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ABSTRACT

Title of Thesis: An Experimental Study of Comparison Between PH-Parametric Pumping and PH-Cycling Zone Adsorption Process on Protein Separation

Vincent P. Jajalla, Master of Science in Chemical Engineering, 1981

Thesis directed by: Dr. Ching-Rong Huang, Professor Department of Chemical Engineering

In this thesis, an experimental investigation was conducted to compare the performance of the continuous pH-parametric pumping and the pH-cycling zone adsorption process on protein separation.

The model system studied was hemoglobin-water on CM-Sepharose CL-6B (cation) exchanger in a single-column system. Various modes of operation were tried on both the pH-parametric pumping and the pH-cycling zone adsorption method. The separation factors for each mode of operation were computed and compared.

It is shown that the continuous pH-parametric pumping, with circulation to insure the pH uniformity inside the column, has the better separation factor than the pH-cycling zone adsorption method.

AN EXPERIMENTAL STUDY OF COMPARISON BETWEEN PH-PARAMETRIC PUMPING AND PH-CYCLING ZONE ADSORPTION PROCESS ON PROTEIN SEPARATION

By

VINCENT P. JAJALLA

A THESIS

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I would like to dedicate this thesis to my wife, Med and to my six-year old daughter, Sheryl Ann.

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I am mostly grateful to the late Dr. H.T. Chen, my thesis advisor whose untimely death was a great loss to the institute. The guidance and help I received from him were invaluable and made this thesis possible.

I am also thankful to Dr. C. R. Huang who succeeded Dr. Chen as my advisor for his help in completing the thesis.

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INTRODUCTION

Chromatography is a physical method of separation in which the components to be separated are destributed between two phases. These phases are the stationary bed of large surface area and the fluid that passes through the stationary bed. The term "chromatography" had its origin in the initial use for separation of colored vegetable extracts but found its application extending rapidly throughout the years to many areas such as separation of gases, liquids, and dissolved solids in liquids. Although it was intrinsically a batch process used mainly in analytical determinations, a continuous chromatographic operation has been successfully developed.

The parametric pumping and the cycling zone adsorption processes are ion exchange chromatographic techniques which induce separation by periodically varying a thermodynamic parameter such as temperature, pressure, or pH. This variation causes a shift in the equilibrium destribution between the two phases involved (the ion exchange medium and the fluid in the column). The only difference between these two techniques is the mode of fluid flow in the column. It is reciprocating flow for the parametric pumping while unidirectional for the cy-

cling zone method.

Parametric pumping was first applied to the separation process in 1966 by the inventor of the batch parametric pumping, the late R. H. Wilhem of Princeton University. Since then, much research work has been done in this area but mostly on thermal and heatless (or pressure cycling) parametric pumps. By contrast, little work has been done on pH-parametric pumps. Such work on the pH-parametric pumps includes the studies of Sabadell and Sweed (1970), Shaffer and Hamrin (1975), and Chen et al (1971, 1972, 1973, 1974, 1975, 1976, 1977, 1979, 1980).

In previous work, Chen et al (1977, 1979) studied pH-parametric pumping on separation of protein mixture and showed the excellent capability of this separation technique.

On the other hand, cycling zone adsorption was first developed by Pigford et al (1969) and later was investigated by Wankat (1973). Other works include Bergman (1971), Gupta and Sweed (1971), and Van der Vlist (1971). BusBice and Wankat (1975) applied pH-cycling zone method to separation of sugars and obtained encouraging results.

In this work, separation of protein mixtures were experimentally investigated using both the pH-parametric pumping and the pH-cycling zone adsorption process. The

purpose of this thesis is to compare both methods experimentally for their separation capability as applied to single-component protein system.

THEORY AND PROCESS DESCRIPTION

Theory of Ion Exchange

An ion exchanger is made of an insoluble matrix which may be based on inorganic compounds, synthetic resins, polysaccharides, etc. This matrix determines the physical characteristic of the ion exchanger such as its mechanical strength, flow properties, behavior toward biological compound and capacity. Covalently bound to this matrix are charged groups associated with mobile counter-ions which can be reversibly exchanged with other similarly charged ions without altering the matrix.

An ion exchanger can either be positively charged or negatively charged. Cation exchanger has negatively charged counter-ion available for exchange while anion exchanger has positively charged counter-ion.

In ion exchange chromatography, separation can be achieved because different substances have different affinities toward the ion exchanger due to variation of their charges. These affinities can be controlled by changes in ionic strength and the pH.

In a brief partial-saturation step, a small amount of the solute mixture to be separated is deposited at the inlet end of a column. Elution (or regeneration) by

an elutant is then carried out. Different solutes generally have different equilibrium distributions between the fluid and the solid phase. Those with a smaller equilibrium factor will advance more rapidly through the bed during elution.

Protein Separation by Ion Exchange

Removal or separation of proteins can not be made by ordinary filtration since their molecules are small enough to pass through the filter medium. A dialytic membrane can remove proteins but can not selectively separate types of normal proteins. One powerful method to achieve this kind of separation is ion exchange chromatography.

Proteins are groups of complex organic compounds that contain amino acids as their basic structural units. They occur in all living matters and are essential for growth and repair of animal tissue. They have high molecular weights. Two types of protein of significant concern are hemoglobin and albumin having molecular weights of 63,000 and 69,000 respectively.

Proteins carry both negatively and positively charged group with a net charge depending on their pH. The intermediate pH at which the net charge is zero is

called the isoelectric point. Above the isoelectric point, the net charge is negative and below, it is positive. Thus they can be bound to a suitable ion exchanger.

The PH-parametric Pumping and PH-cycling Zone Adsorption Processes

This behavior of proteins, coupled with the preferential adsorption ability of the ion exchanger is the basis behind both the pH-parametric pumping and the pH-cycling zone adsorption processes. Both involve a periodically alternating intrinsic variable (pH) to effect an interphase mass transfer in the column. Such mass movement can be made reversible. Only the mode of directing the flow differs between the two. The fluid flow is reciprocating for the parametric pumping while it is unidirectional for the cycling zone. Consequently, the parametric pumping will have one degree of freedom (displacement) more than the cycling zone.

In a single-column pH-parametric pumping, the periodic reciprocating flow of fluid between two feed reservoirs of different pH levels will cause a desired protein having an isoelectric point between these two pH levels to concentrate in one of the reservoirs. A steady state is established after a certain number of repeated cycles where no more protein can be made to transfer. Such transfer can be extended by employing a

multi-column system (columns packed alternately with cation and anion exchangers). Chen et al (1979) described such system and demonstrated a considerably improved separation factor.

In the single-column pH-cycling zone adsorption system, the first feed is introduced into the top of the column for a predetermined period. A desired protein will be adsorbed. For the next period, instead of reversed fluid flow, the second feed is introduced into the top of the column in the same manner as the first. The adsorbed protein in the column will be desorbed and separation will be achieved. As this procedure is repeated for many cycles, a concentration wave is propagated across the column and eventually comes out from the bottom.

EXPERIMENTAL METHOD

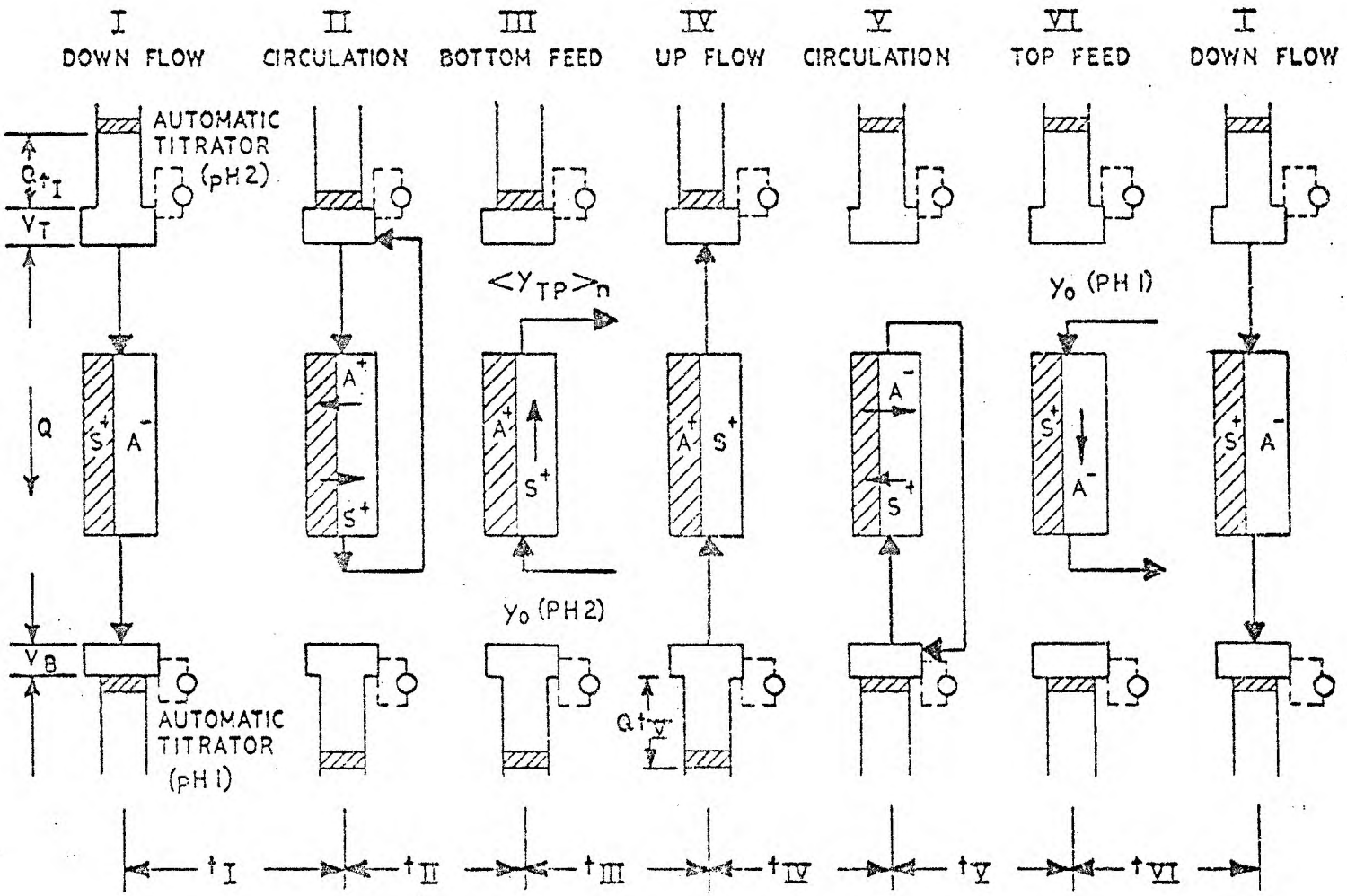
Experimental Procedure for PH-parametric Pumping

The system consists of a jacketed chromatographic column packed with an ionic exchanger. The temperature within the column is kept constant by a refrigeration unit. Two feed reservoirs are attached to each end of the column, one (top) of pH_2 and the other (bottom) of pH_1. Both lines of the reservoirs are hooked up to a pump to induce flow of the fluids through the solid phase in the column in either down-flow or up-flow mode. Moreover, each reservoir is connected to a pH titrator to insure the pH requirement and is constantly agitated by a magnetic stirrer. By alternately varying the flow in a reciprocating manner, a desired protein of isoelectric point between the two pH levels can be separated as shown by Chen et al (1977, 1979).

The pH-parametric pump under study is depicted in a schematic diagram in Figure 1. Originally the column is saturated with fresh feed of pH_1. The flow system involves six different steps which follow:

(1) Feed from the top reservoir is pumped to the top of the column while the displacement goes to the bottom reservoir for time t_I. Adsorption of protein by the ion exchanger is initiated.

(2) Fluid from the top reservoir is circulated



$P_1 < I_A < P_2$; S = COUNTER ION

▨ = CATION EXCHANGER; A = PROTEIN A WITH ISOELECTRIC POINT I_A

SCHEMATIC FLOW DIAGRAM FOR PH-PARAMETRIC PUMP

FIGURE 1

through the column for time t_{II}. Equilibrium is established and adsorption of the protein is thus finalized. (For a no circulation process, this step is omitted.)

(3) Fresh feed of pH_2 is introduced into the column from the bottom, whereas the displaced fluid from the top is collected as sample ($\langle Y_{TP} \rangle_n$) for analysis.

(4) Feed from the bottom reservoir is pumped to the bottom of the column for time t_{IV} while the effluent enriched in protein flows back to the top reservoir.

(5) The fluids from the bottom reservoir are made to circulation through the column for time t_{II}. During this time desorbed protein enters the solution. (This step is also omitted for a no circulation process.)

(6) Fresh feed of pH_1 is pumped to the top of the column and bottom sample ($\langle Y_{TB} \rangle_n$) is collected for time t_{VI}. This completes a cycle. As the cycle is repeated, a steady state will be attained where most of the protein eventually goes to the bottom reservoir.

