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The separation of proteins via parametric pumping and ion exchange

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THE SEPARATION OF PROTEINS VIA PARAMETRIC PUMPING AND ION EXCHANGE

BY

PAUL ALBERT FALCON

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ABSTRACT

A pH parametric pump, coupled with ion exchange, was used in an experimental investigation to separate a protein-water binary mixture. The model system consisted of a hemoglobin-water binary mixture. A pH gradient throughout the parametric pumping column was sustained with the use of hollow fiber devices incorporating phosphate buffer circulation.

Data is presented for both batch and semi-continuous recuperative mode pH parametric pumping. Continuous pH parametric pumping for protein isolation has also been envisioned.

No attempt has been made to optimize this newly developed process; however, certain parameters are suspected of controlling separation, including buffer concentration, half cycle time, packed column height, and reservoir displacement volume. Results show that ratios of bottom product concentration to top product concentration can be sustained at approximately 2.0, using the semi-continuous mode.

ACKNOWLEDGEMENT

I am deeply grateful to Dr. H. T. Chen for his many vital contributions to this work, including the initial conception of the project. I am secondly thankful to Mr. John S. Dell'Osso for his partnership in the lab while obtaining this data. I would thirdly like to thank Ms. Laurel A. Gaines for her assistance in the mechanics of preparing this thesis.

Portions of this work were presented at the 82nd National Meeting of the AIChE, Atlantic City, New Jersey, August, 1976.

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SCOPE

Parametric pumping entails the reciprocating flow of a fluid through a column filled with specialized packing. Associated with this alternating fluid flow is the simultaneous application of an energy field to the column packing and fluid in the column voids. Hence, as fluid flows in one direction through the column, it is exposed to a characteristic energy field. As the fluid return flows in the opposite direction through the column voids, it is exposed to an opposing energy field.

Separation of the fluid into component fractions occurs via the response of each fluid solute to an applied energy field, while simultaneously being exposed to bulk flow. As a fluid is exposed to an energy field while flowing in one direction, one or more of the fluid solutes will adhere to a specialized column packing material. As the fluid flows in the opposite direction, while exposed in an opposite energy field, deabsorption of the one or more solutes occurs. Hence, the solutes are moved in a specific direction along the column axis. This movement causes a solute enrichment of the fluid in the column voids at a specific end of the column or fluid reservoir also located at the end of the column.

Several types of parametric pumping apparatus presently exist. These make use of pressure and temperature to create energy fields to which solutes are exposed.

In pressure parametric pumping, fluids used are gases. The gases are exposed to reciprocating flow in a packed column with synchronous alternating pressures exerted on them while in the column voids. Temperature parametric pumping incorporates the reciprocating flow of liquids with synchronous alternating temperature exerted on them, while in column voids.

In this thesis, parametric pumping theory is extended to the use of a pH field to effect separation of a water-hemoglobin binary system. Recently developed hollow fiber devices will be used to establish an alternating pH field associated with the reciprocating fluid flow through a column packed with SP-Sphadex ion exchanger matrix. Both Batch and Semi-Continuous pH parametric pumping modes will be examined.

CONCLUSIONS AND SIGNIFICANCE

Five batch and nine semi-continuous runs were made. From the experimental data it can be seen that the separation is effected by a net movement of Hemoglobin towards the high pH end of a column packed with SP-Sephadex cationic exchanger resin. A pH gradient between eight and six was achieved at the ends of the column by use of hollow bio-fiber devices buffered with mixtures of Mono- and Di-Sodium Phosphates.

The viability of the process using ion exchange and pH parametric pumping to separate proteins is seen from the separations achieved using the semi-continuous mode. After an initial transient period, Hemoglobin concentration at the acid side of the column is maintained at that of one-half of the feed solution, while at the opposite, basic end of the column, the concentration is equal to the feed.

No attempt to optimize this process has been made, but certain process parameters are suspected of controlling separation. These include displacement rate, displacement volume, pH gradient, packed column height, ionic strength of the system, concentration of buffers employed, and reservoir dead volume.

The following reasons provide the incentive for the investigation and development of a bench-scale pH parametric pumping process. First, parametric pumping offers inherent

advantages of continuous process operation. Second, it offers minimal handling of protein and subsequent protein contamination. Finally, regeneration of the special column adsorbent, which it incorporates, is feasible.

INTRODUCTION

pH protein parametric pumping with ion exchange incorporates the use of a conventional chromatography column with a modified parametric pump apparatus to effect protein separation. Conventional types of column chromatography, used in batch protein isolation, include adsorption, affinity, and ion exchange. The major emphasis in recent investigation of parametric pumping has been mainly restricted to the application of thermal energy to a packed column. The objective of this experimental study, however, has been the coupling of an ion-exchange column with the alternating fluid flow and syncronous alternating pH field of a modified parametric pumping apparatus. The end result is a successful technique to effect protein separation.

