273	1.071	0.203	0.797
6	0.304	0.482	0.241	1.076	0.183	0.817
7	0.282	0.466	0.223	1.050	0.175	0.825
8	0.279	0.463	0.221	1.044	0.175	0.825
9	0.252	0.457	0.199	1.050	0.159	0.841
10	0.250	0.449	0.198	1.029	0.161	0.839
11	0.250	0.436	0.198	0.993	0.166	0.834
12	0.283	0.452	0.224	1.011	0.181	0.819
13	0.273	0.453	0.216	1.022	0.174	0.826
14	0.268	0.446	0.212	1.007	0.174	0.826
15	0.236	0.439	0.187	1.012	0.156	0.844

$(R_{LF})_{403} = 1.263$

$(R_{LF})_{595} = 0.732$

TABLE 20 Bottom Sample Data from Run 10

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.993	0.729	0.950	0.836	0.532	0.468
2	1.244	0.740	1.190	0.624	0.656	0.344
3	1.430	0.741	1.368	0.448	0.753	0.247
4	1.345	0.778	1.287	0.619	0.675	0.325
5	1.496	0.796	1.432	0.518	0.734	0.266
6	1.714	0.860	1.640	0.468	0.778	0.222
7	1.644	0.834	1.573	0.471	0.770	0.230
8	1.493	0.814	1.429	0.567	0.716	0.284
9	1.729	0.869	1.655	0.475	0.777	0.223
10	1.697	0.883	1.624	0.540	0.750	0.250
11	1.796	0.882	1.719	0.443	0.795	0.205
12	1.775	0.834	1.699	0.490	0.776	0.224
13	1.782	0.802	1.705	0.481	0.780	0.220
14	1.789	0.893	1.712	0.475	0.783	0.217
15	1.780	0.752	1.703	0.485	0.778	0.222

$(R_{HF})_{403} = 1.045$

$(R_{HF})_{595} = 0.816$

TABLE 21 Top Sample Data from Run 10

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.661	0.492	0.523	0.616	0.459	0.541
2	0.592	0.536	0.468	0.772	0.377	0.623
3	0.456	0.535	0.361	0.877	0.292	0.708
4	0.329	0.500	0.260	0.897	0.225	0.775
5	0.318	0.490	0.251	0.883	0.221	0.779
6	0.246	0.482	0.194	0.922	0.174	0.826
7	0.200	0.471	0.158	0.932	0.145	0.855
8	0.189	0.420	0.149	0.823	0.153	0.847
9	0.195	0.462	0.154	0.915	0.144	0.856
10	0.325	0.439	0.256	0.760	0.252	0.748
11	0.237	0.465	0.187	0.889	0.174	0.826
12	0.215	0.475	0.169	0.931	0.154	0.846
13	0.164	0.478	0.129	0.977	0.117	0.883
14	0.240	0.500	0.189	0.968	0.163	0.837
15	0.210	0.486	0.158	0.967	0.140	0.860

$(R_{LF})_{403}$ =1.264

$(R_{LF})_{595}$ =0.864

TABLE 22 Bottom and Top Sample Data from Run 11

N	$(R_{sB})_{403}$	F_{Hb_B}	$(R_{sT})_{403}$	F_{Hb_T}	$(BOT/TOP)_{Hb}$
1	1.041	0.971	0.365	0.291	3.336
2	1.028	0.959	0.968	0.771	1.244
3	1.394	1.300	0.599	0.477	2.725
4	0.930	0.868	0.583	0.465	1.867
5	0.883	0.824	0.856	0.682	1.208
6	1.186	1.106	0.713	0.568	1.947
7	1.176	1.097	0.895	0.713	1.539
8	0.649	0.605	0.888	0.708	0.855
9	0.878	0.819	0.931	0.742	1.104
10	0.836	0.780	0.889	0.708	1.102
11	0.844	0.787	1.002	0.798	0.986