The parameters for the pH-parametric pumping are the buffer pH and ionic strength, fluid flow rate,

feeding times (t_{III} and t_{VI}), and displacement and circulation times.

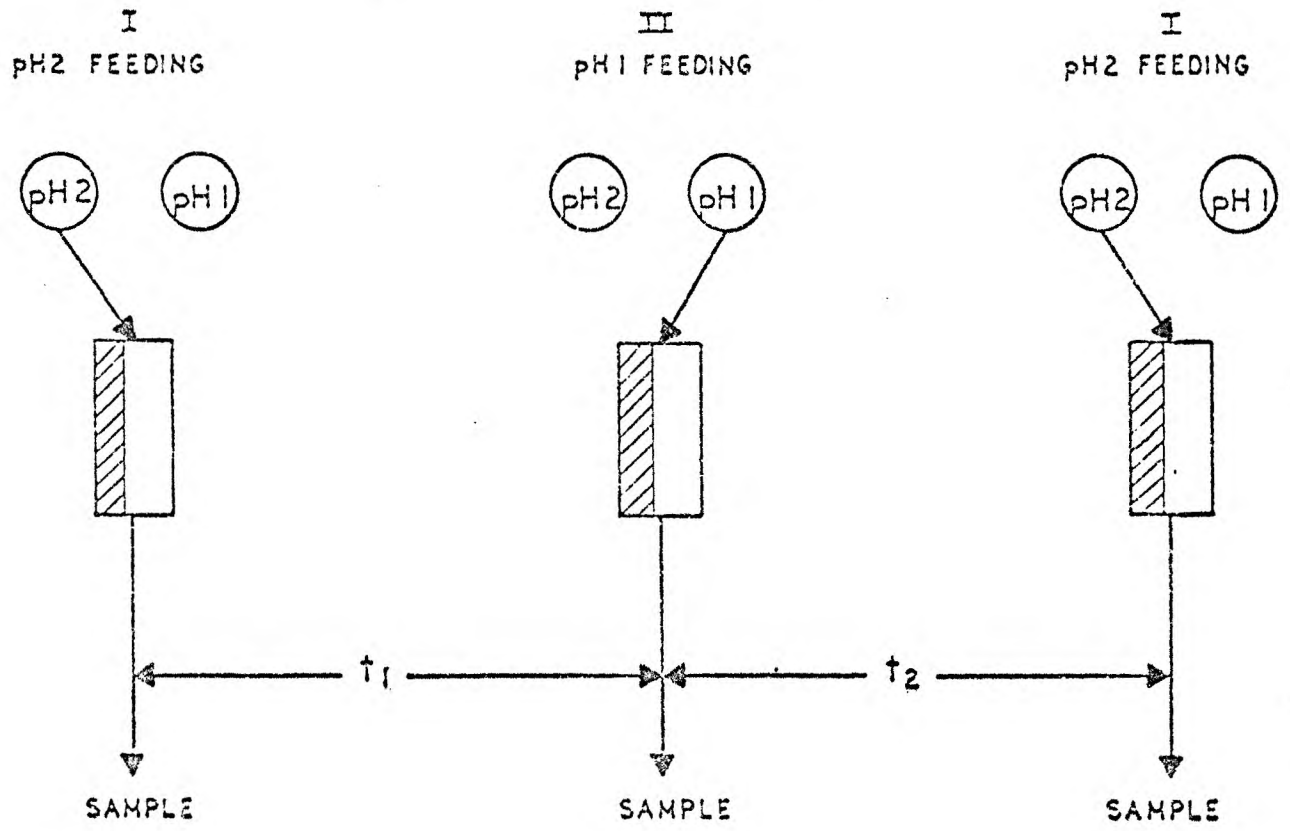
Experimental Procedure for PH-cycling Zone

The same basic apparatus for the pH-parametric pump is used in the pH-cycling zone system. This includes the chromatographic column, pump and two feed reservoirs of pH_1 and pH_2 connected to the top of the column. The two feeds are to be pumped to the top of the column, one at a time, at periodic interval.

A diagram of the steps of the process is presented in Figure 2. It involves two steps only. The first step is to pump feed of pH_2 to the top of the column for time t_1. At pH_2, the protein is taken up by the ionic exchanger. The second step is to switch the flow to feed of pH_1 for time t_2. This allows the stored solute in the solid phase to desorb into the fluid. This step completes the cycle.

For this process, the parameters are the buffer pH and ionic strength, fluid flow rate, and the feeding times (t_1 and t_2). Note that t_1 and t_2 correspond to t_{III} and t_{VI} respectively in the pH-parametric pumping.

As the cycle is continued a steady state of concentration and pH waves propagates across the column and comes out from the bottom of the column. The eluted



SCHEMATIC FLOW DIAGRAM FOR PH-CYCLING ZONE

FIGURE 2

fluids are then taken at regular interval as samples (2 cc. or 3 cc.) for analysis.

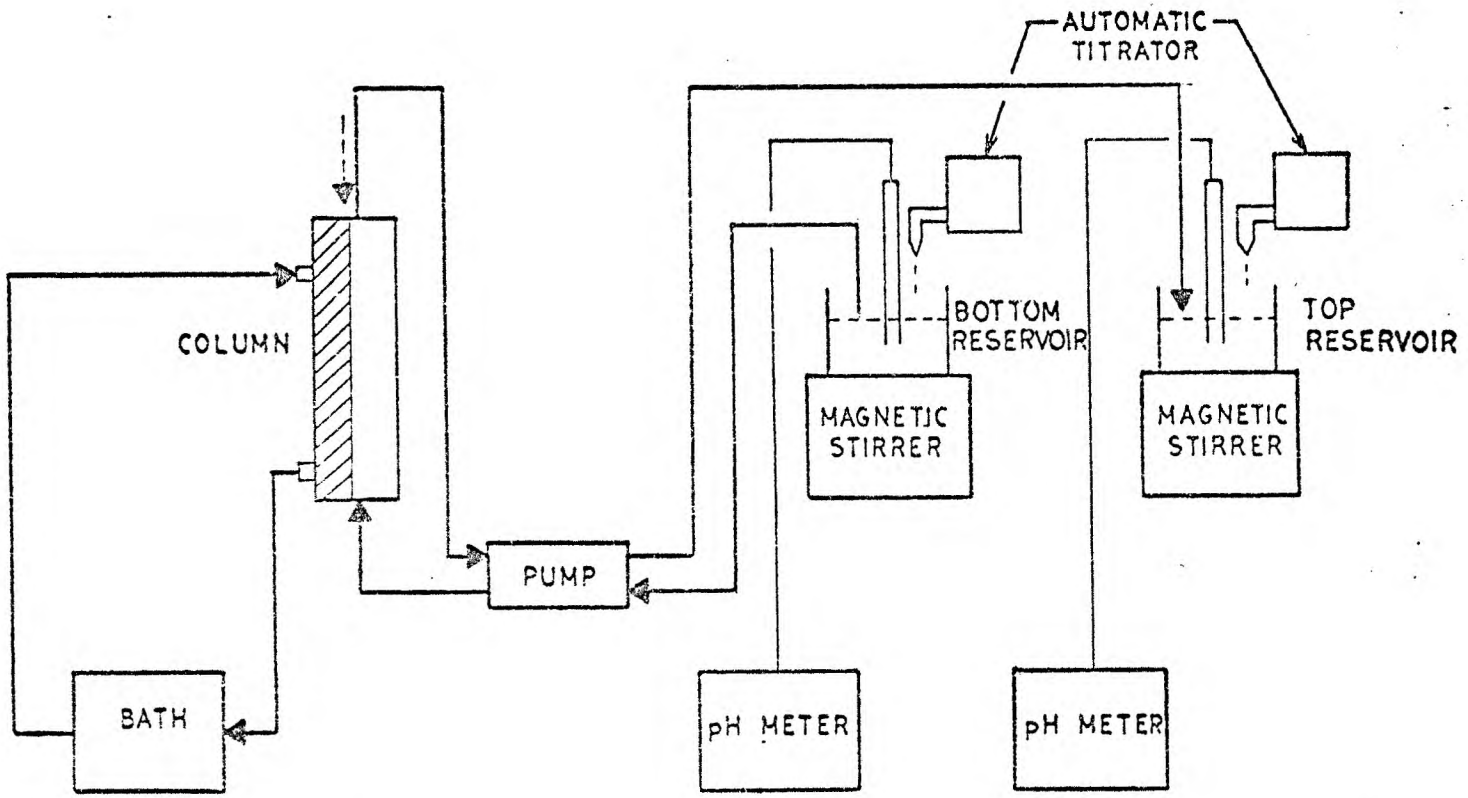
Apparatus Set-up for PH-parametric Pumping

Figure 3 shows a complete apparatus set-up of the pH-parametric pumping. The column consists of a chromatographic tube (a straight, high precision bore, borosilicate glass tube of an inner diameter of 16 ± 0.02 mm.) and a thermostat jacket for controlled temperature work. The column has two end pieces (top and bottom) to hold the chromatographic and jacket tubes in position. Each end piece has an adaptor which consists of a plunger and bed support. The plunger contains the 10 micro nylon net which holds the ion exchanger from going out from the column but not the protein.

Both lines from the top and the bottom of the column are hooked up to a Peristaltic pump made by Pharmacia Fine Chemicals. The flow direction of the pump can be reversed and the rate adjusted from range of zero to five cc./min..

The pH in the two reservoirs is kept constant by a Fisher automatic titrator model 36. Concentration of base and acid used for titrating is 0.5 N.

The cation exchanger used is the gel, CM-Sepharose CL-6B manufactured by Pharmacia Fine Chemicals. This cation exchanger has a capacity of 12 ± 2 milliequivalent



APPARATUS SET-UP FOR PH-PARAMETRIC PUMP

FIGURE 3

weight/100 ml gel bed and particle size of between 40 to 160 μm.

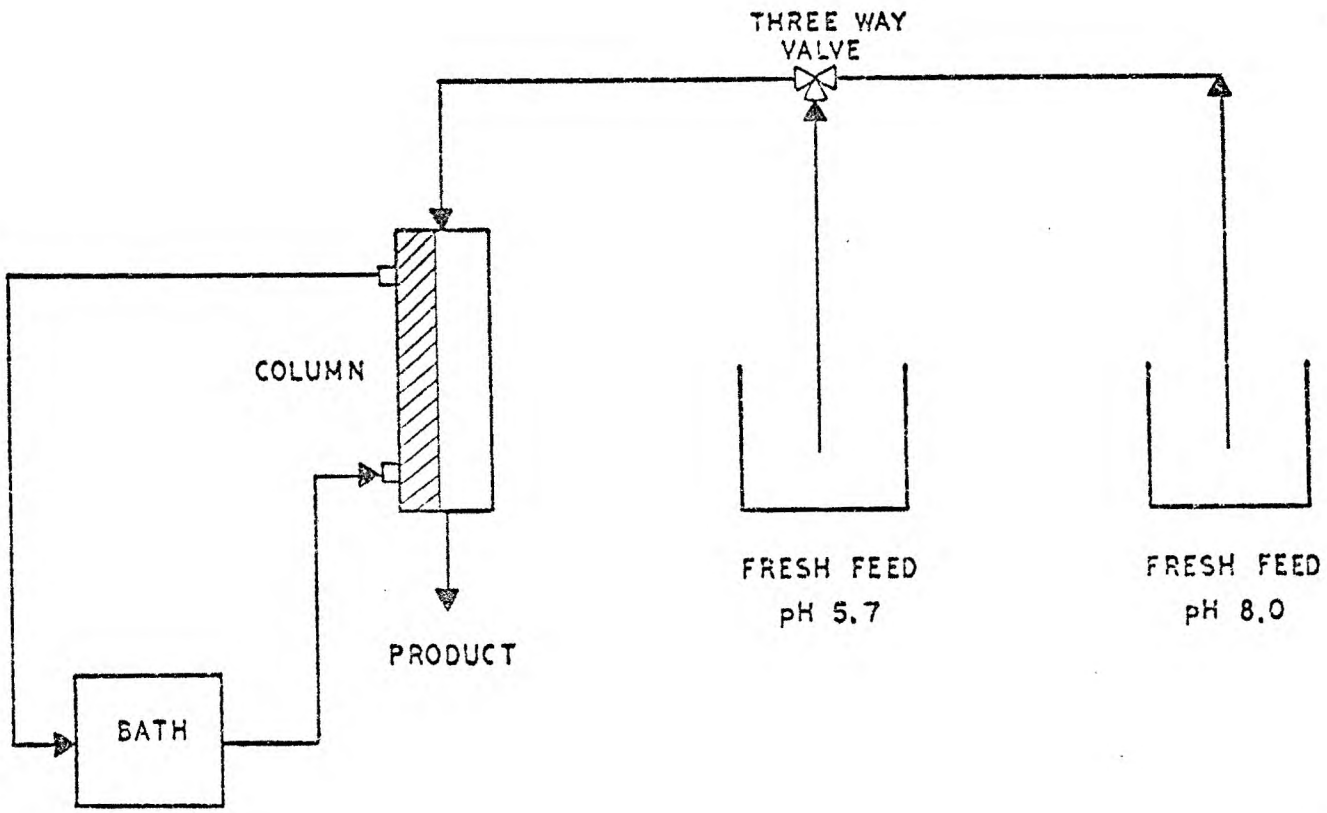
Fresh gel is contained in a bottle with 0.01% merthiolate for preservative. Therefore the gel should be washed thoroughly with distilled water and buffer solution before using it in the experiment.