In an attempt to describe ion exchange chromatography, general remarks on adsorption and affinity chromatography are first presented, followed by ion exchange.

Adsorption chromatography specifically refers to the batch process in which uncharged substances in a suitable solvent are introduced into a packed column of specialized material. The solute is then adsorbed onto the inert surface of the column packing, followed by differential elution from the column packing with an appropriate second solvent.

Affinity chromatography utilizes a column packed with

an insoluble substance, to which is covalently linked a molecule that will bind specifically to other molecules. As in adsorption chromatography, this is a batch process in which the desired solute in solution is introduced into the top of a column packed with specialized packing. As the solution flows with gravity down through the column, the desired solute is attached to specific sites on the covalently bonded molecules. The desired solute, once attached to the column via the covalently bonded molecule, can usually be obtained using an elution solvent of different pH.

Ion exchange chromatography is a batch-type process, employing synthetic resin column packing. The resins may be anionic or cationic substances which exchange positive and negative counter ions respectively for solute molecules at specific solution pH levels.

A protein in solution of low pH, introduced into a column filled with an anionic packing, will exchange itself for positive counter ions on the anionic packing matrix. Displacement of the positive counter ion from the column anionic matrix occurs because of the amphoteric nature of proteins. A protein in solution of high pH will have a net positive charge, and compete for anionic sites on the packing matrix. Protein recovery is next effected by subjecting the packing to a second solvent of high pH. Attached protein is eluted from the column due to the net negative charge of the protein

when exposed to high pH. Positive counter ions, in the second solvent, affix to the vacated anionic sites, generated by the repulsion of the negative protein from the packing matrix. Once the protein leaves the anionic site of the packing solid phase, it is eluted from the packing in the high pH solvent.

The incorporation of cyclic pH changes in a packed column, required in the concept of a continuous ion exchange process for protein separation, led to a parametric pumping type of apparatus. The technique of parametric pumping was first presented by Wilhelm (1966)[1] et al for concentrating NaCl. Refinement of this thermal parametric pumping process was later demonstrated by Wilhelm and Sweed (1968)[2] when separation factors of 10^5 in toluene-n-heptane-silica-gel system, using batch mode operation. Chen, et al (1972,[3] 1973[4]), have obtained separations using both semi continuous and continuous mode parametric pumping for the same system. Chen, et al (1974[5]), have also extended thermal parametric pumping to include multicomponent mixtures using the model system toluene-aniline-n-heptane on silica gel.

Parametric pumping specifically is based on the reciprocating fluid flow across a column of specialized packing. Cotemporaneous to the reciprocating fluid flow is a syncronous alternating energy field exposed to the packed column. In thermal parametric pumping, heat is applied to the specialized

packed column. In the modified pH parametric pumping apparatus used, fluids of alternating pH flow through the packed column.

The resulting advantages of the combination of ion exchange chromatography and pH parametric pumping to separate protein mixtures are three fold. First, it offers inherent advantages of continuous process operation, such as shutdown time, and equipment size as compared to conventional batch type chromatography methods. Second, it offers minimal handling of protein and subsequent protein contamination and denaturation. Finally, regeneration of the incorporated packing material is possible.

The possibilities of coupling ion exchange and pH parametric pumping were first investigated by Sabadell and Sweed (1970)[6]. In their work a chromatographic column of cation exchanger resin was subjected simultaneously to an alternating axial displacement of solution and to an alternating pH gradient. Separations of 15-80 percent for Na^+ and K^+ feed were achieved. The process used was essentially semi-batch mode. pH at the acid end (3.05) was achieved by titrating the reservoir solution with HCl. Feed solution was at Ph 11.75 along with the contents of the opposite basic reservoir.

Results showed the importance of HCl molarity and the rate of infusion of HCl on separation. Little effect of cycle time was found for the cycle time range used, and feed concen-

tration also did not significantly affect separation.

The advantage of this technique is the possibility of processing a feed solution in a semi-continuous mode. Its main drawback, however, is the inability to obtain both concentrated and diluted product fractions without shutdown of the process. The following summary of the separation technique used by the authors indicates the above conclusions.