$(R_{HF})_{403} = 1.072$

$(R_{LF})_{403} = 1.255$

TABLE 23 Bottom and Top Sample Data from Run 12

N	$(R_{sB})_{403}$	F_{Hb_B}	$(R_{sT})_{403}$	F_{Hb_T}	$(BOT/TOP)_{Hb}$
1	1.288	1.204	1.023	0.808	1.490
2	1.303	1.218	0.938	0.741	1.644
3	0.656	0.613	0.811	0.641	0.956
4	0.737	0.689	0.920	0.727	0.948
5	0.625	0.584	0.968	0.765	0.763
6	0.964	0.901	1.130	0.893	1.009
7	1.137	1.063	1.178	0.930	1.143
8	1.115	1.042	0.936	0.739	1.410
9	0.873	0.816	1.040	0.821	0.994
10	0.960	0.897	1.018	0.804	1.116
11	1.163	1.087	1.007	0.795	1.367
12	0.908	0.849	0.991	0.783	1.084
13	0.958	0.895	1.076	0.850	1.053
14	0.761	0.711	1.046	0.826	0.861
15	0.571	0.534	0.922	0.728	0.930

$(R_{HF})_{403} = 1.070$

$(R_{LF})_{403} = 1.266$

TABLE 24 Bottom Sample Data from Run 13

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.464	0.568	0.845	1.038	0.449	0.551
2	0.636	0.630	1.158	0.928	0.555	0.445
3	0.685	0.626	1.248	0.825	0.602	0.398
4	0.778	0.640	1.417	0.702	0.669	0.331
5	0.825	0.641	1.503	0.620	0.708	0.292
6	0.837	0.664	1.525	0.674	0.693	0.307
7	0.891	0.659	1.623	0.559	0.744	0.256
8	0.912	0.684	1.661	0.604	0.844	0.156
9	0.959	0.696	1.747	0.568	0.755	0.245
10	0.930	0.697	1.694	0.614	0.734	0.266
11	0.973	0.694	1.772	0.526	0.771	0.229
12	0.877	0.646	1.597	0.542	0.747	0.253
13	0.982	0.700	1.789	0.529	0.772	0.228
14	0.989	0.709	1.801	0.547	0.767	0.233
15	0.965	0.719	1.758	0.623	0.738	0.262

$(R_{HF})_{403} = 0.549$

$(R_{HF})_{595} = 0.604$

TABLE 25 Top Sample Data from Run 13

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.166	0.436	0.263	1.191	0.181	0.819
2	0.528	0.591	0.838	1.132	0.425	0.575
3	0.342	0.536	0.543	1.244	0.304	0.696
4	0.255	0.506	0.405	1.282	0.240	0.760
5	0.174	0.479	0.276	1.321	0.173	0.827
6	0.147	0.470	0.233	1.334	0.149	0.851
7	0.094	0.459	0.149	1.381	0.097	0.903
8	0.111	0.464	0.176	1.371	0.114	0.886
9	0.094	0.457	0.149	1.374	0.098	0.902
10	0.135	0.475	0.214	1.369	0.135	0.865
11	0.113	0.468	0.179	1.381	0.115	0.885
12	0.021	0.306	0.033	1.287	0.025	0.975
13	0.024	0.333	0.038	1.072	0.034	0.966
14	0.067	0.381	0.106	1.164	0.083	0.917
15	0.038	0.341	0.060	1.076	0.053	0.947

$(R_{LF})_{403} = 0.630$

$(R_{LF})_{595} = 0.600$

DEVELOPMENT OF THE EXPERIMENTAL WORK

Several preliminary runs, listed in Table 1A, were made before the experimental scheme outlined in the Process Description section was adopted for the Parapump system.

For Run 14-16, two columns packed with equal length of ion exchangers were used in series. The ion exchangers were initially saturated with low pH feed solution (pH = P_1). The cycle started with the upflow of the high pH fluid into the column. The separation attained was not satisfactory.

Therefore, runs were made with the ion exchanger initially saturated with high pH feed solution (pH = P_2), for Run 17 and 18.

Ion exchangers of shorter length were used to reduce the experimental cycle time, for Run 1 - 13.