Apparatus Set-up for PH-cycling Zone

The set-up for pH-cycling zone is simpler than the pH-parametric pumping. No titrator is needed since feed is always fresh. This system is represented in Figure 4. The apparatus used are basically the same as those in the pH-parametric pumping described in the preceding section.

Preparation and Packing of Gel in the Column

Unlike Sephadex ion exchangers which are supplied as powders and are to be swollen in a buffer solution for a day, Sepharose ion exchanger are supplied preswollen. Therefore no swelling is required. After washing the gel with distilled water, the required amount of ion exchanger is stirred into an excess of starting buffer. Vigorous stirring should be avoided since it might damage the beads of the gel. The starting buffer must contain the same ion as that originally present



APPARATUS SET-UP FOR PH-PARAMETRIC PUMP

FIGURE 4

in the ion exchanger. The supernatant liquid is removed and replaced with fresh buffer several times. This procedure insures that the charged groups in the ion exchanger are completely titrated with the counter-ions of the buffer. Instead of decantation, the ion exchanger can be washed extensively through a Buchner funnel.

As with any other chromatographic technique, packing the gel in the column is a critical stage in an ion exchange experiment. A poorly packed column gives a poor and uneven flow, zone broadening, and loss of resolution. The swollen ion exchanger is mixed with the starting buffer to form a fairly thick slurry but not so thick to retain air bubbles. The column is mounted vertically on a suitable stand and the gel suspension is poured slowly into the column by using a glass rod as a guide. This prevents formation of air bubbles. The gel is allowed to settle and the excess liquid removed. To settle the gel to desired porosity, a slight operating pressure is applied in the column by running the pump at 1 cc./min.. (Note that 1 cc./min. is the flow rate of all the experiments). When placing the plunger on the top of the column, care must be observed so as not to create bubbles in the settled gel.

After the column has been completey packed with the ion exchanger, the starting buffer is run again

through the bed to equilibrate and stabilize the ion exchanger.

Preparation of the Buffers

A tris (hydroxymethyl) aminomethane-maleate (tris-maleate) buffer is used. First, stock solutions are prepared which consist of:

a) A: 0.2 M solution of tris acid maleate (24.2 gm of tris (hydroxymethyl) aminomethane + 23.2 gm of maleic acid or 19.6 gm of maleic anhydride to 1000 ml.)

b) B: 0.2 M NaOH

For experiments requiring a buffer concentration lesser than 0.2 M and the presence of NaCl, the stock solutions are diluted with distilled water and the required amount of NaCl is added.

Stock solution A is gradually added to stock solution B in a beaker equipped with a pH meter until the desired pH is reached. The pH meter insures that the correct buffer pH is really obtained. Mixing can be facilitated by a use of a magnetic stirrer. The buffers are then stored in the refrigerator at $5^{o}C$.

Solutions over five-days old are discarded because of possible microbial degradation of the solutions. Extreme cleanliness must be observed in preparing the

buffer solutions.

Preparation of the Feeds

All the feeds are made of 0.02% hemoglobin in buffer solution (one for the low pH and another for the high pH).

0.02 gm of hemoglobin is weighed and dissolved in 100 ml of the buffer solution. Magnetic stirring is applied to dissolve the protein fast and completely. The solution is then filtered through a no. 4 Whatman filter paper and is kept in the refrigerator at $5^{o}C$. Worthington human hemoglobin is used.

System Selection

The model system selected is hemoglobin-water mixture on CM-Sepharose CL-6B (cation) exchanger in a single-column system.

For the choice of a buffer system, a number of experiments were conducted. Note that a same buffer system will have the same effect in both the pH-parametric pumping and the pH-cycling zone method. Regardless of the process used, a bad buffer will result to a poor performance while a good one will always perform well, although in varying degrees for different processes. Therefore, the choice of buffer is not a critical fac-

tor and finding the buffer system of maximum performance is not necessary. A fairly good choice is applicable for the purpose of comparing the pH-parametric pumping and the pH-cycling zone adsorption process.

Analysis of Samples

Samples were analyzed by a Spectrophotometer model 400 made by Bausch and Lomb. For hemoglobin analysis a wavelength of 403 and a tungsten lamp were used.

Analysis of hemoglobin can also be made by using a Bio-Rad reagent. In this case the reading is taken at wavelength of 595 of tungsten lamp in the Spectrophotometer. An illustration of sample calculations are given in the APPENDIX A. Bio-Rad reagent preparation is described in the APPENDIX B.

Calculation of the Concentration of Protein (Y_H)

Calculation of the concentration of hemoglobin (Y_H) from the readings obtained from the Spectrophotometer involves the equation if wavelength of 403 is used:

$$Y_H = \frac{R_s^{403} - R_b^{403}}{R_f^{403} - R_b^{403}} \qquad (3\text{-}1)$$

where Y_H = the ratio of hemoglobin concentration of product to feed.

R_s^{403} = 403 reading of product sample.

R_b^{403} = 403 reading of the buffer.

R_f^{403} = 403 reading of the fresh feed.

For wavelength of 595, calculation requires the equation:

$$Y_H = \frac{R_s^{595} - R_b^{595}}{R_f^{595} - R_b^{595}} \qquad (3\text{-}2)$$

where R_s^{595} = 595 reading of the product sample.

R_b^{595} = 595 reading of the buffer.

R_f^{595} = 595 reading of the fresh feed.

Readings taken at 403 are dependent on pH while those at 595 are unaffected. Therefore for 403 readings, the pH of the buffer, feed and product sample should be the same in order for equation (3-1) to be consistent. Otherwise, a correction factor for different pH is required.

The equation

$$Y_H = \frac{R_s^{403} - R_b^{403}}{R_f^{403} - R_b^{403}} \times \frac{k_f / k_{pH=6}}{k_s / k_{pH=6}} \qquad (3\text{-}3)$$

is used for unequal pH where

$$\frac{k_f / k_{pH=6}}{k_s / k_{pH=6}} = \text{over-all pH correction factor}$$

$k_f / k_{pH=6}$ = correction factor for feed at its pH.

$k_s / k_{pH=6}$ = correction factor for sample at its pH.

The value of $k / k_{pH=6}$ can be found from the graph in Figure 5 where correction factor vs. pH is plotted for 0.1 M Phosphate buffer. This curve is constant for a variety of buffers including Tris maleate buffer.

Note that if pH of feed and product sample are equal, $(k_f / k_{pH=6})/(k_s / k_{pH=6}) = 1$ and equation (3-3) becomes equation (3-1).

Calculation of the Separation Factor (SF)

Separation factor (SF) which is the ratio of the average bottom (or high) product concentration to the average top (or low) product concentration is calculated by the equation

$$SF = \frac{\langle Y_{BP} \rangle_n}{\langle Y_{TP} \rangle_n} \qquad (3\text{-}4)$$

where $\langle Y_{BP} \rangle_n$ = the average bottom (or high) product concentration, and

$\langle Y_{TP} \rangle_n$ = the average top (or low) product concentration.

$\langle Y_{BP} \rangle_n$ is equal to Y_H of the bottom product while $\langle Y_{TP} \rangle_n$ is equal to Y_H of the top product.

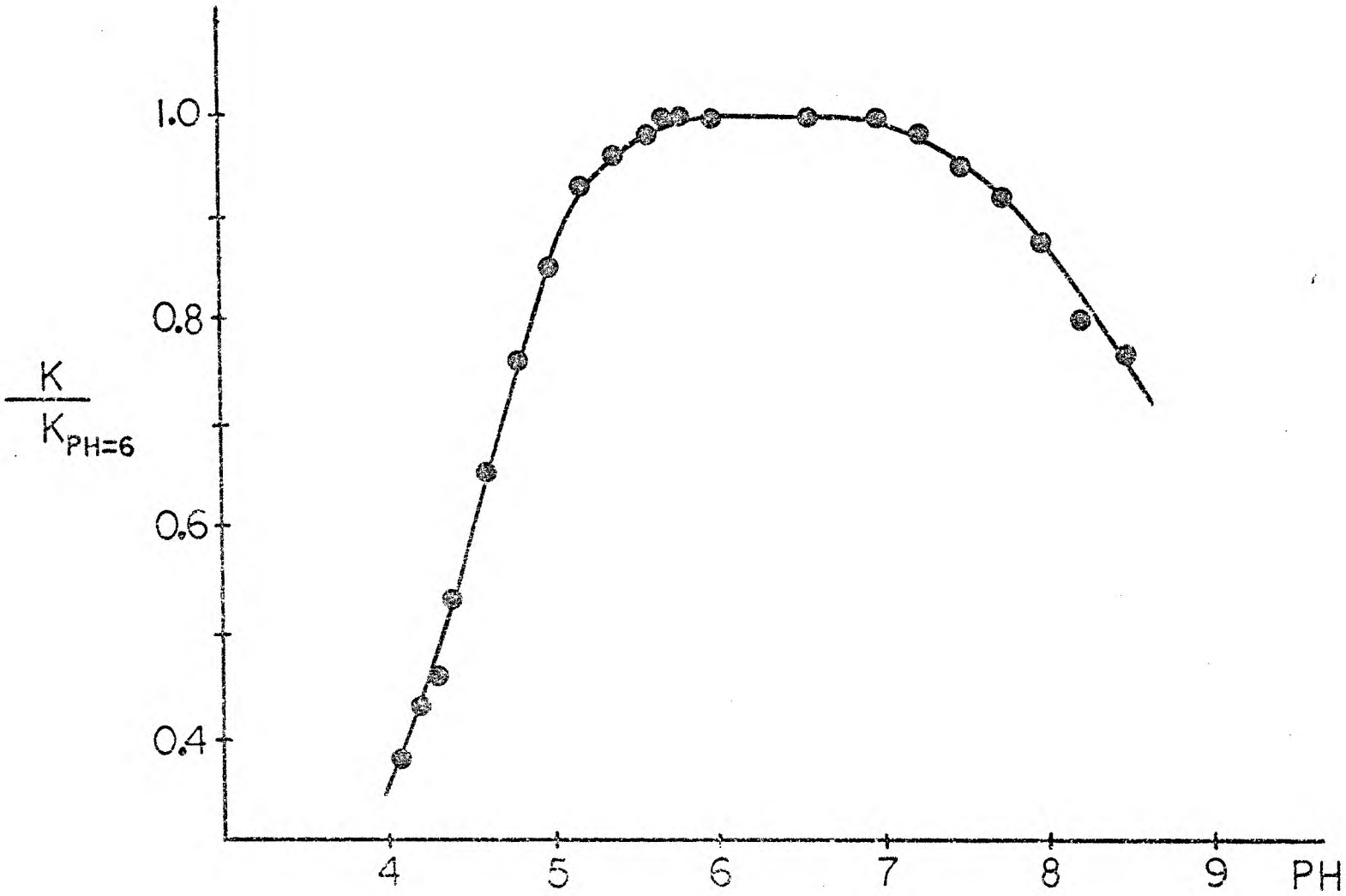


FIGURE 5

DISCUSSIONS AND RESULTS

Choice of a Buffer System

Three runs of experiments were conducted at different buffer pH and ionic strength, and NaCl concentration. The pH-cycling zone adsorption process was used in these experiments to determine which buffer system is suitable for use in the experiments. It is obvious that the same buffer system will have the same effect on both the pH-parametric pumping and the pH-cycling zone method.

Choice of the buffer systems in the three runs were based from the previous work of Chen et al.

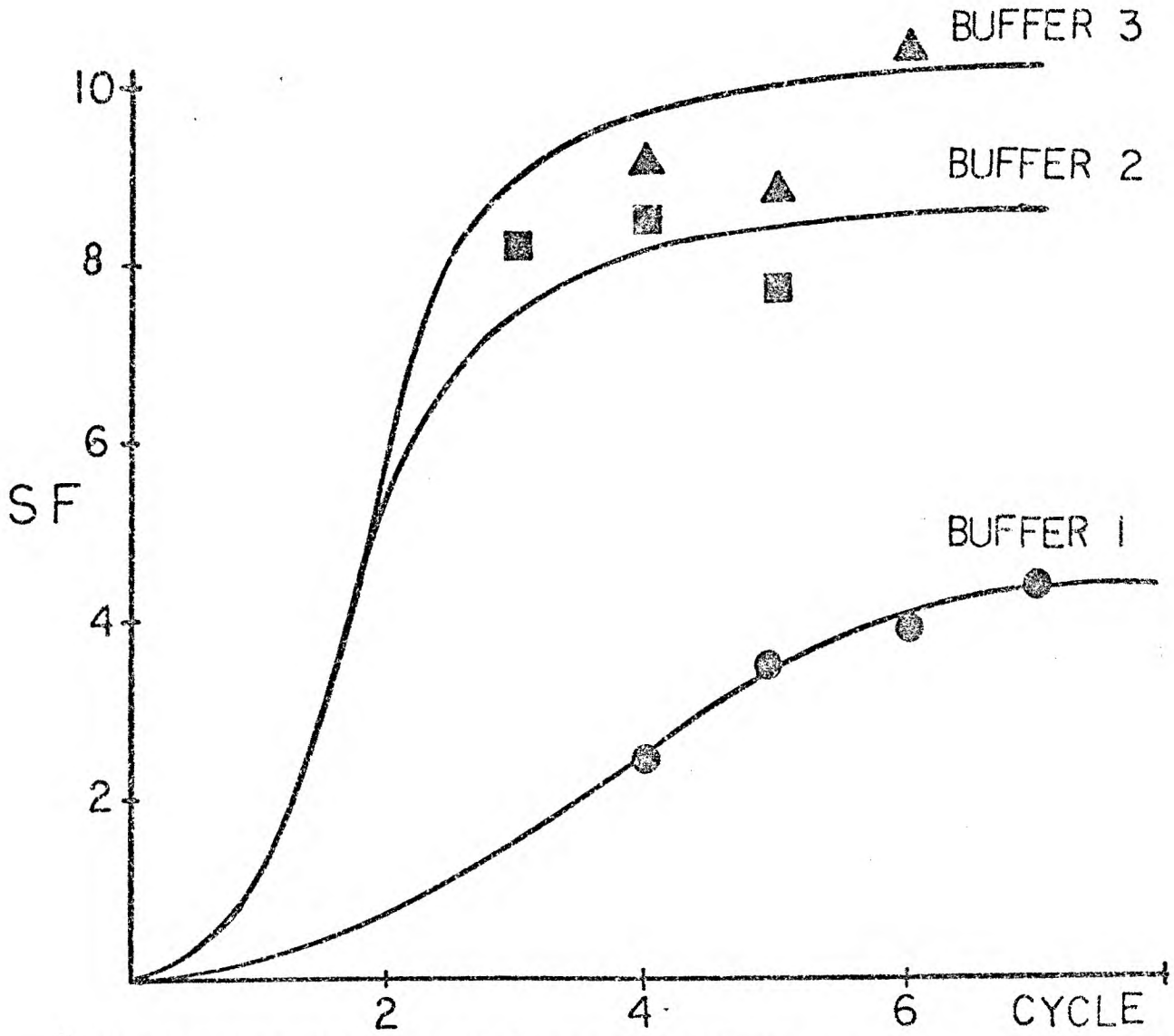
The separation factor (SF) was computed for each run. Figure 6 shows the graph of separation factor vs. cycle for every buffer system. Buffer 3 has the highest separation factor among the three runs, thus the buffer systems are:

Buffer of pH 5.7 - 0.05 M Tris maleate buffer

Buffer pf pH 8.0 - 0.2 M Tris maleate buffer + 0.1 M NaCl.

Comparison Between the PH-parametric Pumping and the PH-cycling Zone Adsorption Process

All the experiments presented in this section were conducted on buffer of ionic strength and salt concen-



● Buffer 1: Buffer of pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl
Buffer of pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl
Feeding mode: t_1= 4 min.
t_2= 4 min.

■ Buffer 2: Buffer of pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl
Buffer of pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl
Feeding mode: t_1= 12 min.
t_2= 12 min.

▲ Buffer 3: Buffer of pH 8.0 - 0.2 M Tris maleate + 0.1 NaCl
Buffer of pH 5.7 - 0.05 M Tris maleate
Feeding mode: t_1= 12 min.
t_2= 4 min.

FIGURE 6

tration of 0.05 M Tris maleate of pH_2=5.7 and 0.2 M Tris maleate + 0.1 M NaCl of pH_1=8.0. Feed was 0.02% hemoglobin and cation exchanger (R^-) was used. Column height was 8 cm.. A low fluid flow rate of 1 cc./min. was selected. This choice is based on recommendation made from the previous work of Chen et al. It was found that this is the optimum rate where adsorption or desorption of protein is sufficiently high, and axial mass transfer and pH gradient across the column are minimal.

Variation of the pH-parametric pumping or the pH-cycling zone method was made by varying the times (t_1 and t_2 for cycling zone, and t_{III} and t_{VI} for the parametric pumping) of fresh feed introduction into the column. Varying the feeding times could make a significant effect on the separation factor.

Runs 1 and 2 were experiments on feeding times of t_1= 12 min. (or t_{III}= 12 min.) and t_2= 4 min. (or t_{VI}= 4 min.). Runs 3, 4, and 5 were on feeding of equal times (t_1= t_2= 4 min., or t_{III}= t_{VI}= 4 min.). Their experimental data are tabulated in Tables 1 to 5 and their corresponding graphs in Figures 7 to 11.

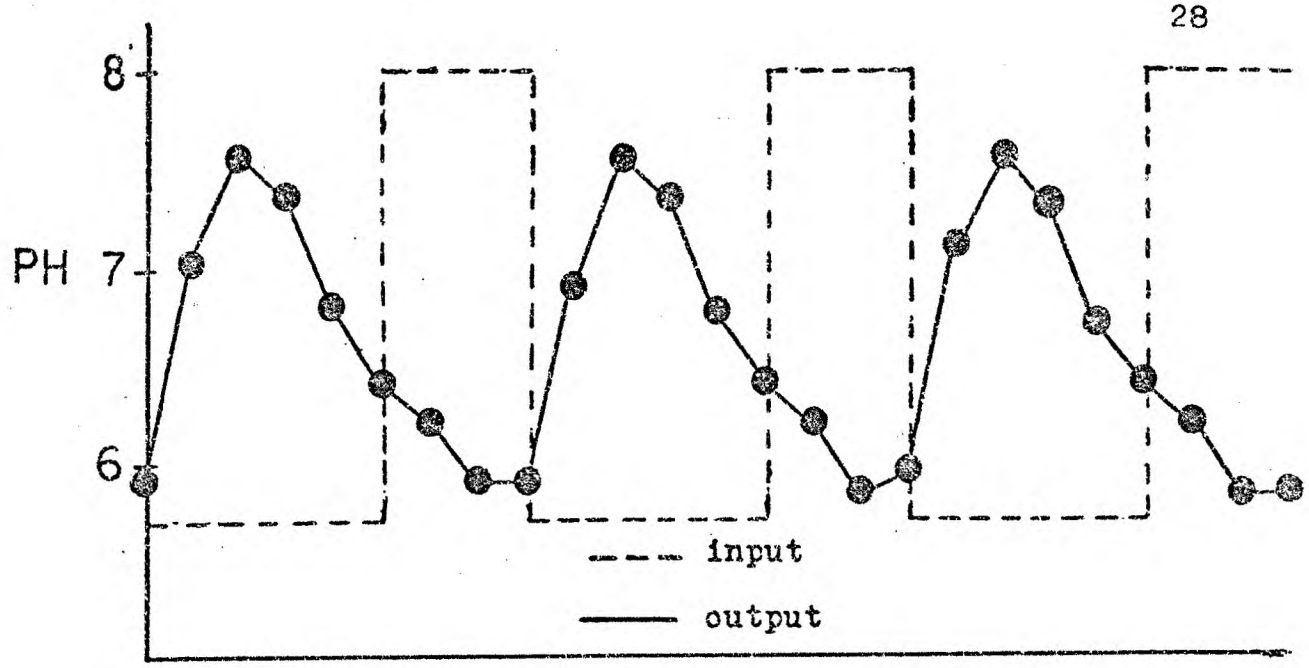
Unequal Feeding Mode

Table 1 shows the experimental data of Run 1 of

pH-cycling zone with feeding times of t_1 and t_2 at 12 min. and 4 min. respectively. This process corresponds to the diagram shown in Figure 2, page 12. Feed of pH_2= 5.7 was introduced to the top of the column for 12 minutes and later switched to feed of pH_1=8.0 for 4 minutes and this procedure was repeated for many cycles. Product samples of 2 cc. were continuously taken at the outlet and analyzed. At pH=5.7, hemoglobin is adsorbed by the cation exchanger and released at pH=8.0. The data of concentration of the product (Y_H) and pH were plotted against cycle in Figure 7. Output pH was also measured to note relation between pH and concentration since adsorption and desorption depend on pH. PH breakthrough curve was plotted together with Y_H-cycle curve in Figure 7. The concentration wave did not attain a steady state until the fourth cycle in which 36 cc. of feed (approximately two void volumes of the column) had been pumped.

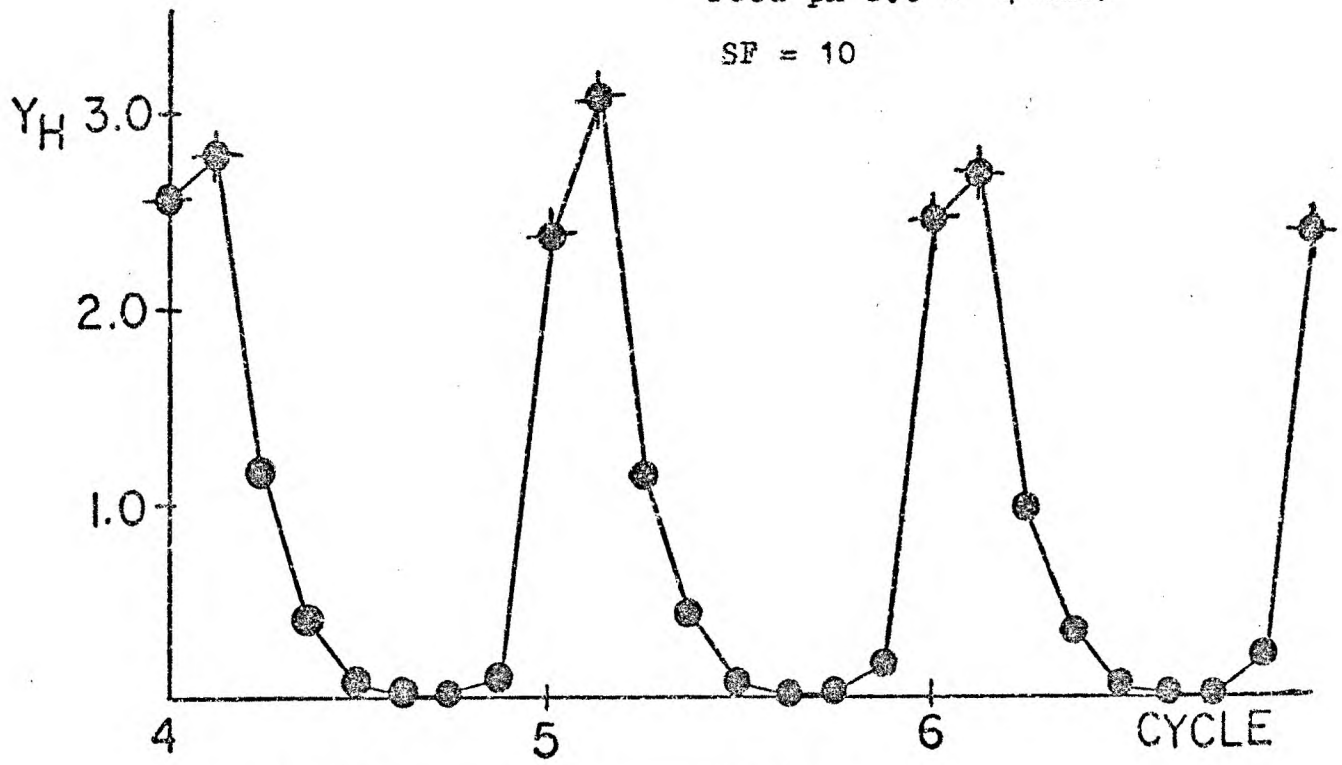
The separation factor (SF) for this mode of operation was calculated to be 10.

The same feeding mode (t_{III}= 12 min. and t_{VI}= 4 min.) was tried on the continuous pH-parametric pumping system (Run 2) with no circulation (t_{II}= t_V= 0). The flow diagram is shown in Figure 1, page 9. Displacement time (t_I or t_{IV}) was equal to 18 minutes. Dead vol-



RUN 1
pH-cycling zone
Feeding mode:
Feed pH 5.7 = 12 min.
Feed pH 8.0 = 4 min.