Table 1A: Experimental Parameter for preliminary runs

Run	Feed (Weight %) Haemoglobin	Feed (Weight %) Albumin	Displacement Rate, Q cm^3/s	Feed Volume (cm^3) Bottom Qt_{II}	Feed Volume (cm^3) Top Qt_{IV}	Reservoir Displacement $Qt_I = Qt_{II}$ (cm^3)	Ionic onc. Molarity, M Bottom Buffer	Ionic onc. Molarity, M Top Buffer	Column length, cm X number of columns
14	0.01	--	16.67×10^{-3}	4	4	34	0.2	0.15	10.5 X 2
15	--	0.02	16.67×10^{-3}	4	4	34	0.2	0.15	11.0 X 2
16	0.01	--	33.34×10^{-3}	4	4	34	0.2	0.15	11.0 X 2
17	0.01	0.02	16.67×10^{-3}	5	5	30	0.2	0.15	11.5 X 2
18	0.02	0.02	16.67×10^{-3}	5	5	30	0.2	0.15	20.0 X 1

TABLE 26 Bottom and Top Sample Data from Run 14

N	$(R_{sB})_{403}$	F_{Hb_B}	$(R_{sT})_{403}$	F_{Hb_T}
1	0.498	1.004	0.264	0.495
2	0.596	1.202	0.234	0.439
3	0.576	1.161	0.216	0.405
4	0.564	1.137	0.201	0.377
5	0.545	1.098	0.196	0.368
6	0.531	1.071	0.186	0.349
7	0.521	1.050	0.173	0.325
8	0.523	1.054	0.202	0.379
9	0.499	1.006	0.190	0.356
10	0.499	1.006	0.180	0.338
11	0.497	1.002	0.174	0.326
12	0.495	0.998	0.172	0.323

$(R_{HF})_{403} = 0.496$

$(R_{LF})_{403} = 0.533$

TABLE 27 Bottom and Top Sample Data from Run 15

N	$(R_{sB})_{595}$	F_{Alb_B}	$(R_{sT})_{595}$	F_{Alb_T}
1	0.682	1.041	0.542	0.903
2	0.678	1.035	0.571	0.951
3	0.656	1.002	0.543	0.905
4	0.641	0.979	0.557	0.928
5	0.628	0.959	0.608	1.013
6	0.657	1.003	0.617	1.028
7	0.660	1.008	0.603	1.005
8	0.666	1.017	0.605	1.008
9	0.681	1.040	0.608	1.013
10	0.675	1.031	0.606	1.010

$(R_{HF})_{595} = 0.655$

$(R_{LF})_{595} = 0.600$

TABLE 28 Bottom and Top Sample Data from Run 16

N	$(R_{sB})_{403}$	F_{Hb_B}	$(R_{sT})_{403}$	F_{Hb_T}
1	0.424	0.902	0.150	0.253
2	0.509	1.083	0.250	0.421
3	0.520	1.106	0.275	0.463
4	0.508	1.081	0.282	0.475
5	0.467	0.994	0.278	0.468
6	0.425	0.904	0.287	0.483
7	0.419	0.891	0.303	0.510
8	0.412	0.877	0.317	0.534
9	0.410	0.872	0.330	0.555
10	0.394	0.838	0.337	0.567

$(R_{HF})_{403} = 0.470$

$(R_{LF})_{403} = 0.594$

TABLE 29 Bottom Sample Data from Run 17

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.436	0.547	0.899	0.900	0.500	0.500
2	0.438	0.548	0.903	0.900	0.501	0.499
3	0.441	0.566	0.909	0.953	0.488	0.512
4	0.464	0.578	0.957	0.944	0.503	0.497
5	0.477	0.608	0.983	1.017	0.492	0.508
6	0.487	0.591	1.004	0.940	0.516	0.484
7	0.491	0.584	1.012	0.909	0.527	0.473
8	0.492	0.608	1.014	0.986	0.507	0.493
9	0.502	0.587	1.035	0.896	0.536	0.464
10	0.512	0.612	1.056	0.957	0.525	0.475
11	0.506	0.614	1.043	0.977	0.516	0.484
12	0.509	0.618	1.049	0.984	0.516	0.484
13	0.508	0.617	1.047	0.983	0.516	0.484