SF = 10



● - low product concentration

✦ - high product concentration

FIGURE 7

umes V_T and V_B were both equal to 5 cc..The data were tabulated in Table 2 and plotted in Figure 8. Separation factor was calculated for every cycle and plotted against cycle. At the ninth cycle, the separation factor peaked and remained more or less constant. This means that the two reservoirs had reached a steady state whereby no more significant protein transfer was possible. The top product concentration ($\langle Y_{TP} \rangle_n$) went down to about 0.16 while the bottom ($\langle Y_{BP} \rangle_n$) was up to about 2.30. Figure 8 also shows the concentration (Y_H) for top and bottom product plotted against cycle. For this system the separation factor is about 14. This is slightly better than that of the cycling zone which is 10.

Unequal feeding mode seems to give the cycling zone a disadvantage because a short feed of pH_1=8.0 means a short desorption time. Obviously, it will affect the separation factor adversely while it tends to improve, or maintain no effect on the parametric pumping. Subsequent experiments were run on equal feeding times.

Equal Feeding Mode

Table 3 shows the experimental data (Run 3) of pH-cycling zone method with equal feeding times (t_1= t_2= 4 min.). Feed of pH_2= 5.7 was introduced first to the top

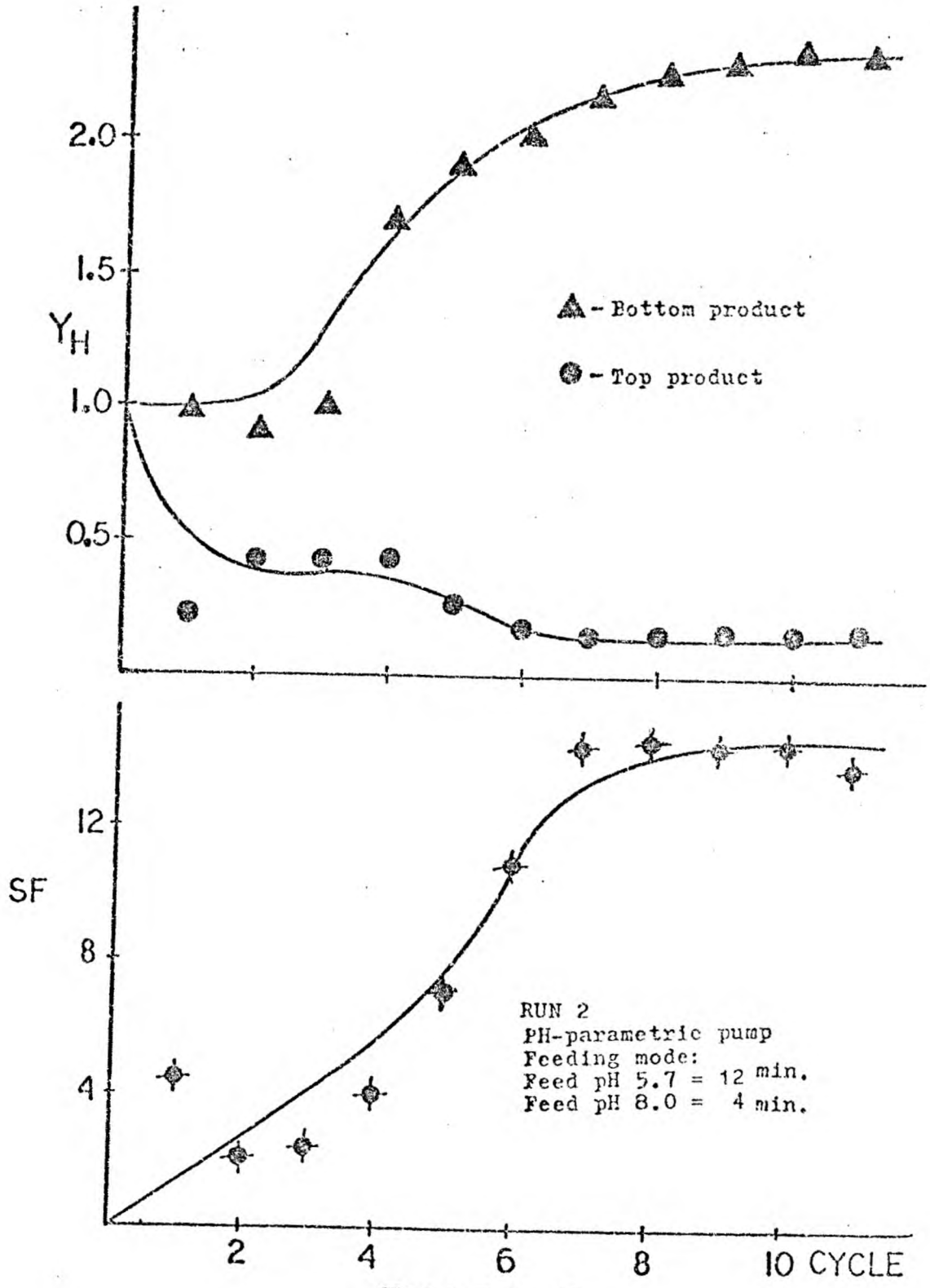

2.0
1.5
Y_H
1.0
0.5
▲ - Bottom product
● - Top product
12
SF
8
4
RUN 2
PH-parametric pump
Feeding mode:
Feed pH 5.7 = 12 min.
Feed pH 8.0 = 4 min.
2
4
6
8
10 CYCLE

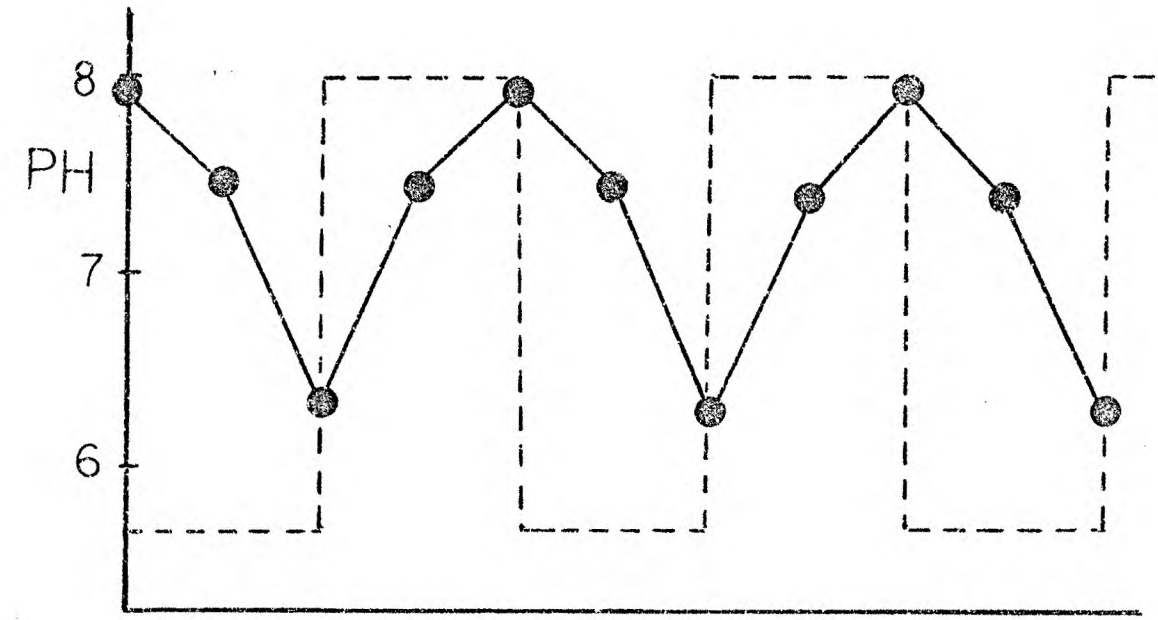
FIGURE 8

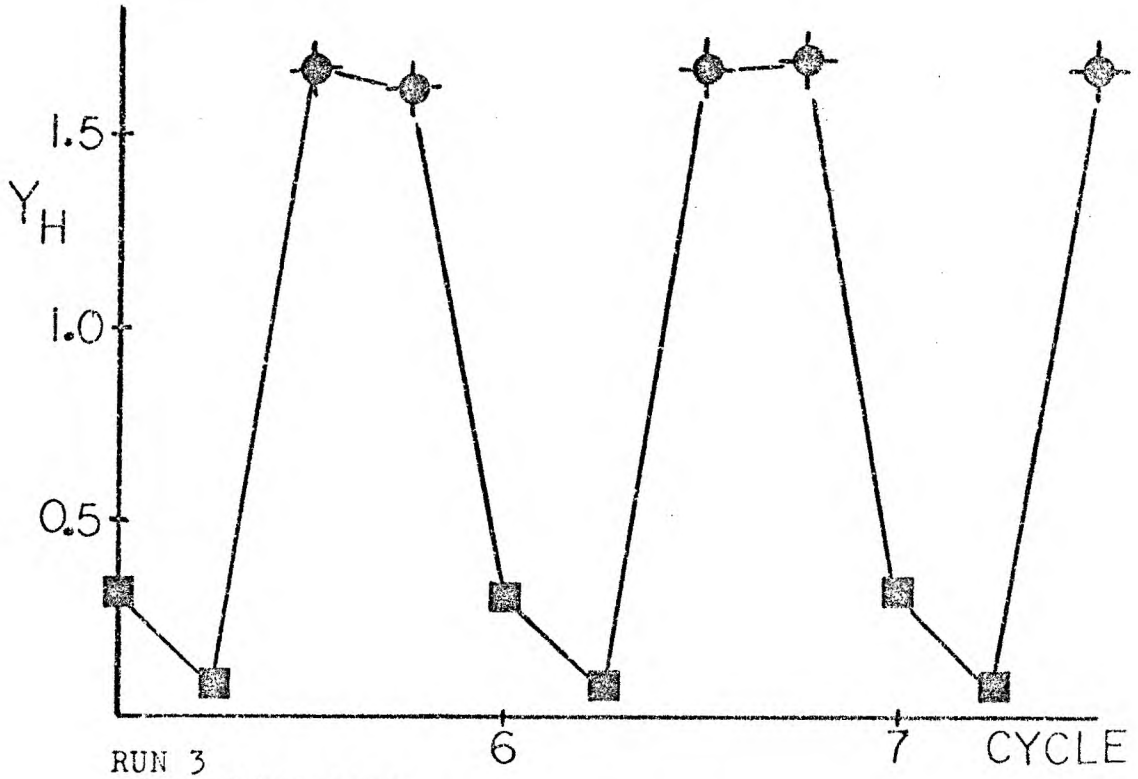
of the column for 4 minutes followed by feed of pH_1= 8.0 for another 4 minutes. Because of a shorter feeding time, the concentration wave took a longer time to attain a steady state. This was about in the fifth cycle.

Figure 9 shows the graphs of pH vs. cycle and concentration of product (Y_H) vs. cycle. The computed separation factor is about 9 which is smaller the separation factor of Run 1. This could be accounted to a shorter feed (pH_2= 5.7) resulting to a lesser amount of hemoglobin adsorbed than in Run 1.

Equal feeding mode (t_{III}= t_{VI}= 4 min.) was applied to the pH-parametric pumping with no circulation (t_{II}= t_V= 0). Displacement time (t_I or t_{IV}) was still 18 minutes. Dead volumes V_T and V_B were still 5 cc. each. The experimental data were tabulated in Table 4 and the curves Y_H vs. cycle and SF vs. cycle were plotted in Figure 10. At the steady state, the bottom product concentration went up only to 1.6 while the top went down to 0.20. The separation factor is computed to be 9 which is no better than the pH-cycling zone.

Theoritically, a pH-parametric pumping without circulation should yield a high separation factor. But in reality, it seldom happens this way. There is always the possibility that not all fluid of pH above 6.9 is out from the column after the down-flow step because





RUN 3
PH-cycling zone
Feeding mode: Feed pH 5.7 = 4 min.
Feed pH 8.0 = 4 min.
SF = 9

- low product concentration
- high product concentration

FIGURE 9

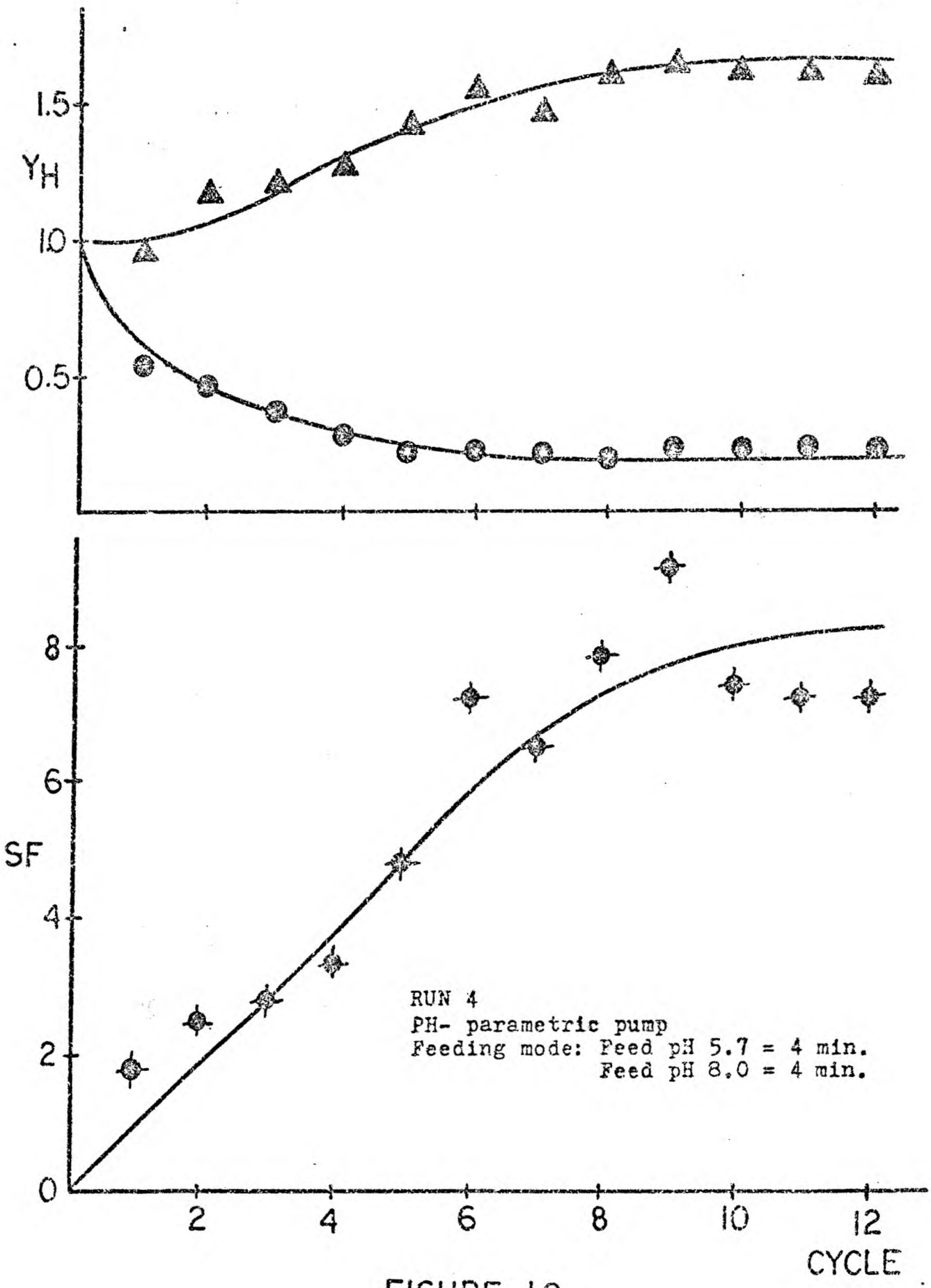

1.5
Y_H
1.0
0.5
8
6
SF
4
2
0
2
4
6
8
10
12
CYCLE
RUN 4
PH- parametric pump
Feeding mode: Feed pH 5.7 = 4 min.
Feed pH 8.0 = 4 min.

FIGURE 10

of a formation of a pH gradient between two pH levels. During the up-flow step, a residual fluid of pH above 6.9 will, on its way up, desorbs some hemoglobin at the top of the column. The desorbed protein is carried back to the top reservoir. Thus, the separation factor will be lower than it is supposed to be. An additional step of circulation can resolve this problem.

Equal Feeding Mode With Circulation

The same condition of feeding (t_{III}= t_{VI}= 4 min.) was again tried on the pH-parametric pumping but with the circulations (t_{II}= t_{V}= 18 min.). These steps insure pH uniformity inside the column and prevent adsorbed proteins in the column from going back to the top reservoir during the up-flow step. This is Run 5 and the data are shown in Table 5. Hemoglobin concentration (Y_H) vs. cycle is plotted in Figure 11 which shows a much improvement on the curve. The second graph in Figure 11 (separation factor vs. cycle) shows a separation factor of 70 at steady state.

A summary of the results is given below.

Process	Mode of Feeding	circulation	SF
1. pH-cycling zone	t_1= 12 min.		10
	t_2= 4 min.		

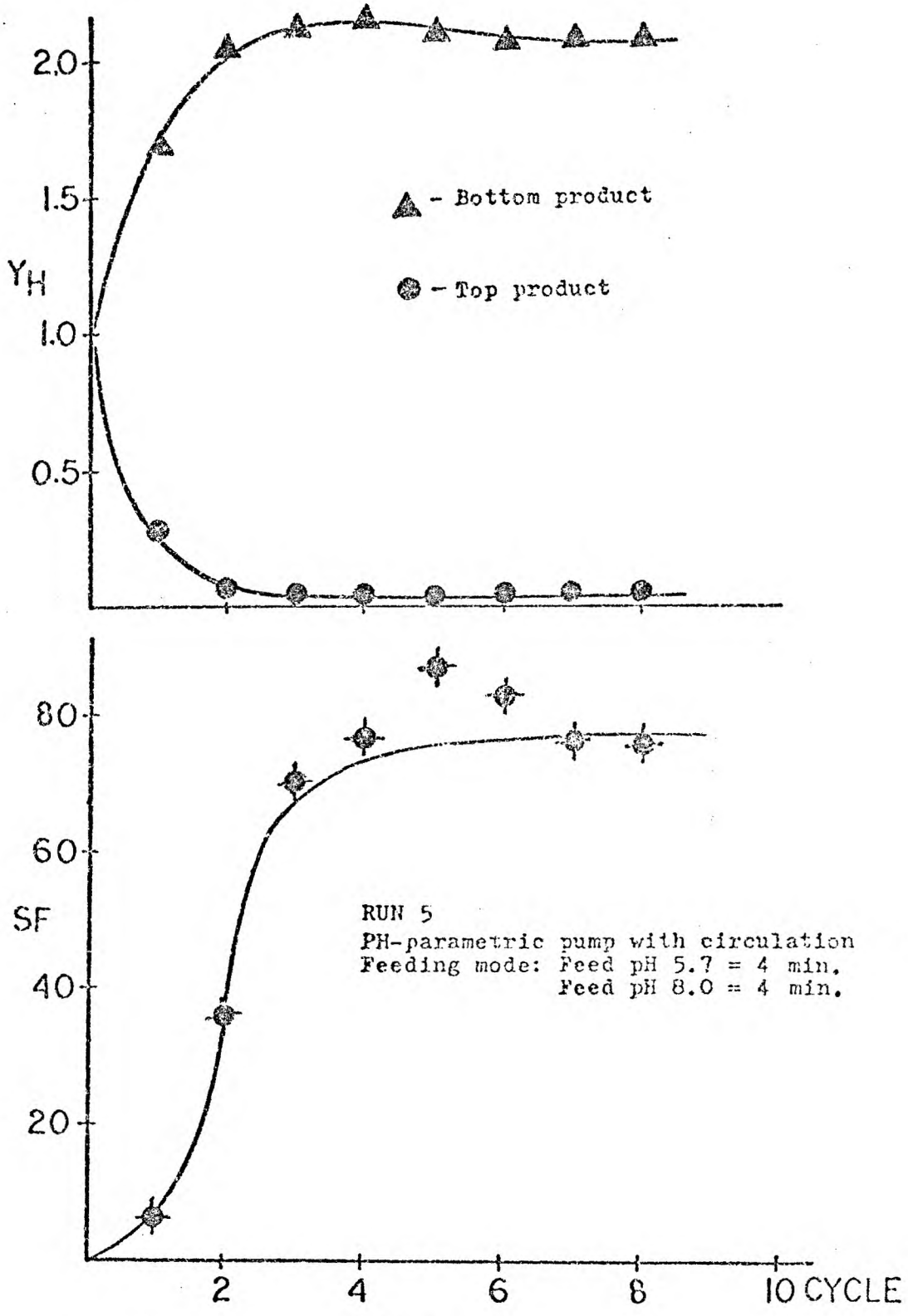

2.0
1.5
Y_H
1.0
0.5
▲ - Bottom product
● - Top product
80
60
SF
40
20
RUN 5
PH-parametric pump with circulation
Feeding mode: Feed pH 5.7 = 4 min.
Feed pH 8.0 = 4 min.
2
4
6
8
10 CYCLE

FIGURE 11

(continued)

Process	Mode of Feeding	circulation	SF
2. pH-parametric pumping	t_{III}= 12 min.	none	14
	t_{VI} = 4 min.		
3. pH-cycling zone	t_1 = 4 min.		9
	t_2 = 4 min.		
4. pH-parametric pumping	t_{III}= 4 min.	none	9
	t_{VI} = 4 min.		
5. pH-parametric pumping	t_{III}= 4 min.	18 min.	70
	t_{VI} = 4 min.		

CONCLUSIONS

The experimental data provided a fairly clear picture between the performance of the pH-parametric pumping and the pH-cycling zone adsorption process. The comparison was purely based on separation factor. It is shown that the pH-parametric pumping with circulation has the best separation factor than the pH-parametric pumping without circulation and the pH-cycling zone adsorption method.

The pH-cycling zone technique has a satisfactory capability on protein separation as demonstrated by the experiments but seems to appeal more on its simplicity. Although, it is predicted by theory that the pH-parametric pumping without circulation should do much better than the pH-cycling zone, this is not so because of the formation of a pH gradient between the two feed mixtures in the column. As shown in the experiments, the separation factors obtained were just about equal as in the pH-cycling zone adsorption process regardless of the type of feeding mode applied. However, by adopting an additional step of circulation in the pH-parametric pumping each after down-flow and up-flow step insuring the pH uniformity inside the column, a more efficient process is obtained wherein protein se-

paration is greatly enhanced. This makes the pH-parametric pumping a very powerful technique in protein separation.

The simplicity fo the pH-cycling zone is an attractive feature of the system over the pH-parametric pumping where high purity yield is not of primary importance. For the pH-cycling zone method, the separation looks retively easy. However, for the pH-parametric pumping, the high purity yield makes up for its complexity and warrants its choice over the pH-cycling zone adsorption process.

APPENDIX A

(Sample Calculation of Protein Concentration)

Sample Calculation of Protein Concentration

For 403 readings where pH of buffer, feed and product sample are equal, equation (3-1), page 21 is used.

EXAMPLE:

From Table 2 (Run 2), feed of pH_2=5.7, first cycle,

R_s^{403}= 0.206

R_b^{403}= 0.000

R_f^{403}= 0.949

$$\text{Therefore, } Y_H = \frac{R_s^{403} - R_b^{403}}{R_f^{403} - R_b^{403}}$$

$$= \frac{0.206 - 0.000}{0.949 - 0.000}$$

$$= 0.217$$

For readings of 595 wavelength, equation (3-2), page 21 is used.

EXAMPLE:

From table 6, feed of pH_2=5.7, third cycle,

R_b^{595}= 0.428

R_f^{595}= 0.656

R_s^{595}= 0.458

$$\text{Therefore, } Y_H = \frac{R_s^{595} - R_b^{595}}{R_f^{595} - R_b^{595}}$$

$$= \frac{0.458 - 0.428}{0.656 - 0.428}$$

$$= 0.132$$

For 403 readings with unequal pH, equation (3-3), page 21 is used.

EXAMPLE:

From Table 3 (Run3), feed of pH_1=8.0, fifth cycle,

R_b^{403}= 0.000

R_f^{403}= 0.805 for pH at 8.0

$k_f / k_{pH=6}$ for pH at 8.0 (Figure 5, page 23) = 0.87

R_s^{403}= 0.260 for pH at 7.9

$k_s / k_{pH=6}$ for pH at 7.9 (Figure 5, page 23) = 0.90

$$\text{Therefore, } Y_H = \frac{R_s^{403} - R_b^{403}}{R_f^{403} - R_b^{403}} \times \frac{k_f / k_{pH=6}}{k_s / k_{pH=6}}$$

$$= \frac{0.260 - 0.000}{0.805 - 0.000} \times \frac{0.87}{0.90}$$

$$= 0.312$$

APPENDIX B

(Bio-Rad Reagent Preparation)

Bio-Rad Reagent Preparation

The Bio-Rad Protein Assay is a dye-binding assay based on the differential color change of a dye in response to various concentration of protein. This assay is recommended because it is easy to use.

One part dye reagent concentrate is diluted with four parts distilled water and filtered through Whatman No. 1 filter paper or equivalent. The resulting solution is stored at room temperature.

Test on hemoglobin is made by mixing 0.1 cc. of sample to 3 cc. of the diluted Bio-Rad reagent. It is normally required to let the mixture stand for ten minutes to allow the reagent to react to the protein prior to testing in the Spectrophotometer.