$(R_{HF})_{403} = 0.485$

$(R_{HF})_{595} = 0.608$

TABLE 30 Top Sample Data from Run 17

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.529	0.561	0.885	0.933	0.487	0.513
2	0.540	0.579	0.903	0.974	0.481	0.519
3	0.543	0.580	0.908	0.940	0.491	0.509
4	0.544	0.576	0.911	0.956	0.488	0.512
5	0.532	0.545	0.890	0.877	0.504	0.496
6	0.515	0.554	0.861	0.934	0.480	0.520
7	0.492	0.556	0.823	0.979	0.457	0.543
8	0.480	0.547	0.803	0.970	0.453	0.547
9	0.497	0.555	0.801	0.998	0.445	0.555
10	0.470	0.554	0.786	1.010	0.438	0.562
11	0.475	0.555	0.794	1.005	0.441	0.559
12	0.470	0.562	0.786	1.036	0.431	0.569
13	0.471	0.560	0.788	1.027	0.434	0.566

$(R_{LF})_{403} = 0.598$

$(R_{LF})_{595} = 0.617$

TABLE 31 Bottom Sample Data from Run 18

N	$(R_{sB})_{403}$	$(R_{sB})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	0.779	0.622	0.807	0.923	0.466	0.534
2	0.800	0.627	0.829	0.915	0.475	0.525
3	0.836	0.647	0.866	0.934	0.481	0.519
4	0.842	0.654	0.873	0.946	0.480	0.520
5	0.912	0.683	0.945	0.955	0.497	0.503
6	0.927	0.686	0.961	0.947	0.504	0.496
7	0.959	0.700	0.993	0.954	0.510	0.490
8	0.972	0.707	1.007	0.960	0.512	0.488
9	1.098	0.759	1.138	0.973	0.539	0.461
10	1.105	0.769	1.145	0.994	0.535	0.465
11	1.124	0.778	1.165	0.999	0.538	0.462
12	0.933	0.703	0.967	0.988	0.495	0.505
13	1.111	0.765	1.151	0.977	0.541	0.459
14	1.097	0.766	1.137	0.994	0.534	0.466
15	1.120	0.778	1.161	1.003	0.537	0.463
16	1.116	0.780	1.156	1.014	0.533	0.467
17	1.155	0.784	1.197	0.984	0.549	0.451

$(R_{HF})_{403} = 0.965$

$(R_{HF})_{595} = 0.719$

TABLE 32 Top Sample Data from Run 18

N	$(R_{sT})_{403}$	$(R_{sT})_{595}$	F_{Hb}	F_{Alb}	W_{Hb}	W_{Alb}
1	1.150	0.666	0.966	0.929	0.510	0.490
2	1.028	0.628	0.863	0.924	0.483	0.517
3	0.925	0.598	0.777	0.924	0.457	0.543
4	0.915	0.597	0.768	0.930	0.452	0.548
5	0.892	0.594	0.749	0.941	0.443	0.557
6	0.890	0.594	0.747	0.943	0.442	0.558
7	0.871	0.583	0.731	0.928	0.441	0.559
8	0.880	0.573	0.739	0.891	0.453	0.547
9	0.838	0.576	0.704	0.935	0.430	0.570
10	0.879	0.580	0.738	0.912	0.447	0.553
11	0.825	0.560	0.693	0.900	0.435	0.565
12	0.854	0.542	0.717	0.825	0.465	0.535
13	0.852	0.551	0.715	0.853	0.456	0.544
14	0.834	0.561	0.700	0.896	0.439	0.561
15	0.842	0.536	0.707	0.818	0.464	0.536
16	0.808	0.535	0.678	0.844	0.445	0.555
17	0.802	0.538	0.674	0.857	0.440	0.560

$(R_{LF})_{403} = 1.190$

$(R_{LF})_{595} = 0.703$