APPENDIX C

(Tables of the Experimental Data)

TABLE 1

Run 1 — Lab. Book No. 3

Lab. Exp. No. J050

Process: PH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.05 M Tris maleate

pH 8.0 - 0.2 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. — Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7= 12 min. — Bath Temp.: 5°C

pH 8.0= 4 min. — Exchanger: Cation

Cycle/Sample	pH	R^{403}	$k/k_{pH=6}$	Y_H
Buffer 8.0		0.000		
Feed 8.0		0.753	0.995	
Buffer 5.7		0.000		
Feed 5.7		0.904	0.880	
4th cycle				
1	5.9	2.614	1.0	2.545
2	7.0	2.092	1.0	2.764
3	7.55	0.822	0.95	1.148
4	7.35	0.286	0.965	0.392
5	6.8	0.081	1.0	0.079
6	6.4	0.027	1.0	0.026
7	6.2	0.017	1.0	0.017
8	5.9	0.096	1.0	0.093

TABLE 1 (continued)

Cycle/Sample	pH	R^{403}	$k/ k_{pH=6}$	Y_H
5th cycle				
1	5.9	2.398	1.0	2.334
2	6.9	2.314	1.0	3.058
3	7.55	0.829	0.975	1.123
4	7.35	0.306	0.965	0.419
5	6.75	0.073	1.0	0.071
6	6.4	0.024	1.0	0.024
7	6.2	0.021	1.0	0.020
8	5.85	0.177	0.995	0.173
6th cycle				
1	5.95	2.496	1.0	2.429
2	7.1	2.006	0.995	2.664
3	7.55	0.684	0.95	0.951
4	7.3	0.242	0.975	0.313
5	6.7	0.064	1.0	0.062
6	6.4	0.023	1.0	0.022
7	6.2	0.017	1.0	0.017
8	5.85	0.115	0.995	0.113

TABLE 2

Run 2 — Lab. Book No. 3

Lab. Exp. No. J051

Process: PH-parametric Pumping

Buffer Ionic Strength: pH 5.7 - 0.05 M Tris maleate

pH 8.0 - 0.2 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. — Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 12 min. — Bath Temp.: 5°C

pH 8.0 = 4 min. — Exchanger: Cation

Displacement: 18 cc. — Dead Volume: 5 cc.

Circulation: none

Cycle/Sample	pH	R^{403}	Y_H	SF
Buffer 5.7		0.000		
Feed 5.7		0.949		
Buffer 8.0		0.000		
Feed 8.0		0.792		
TOP PRODUCT				
1	5.85	0.206	0.217	4.50
2	5.75	0.407	0.429	2.11
3	5.7	0.402	0.424	2.33
4	5.75	0.413	0.435	3.90
5	5.7	0.254	0.268	7.04

TABLE 2 (continued)

Cycle/Sample	pH	R^{403}	Y_H	SF
6	5.75	0.178	0.187	10.78
7	5.7	0.143	0.151	14.21
8	5.7	0.146	0.154	14.45
9	5.75	0.152	0.160	14.22
10	5.7	0.154	0.162	14.40
11	5.7	0.161	0.169	13.62
BOTTOM PRODUCT				
1	7.9	0.773	0.976	
2	7.85	0.716	0.904	
3	7.8	0.783	0.989	
4	7.8	1.343	1.696	
5	7.85	1.495	1.888	
6	7.85	1.595	2.014	
7	7.8	1.699	2.145	
8	7.85	1.762	2.225	
9	7.8	1.802	2.275	
10	7.8	1.840	2.323	
11	7.8	1.823	2.302	

TABLE 3

Run 3 Lab. Book No. 3

Lab. Exp. No. J052

Process: PH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.05 M Tris maleate

pH 8.0 - 0.2 M Tris maleate +

0.1 M NaCl

Flow Rate: 1 cc./ min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 4 min. Bath Temp.: 5°C

pH 8.0 = 4 min. Exchanger: Cation

Cycle/Sample	pH	R^{403}	$k/ k_{pH=6}$	Y_H
Buffer 5.7		0.000		
Feed 5.7		0.911		
Buffer 8.0		0.000		
Feed 8.0		0.805		
5th cycle				
1	7.9	0.260	0.90	0.312
2	7.45	0.063	0.96	0.076
3	6.3	1.529	1.0	1.661
4	7.4	1.444	0.97	1.608
6th cycle				
1	7.9	0.254	0.90	0.305
2	7.4	0.061	0.97	0.068
3	6.25	1.532	1.0	1.660

TABLE 3 (continued)

Cycle/Sample	pH	R^{403}	$k/ k_{pH=6}$	Y_H
4	7.35	1.516	0.97	1.689
7th cycle				
1	7.9	0.264	0.90	0.317
2	7.35	0.064	0.97	0.071
3	6.25	1.526	1.0	1.658
4	7.35	1.509	0.97	1.681
8th cycle				
1	7.9	0.262	0.90	0.315
2	7.35	0.060	0.97	0.068
3	6.25	1.546	1.0	1.680
4	7.35	1.516	0.97	1.689

TABLE 4

Run 4 — Lab. Book No. 3

Lab. Exp. No. J054

Process: PH-parametric Pumping

Buffer Ionic Strength: pH 5.7 - 0.05 M Tris maleate

pH 8.0 - 0.2 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./ min. — Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 4 min. — Bath Temp.: 5°C

pH 8.0 = 4 min. — Exchanger: Cation

Displacement: 18 cc. — Dead Volume: 5 cc.

Circulation: none

Cycle/Sample	R^{403}	Y_H	SF
Buffer 5.7	0.000		
Feed 5.7	0.907		
Buffer 8.0	0.000		
Feed 8.0	0.782		
TOP PRODUCT			
1	0.475	0.534	1.8
2	0.414	0.456	2.54
3	0.394	0.434	2.89
4	0.342	0.377	3.3
5	0.264	0.291	4.8

TABLE 4 (continued)

Cycle/Samle	R^{403}	Y_H	SF
6	0.196	0.216	7.2
7	0.200	0.226	6.5
8	0.186	0.205	7.8
9	0.163	0.180	9.1
10	0.198	0.218	7.4
11	0.204	0.225	7.2
12	0.201	0.232	7.2
BOTTOM PRODUCT			
1	0.745	0.953	
2	0.907	1.160	
3	0.939	1.201	
4	0.986	1.261	
5	1.097	1.403	
6	1.208	1.545	
7	1.145	1.464	
8	1.251	1.600	
9	1.279	1.636	
10	1.262	1.614	
11	1.258	1.609	
12	1.241	1.587	

TABLE 5

Run 5 — Lab. Book No. 3

Lab. Exp. No. J055

Process: PH-parametric Pumping

Buffer Ionic Strength: pH 5.7 - 0.05 M Tris maleate

pH 8.0 - 0.2 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. — Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 4 min. — Bath Temp.: 5°C

pH 8.0 = 4 min. — Exchanger: Cation

Displacement: 18 cc. — Dead Volume: 10 cc.

Circulation: 18 min.

Cycle/Sample	R^{403}	Y_H	SF
Buffer 5.7	0.000		
Feed 5.7	0.952		
Buffer 8.0	0.000		
Feed 8.0	0.751		
TOP PRODUCT			
1	0.262	0.275	6.16
2	0.054	0.057	35.6
3	0.029	0.030	70.3
4	0.027	0.028	76.5
5	0.023	0.024	87.4

TABLE 5 (continued)

Cycle/Sample	R^{403}	Y_H	SF
6	0.024	0.025	82.6
7	0.026	0.027	76.3
8	0.026	0.027	75.9
BOTTOM PRODUCT			
1	1.273	1.695	
2	1.273	2.031	
3	1.584	2.109	
4	1.608	2.141	
5	1.575	2.097	
6	1.551	2.065	
7	1.548	2.061	
8	1.540	2.051	

TABLE 6

Lab. Book No. 2 Exp. No. J032

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 12 min. Bath Temp.: 5°C

pH 8.5 = 12 min. Exchanger: Cation

Cycle/Sample	pH	R^{595}	Y_H
Buffer 5.7		0.428	
Feed 5.7		0.656	
Buffer 8.5		0.419	
Feed 8.5		0.632	
3rd cycle			
1	5.8	0.458	0.132
2	5.8	0.467	0.171
3	5.85	0.803	1.645
4	7.3	0.989	2.461
5	7.8	0.663	1.146
6	8.0	0.485	0.310

TABLE 6 (continued)

Cycle/Sample	pH	R^{595}	Y_H
7	7.8	0.449	0.141
8	6.2	0.435	0.075
4th cycle			
1	5.8	0.438	0.044
2	5.8	0.460	0.140
3	5.85	0.769	1.496
4	7.4	1.008	2.544
5	7.85	0.661	1.136
6	7.95	0.496	0.362
7	7.8	0.445	0.122
8	6.2	0.439	0.094
5th cycle			
1	5.85	0.445	0.075
2	5.75	0.458	0.132
3	5.8	0.785	1.566
4	7.4	1.012	2.561
5	7.85	0.673	1.192
6	7.95	0.501	0.385
7	7.85	0.452	0.155
8	6.2	0.439	0.094

TABLE 7

Lab. Book No. 2 Exp. No. J033

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Albumin

Feeding Mode: pH 5.7 = 12 min. Bath Temp.: 5°C

pH 8.5 = 12 min. Exchanger: Cation

Cycle/Sample	pH	R^{595}	Y_A
Buffer 5.7		0.442	
Feed 5.7		0.797	
Buffer 8.5		0.440	
Feed 8.5		0.801	
3rd cycle			
1	5.8	0.789	0.977
2	5.7	0.779	0.949
3	5.95	0.789	0.977
4	7.8	0.764	0.907
5	7.95	0.786	0.958
6	8.1	0.751	0.861

TABLE 7 (continued)

Cycle/Sample	pH	R^{595}	Y_A
7	7.5	0.782	0.947
8	6.0	0.776	0.931
4th cycle			
1	5.8	0.780	0.952
2	5.75	0.784	0.963
3	6.0	0.774	0.935
4	7.75	0.768	0.918
5	7.95	0.763	0.895
6	8.1	0.761	0.869
7	7.6	0.766	0.903
8	5.95	0.785	0.956
5th cycle			
1	5.75	0.762	0.901
2	5.7	0.766	0.913
3	5.95	0.755	0.882
4	7.85	0.778	0.946
5	8.05	0.754	0.870
6	8.1	0.769	0.911
7	7.65	0.763	0.895
8	5.95	0.740	0.831

TABLE 8

Lab. Book No. 2 Exp. No. J034

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 4.0 - 0.1 M NaAc-HAC + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Albumin

Feeding Mode: pH 5.7 = 12 min. Bath Temp.: 5°C

pH 4.0 = 12 min. Exchanger: Anion

Cycle/Sample	pH	R^{595}	Y_A
Buffer 4.0		0.433	
Feed 4.0		0.758	
Buffer 5.7		0.435	
Feed 5.7		0.781	
3rd cycle			
1	5.05	0.695	0.751
2	5.35	0.669	0.676
3	5.4	0.702	0.772
4	4.65	0.929	1.428
5	4.3	0.814	1.095
6	4.5	0.681	0.711

TABLE 8 (continued)

Cycle/Sample	pH	R^{595}	Y_A
7	4.5	0.668	0.673
8	4.65	0.674	0.691
4th cycle			
1	5.05	0.704	0.777
2	5.35	0.678	0.702
3	5.35	0.720	0.824
4	4.65	0.947	1.480
5	4.3	0.837	1.162
6	4.5	0.686	0.725
7	4.55	0.661	0.653
8	4.65	0.649	0.618
5th cycle			
1	5.05	0.701	0.969
2	5.35	0.688	0.731
3	5.35	0.721	0.827
4	4.68	0.976	1.564
5	4.3	0.809	1.081
6	4.5	0.695	0.751
7	4.55	0.669	0.676
8	4.65	0.676	0.697

TABLE 9

Lab. Book No. 2 Exp. No. J035

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 4.0 - 0.1 M NaAc-HAC + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 12 min. Bath Temp.: 5°C

pH 4.0 = 12 min. Exchanger: Anion

Cycle/Sample	pH	R^{595}	Y_H
Buffer 4.0		0.423	
Feed 4.0		0.760	
Buffer 5.7		0.430	
Feed 5.7		0.730	
3rd cycle			
1	5.0	0.714	0.947
2	5.25	0.761	1.103
3	5.2	0.768	1.125
4	4.85	0.732	1.007
5	4.5	0.709	0.849
6	4.45	0.669	0.730

TABLE 9 (continued)

Cycle/Sample	pH	R^{595}	Y_H
7	4.5	0.670	0.733
8	4.65	0.688	0.786
4th cycle			
1	5.0	0.739	1.030
2	5.2	0.749	1.063
3	5.25	0.778	1.160
4	4.9	0.777	1.158
5	4.5	0.713	0.861
6	4.45	0.679	0.760
7	4.55	0.668	0.725
8	4.65	0.691	0.795
5th cycle			
1	4.95	0.741	1.035
2	5.25	0.770	1.133
3	5.25	0.781	1.170
4	4.85	0.751	1.070
5	4.45	0.718	0.875
6	4.4	0.663	0.712
7	4.5	0.674	0.745
8	4.7	0.695	0.807

TABLE 10

Lab. Book No. 3 Exp. No. J043

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 4.0 - 0.1 M NaAc-HAC + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Albumin

Feeding Mode: pH 5.7 = 12 min. Bath Temp.: 5°C

pH 4.0 = 12 min. Exchanger: Cation

Cycle/Sample	pH	R^{595}	Y_A
Buffer 4.0		0.416	
Feed 4.0		0.745	
Buffer 5.7		0.411	
Feed 5.7		0.779	
3rd cycle			
1	4.4	1.071	1.991
2	4.7	0.948	1.617
3	4.65	0.779	1.103
4	4.55	0.575	0.483
5	4.5	0.478	0.182
6	4.5	0.458	0.128

TABLE 10 (continued)

Cycle/Sample	pH	R^{595}	Y_A
7	4.4	0.437	0.071
8	4.25	0.431	0.054
4th cycle			
1	4.45	1.130	2.170
2	4.7	0.954	1.635
3	4.6	0.789	1.134
4	4.55	0.611	0.593
5	4.5	0.503	0.250
6	4.45	0.477	0.179
7	4.35	0.462	0.139
8	4.3	0.458	0.128
5th cycle			
1	4.45	1.124	2.152
2	4.7	0.960	1.653
3	4.6	0.793	1.146
4	4.55	0.603	0.568
5	4.5	0.511	0.272
6	4.5	0.489	0.212
7	4.35	0.473	0.168
8	4.3	0.455	0.120

TABLE 11

Lab. Book No. 3 Exp. No. J044

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl

pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 8.5 = 12 min. Bath Temp.: 5°C

pH 5.7 = 12 min. Exchanger: Anion

Cycle/Sample	pH	R^{403}	$k/k_{pH=6}$	Y_H
Buffer 8.5		0.001		
Feed 8.5		0.640	0.77	
Buffer 5.7		0.002		
Feed 5.7		0.912	0.99	
3rd cycle				
1	7.15	0.770	0.99	0.936
2	6.7	0.821	1.0	0.988
3	6.4	0.872	1.0	1.050
4	6.3	0.892	1.0	1.074
5	6.25	0.896	1.0	0.972
6	6.6	0.867	1.0	0.940

TABLE 11 (continued)

Cycle/Sample	pH	R^{403}	$k/ k_{pH=6}$	Y_H
7	6.95	0.816	1.0	0.885
8	7.05	0.766	0.995	0.834
4th cycle				
1	7.15	0.744	0.99	0.904
2	6.5	0.811	1.0	0.976
3	6.25	0.848	1.0	1.021
4	6.2	0.880	1.0	1.059
5	6.2	0.886	1.0	0.961
6	6.5	0.859	1.0	0.931
7	7.05	0.798	0.995	0.869
8	7.25	0.757	0.98	0.837
5th cycle				
1	7.4	0.739	0.97	0.917
2	7.25	0.780	0.98	0.958
3	6.55	0.850	1.0	1.023
4	6.35	0.879	1.0	1.058
5	6.25	0.881	1.0	0.955
6	6.3	0.841	1.0	0.912
7	6.7	0.792	1.0	0.859
8	7.1	0.753	0.995	0.820

TABLE 12

Lab. Book No. 3 Exp. No. J045

Process: One column pH-parametric pumping

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 12 min. Bath Temp.: 5°C

pH 8.5 = 12 min. Exchanger: Cation

Displacement: 15 cc. Dead Volume: 10 cc.

Circulation: none

Cycle/Sample	R^{403}	Y_H	SF
Buffer 5.7	0.000		
Feed 5.7	0.646		
Buffer 8.5	0.000		
Feed 8.5	0.563		
TOP PRODUCT			
1	0.551	0.849	1.35
2	0.422	0.660	1.97
3	0.294	0.457	2.55
4	0.230	0.360	3.68
5	0.206	0.321	3.97

TABLE 12 (continued)

Cycle/Sample	R^{403}	Y_H	SF
6	0.234	0.364	3.56
7	0.210	0.325	4.72
8	0.207	0.322	5.54
9	0.193	0.300	5.80
10	0.197	0.306	5.74
BOTTOM PRODUCT			
1	0.639	1.143	
2	0.788	1.531	
3	0.794	1.543	
4	0.787	1.501	
5	0.807	1.539	
6	0.834	1.591	
7	0.828	1.580	
8	0.793	1.512	
9	0.828	1.560	
10	0.812	1.549	

TABLE 13

Lab. Book No. 3 Exp. No. J046

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 4 min. Bath Temp.: 5°C

pH 8.5 = 4 min. Exchanger: Cation

Cycle/Sample	pH	R^{403}	$k/ k_{pH=6}$	Y_H	SF
Buffer 5.7		0.000			
Feed 5.7		0.886	0.99		
Buffer 8.5		0.000			
Feed 8.5		0.763	0.77		
4th cycle					
1	7.7	0.660	0.93	0.716	
2	6.55	0.251	1.0	0.280	
3	6.0	0.820	1.0	0.916	
4	6.9	1.644	1.0	1.659	2.5
5th cycle					
1	7.6	0.504	0.945	0.538	

TABLE 13 (continued)

Cycle/Sample	pH	R^{403}	$k/ k_{pH=6}$	Y_H	SF
2	6.3	0.230	1.0	0.257	
3	6.0	1.152	1.0	1.287	
4	7.3	1.415	0.975	1.465	3.5
6th cycle					
1	7.5	0.426	0.955	0.450	
2	6.2	0.247	1.0	0.276	
3	6.05	1.513	1.0	1.691	
4	7.6	1.091	0.945	1.165	3.9
7th cycle					
1	7.25	0.363	0.98	0.374	
2	6.1	0.276	1.0	0.308	
3	6.2	1.760	1.0	1.967	
4	7.7	0.937	0.93	1.017	4.4
8th cycle					
1	7.15	0.299	0.99	0.306	
2	6.1	0.266	1.0	0.297	
3	6.2	1.786	1.0	1.996	
4	7.7	0.947	0.93	1.028	5.0
9th cycle					
1	7.15	0.302	0.99	0.308	
2	6.1	0.266	1.0	0.297	
3	6.15	1.780	1.0	1.989	
4	7.7	0.965	0.93	1.047	5.0

TABLE 14

Lab. Book No. 3 Exp. No. J047

Process: One column pH-parametric pumping

Buffer Ionic Strength: pH 5.7 - 0.1 M Tris maleate + 0.1 M NaCl

pH 8.5 - 0.1 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 4 min. Bath Temp.: 5°C

pH 8.5 = 4 min. Exchanger: Cation

Displacement: 15 cc. Dead Volume: 10 cc.

Circulation: none

Cycle/Sample	pH	R^{403}	Y_H	SF
Buffer 5.7		0.000		
Feed 5.7		0.775		
Buffer 8.5		0.000		
Feed 8.5		0.802		
TOP PRODUCT				
1	5.7	0.674	0.870	1.1
2	5.7	0.625	0.806	1.2
3	5.7	0.576	0.743	1.5
4	5.65	0.469	0.605	1.9

TABLE 14 (continued)

Cycle/Sample	pH	R^{403}	Y_H	SF
5	5.8	0.487	0.628	1.9
6	5.75	0.459	0.592	2.0
7	5.7	0.406	0.524	2.3
8	5.7	0.412	0.532	2.3
9	5.7	0.406	0.524	2.3
10	5.7	0.425	0.548	2.2
11	5.7	0.436	0.563	2.1
BOTTOM PRODUCT				
1	8.4	0.799	0.996	
2	8.4	0.849	1.058	
3	8.4	0.874	1.090	
4	8.35	0.922	1.150	
5	8.3	0.961	1.198	
6	8.4	0.952	1.197	
7	8.4	0.981	1.223	
8	8.4	0.968	1.207	
9	8.4	0.954	1.189	
10	8.4	0.961	1.198	
11	8.4	0.966	1.205	

TABLE 15

Lab. Book No. 3 Exp. No. J048

Process: One column pH-parametric pumping

Buffer Ionic Strength: pH 5.7 - 0.05 M Tris maleate

pH 8.0 - 0.2 M Tris maleate + 0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 8 min. Bath Temp.: 5°C

pH 8.0 = 8 min. Exchanger: Cation

Displacement: 15 cc. Dead Volume: 10 cc.

Circulation: none

Cycle/Sample	pH	R^{403}	Y_H	SF
Buffer 5.7		0.000		
Feed 5.7		0.972		
Buffer 8.0		0.000		
Feed 8.0		0.752		
TOP PRODUCT				
1	5.7	0.291	0.299	3.6
2	5.7	0.309	0.318	3.9
3	5.75	0.407	0.419	3.3
4	5.7	0.361	0.371	3.9
5	5.7	0.331	0.341	3.9

TABLE 15 (continued)

Cycle/Sample	pH	R^{403}	Y_H	SF
6	5.7	0.258	0.265	5.4
7	5.8	0.226	0.233	6.9
8	5.7	0.193	0.199	8.5
9	5.8	0.172	0.177	10.9
10	5.7	0.164	0.169	10.1
11	5.75	0.160	0.165	10.1
12	5.7	0.160	0.165	9.9
BOTTOM PRODUCT				
1	8.0	0.837	1.088	
2	7.9	0.984	1.250	
3	7.75	1.109	1.379	
4	7.7	1.166	1.442	
5	7.7	1.070	1.323	
6	7.8	1.150	1.437	
7	7.85	1.273	1.609	
8	7.75	1.363	1.694	
9	7.7	1.570	1.941	
10	7.8	1.368	1.710	
11	7.85	1.318	1.666	
12	7.8	1.302	1.627	

TABLE 16

Lab. Book No. 3 Exp. No. J049

Process: One column pH-cycling zone

Buffer Ionic Strength: pH 5.7 - 0.05 M Tris maleate

pH 8.0 - 0.2 M Tris maleate +

0.1 M NaCl

Flow Rate: 1 cc./min. Feed: 0.02% Hemoglobin

Feeding Mode: pH 5.7 = 8 min. Bath Temp.: 5°C

pH 8.0 = 8 min. Exchanger: Cation

Cycle/Sample	pH	R^{403}	$k/k_{pH=6}$	Y_H	SF
Buffer 5.7		0.000			
Feed 5.7		0.934	0.995		
Buffer 8.0		0.000			
Feed 8.0		0.788	0.890		
4th cycle					
1	7.65	0.185	0.935	0.223	
2	7.65	0.052	0.935	0.063	
3	7.15	0.023	0.99	0.025	
4	6.5	0.018	1.0	0.019	
5	6.1	0.316	1.0	0.337	
6	6.35	3.420	1.0	3.643	
7	7.5	1.798	0.955	2.126	

TABLE 16 (continued)

Cycle/Sample	pH	R^{403}	$k/ k_{pH=6}$	Y_H	SF
8	7.55	0.611	0.975	0.708	21.7
5th cycle					
1	7.65	0.173	0.935	0.209	
2	7.65	0.046	0.935	0.056	
3	7.15	0.023	0.99	0.025	
4	6.55	0.018	1.0	0.019	
5	6.05	0.328	1.0	0.349	
6	6.3	3.670	1.0	3.910	
7	7.55	1.805	0.975	2.091	
8	7.6	0.563	0.945	0.673	22.7
6th cycle					
1	7.65	0.166	0.935	0.201	
2	7.65	0.043	0.935	0.052	
3	7.15	0.021	0.99	0.023	
4	6.55	0.016	1.0	0.017	
5	6.05	0.366	1.0	0.390	
6	6.35	3.610	1.0	3.846	
7	7.55	1.803	0.975	2.089	
8	7.55	0.548	0.975	0.635	23.8

NOMENCLATURE

I	= isoelectric point
IS_1	= ionic strength in the bottom reservoir
IS_2	= ionic strength in the top reservoir
$k/ k_{pH=6}$	= pH correction factor
pH_1	= high level pH
pH_2	= low level pH
Q	= reservoir displacement rate, cc./s
R^-	= cation exchanger
R^+	= anion exchanger
R_b^{403}	= 403 reading of the buffer
R_f^{403}	= 403 reading of the feed
R_s^{403}	= 403 reading of the sample
R_b^{595}	= 595 reading of the buffer
R_f^{595}	= 595 reading of the feed
R_s^{595}	= 595 reading of the sample
SF	= separation factor. It is the ratio of the average bottom (or high) product concentration to the average top (or low) product concentration.
t_1	= duration of feed pH_2
t_2	= duration of feed pH_1
t_i	= duration of step where i= I, II,.....VI

V_B = dead volume of bottom reservoir

V_T = dead volume of top reservoir

y_o = concentration of solute in fresh feed

Y_H = concentration ratio of hemoglobin in product to feed

$\langle Y_{BP} \rangle_n$ = average concentration of hemoglobin in the bottom reservoir

$\langle Y_{TP} \rangle_n$ = average concentration of hemoglobin in the top reservoir

Subscript

1 = up-flow or high pH level

2 = down-flow or low pH level

BP = bottom product

B = bottom reservoir

TP = top product

T = top reservoir

H = hemoglobin

n = n th cycle

o = initial or original condition

b = buffer

f = feed

s = samle

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