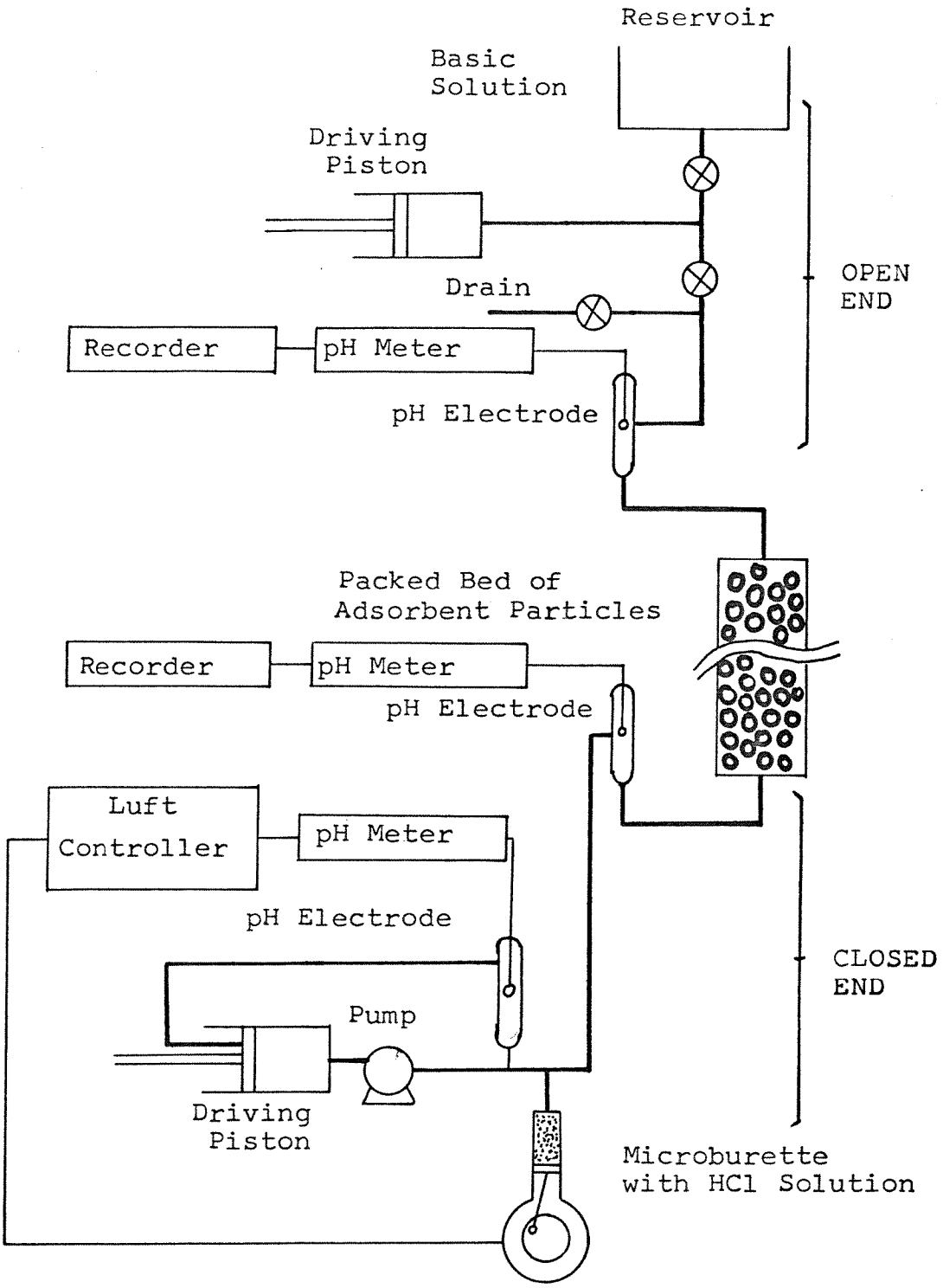
A schematic of the apparatus used by Sabadell and Sweed is Figure 1.

Bottom concentrated products could only be obtained from the acid end of the column by stopping an experimental run. This is due to the use of a closed fluid circuit in conjunction with the acid reservoir to titrate fluid exiting the bottom of the column. This titration of the bottom reservoir fluid with concentrated HCl sustained the low pH of 3.05 required in attempting to concentrate the Na^+ and K^+ ions.

Before a semi-batch cycle was run, the packed column resin voids were filled with pH = 11.75 NaOH, KCl aqueous solution. This same high pH solution in equilibrium with the column packing is placed in the top reservoir. The bottom reservoir and closed loop segment of the column were filled with low pH = 3.05 aqueous solution of same Na^+ and K^+ concentration, as in the top reservoirs and column voids.

The mechanics involved in making a semi-batch parametric

FIGURE 1
pH Parametric Pumping Apparatus
Used by Sabadell and Sweed (1970)[6]



pumping run are as follows. On the first downward cycle, aqueous Na^+ K^+ solution, pH = 11.75, fills the column. The interstitial column fluid is pushed into the bottom reservoir, where it flows into the auxiliary titration loop. The rate of circulation of this low pH fluid through the auxiliary titration loop was 12 to 15 times greater than the bottom column effluent rate. Hence, the fluid in the bottom reservoir is adjusted to a pH = 3.05.

On the return upward half cycle, the fluid in the bottom, low pH, reservoir is displaced back into the column voids, simultaneously pushing the interstitial column fluid out an exit valve. The top reservoir is filled contemporaneous to the upward half cycle.

The mechanism for concentratin of Na^+ and K^+ is as follows. When flow is on an upward half cycle, the fluid with low pH, rich in H^+, replaces some of the alkali metal cations, already fixed on the solid matrix in preparation of the column packing or fixed during the previous half cycle. The overall reaction used in this pH parametric pumping is

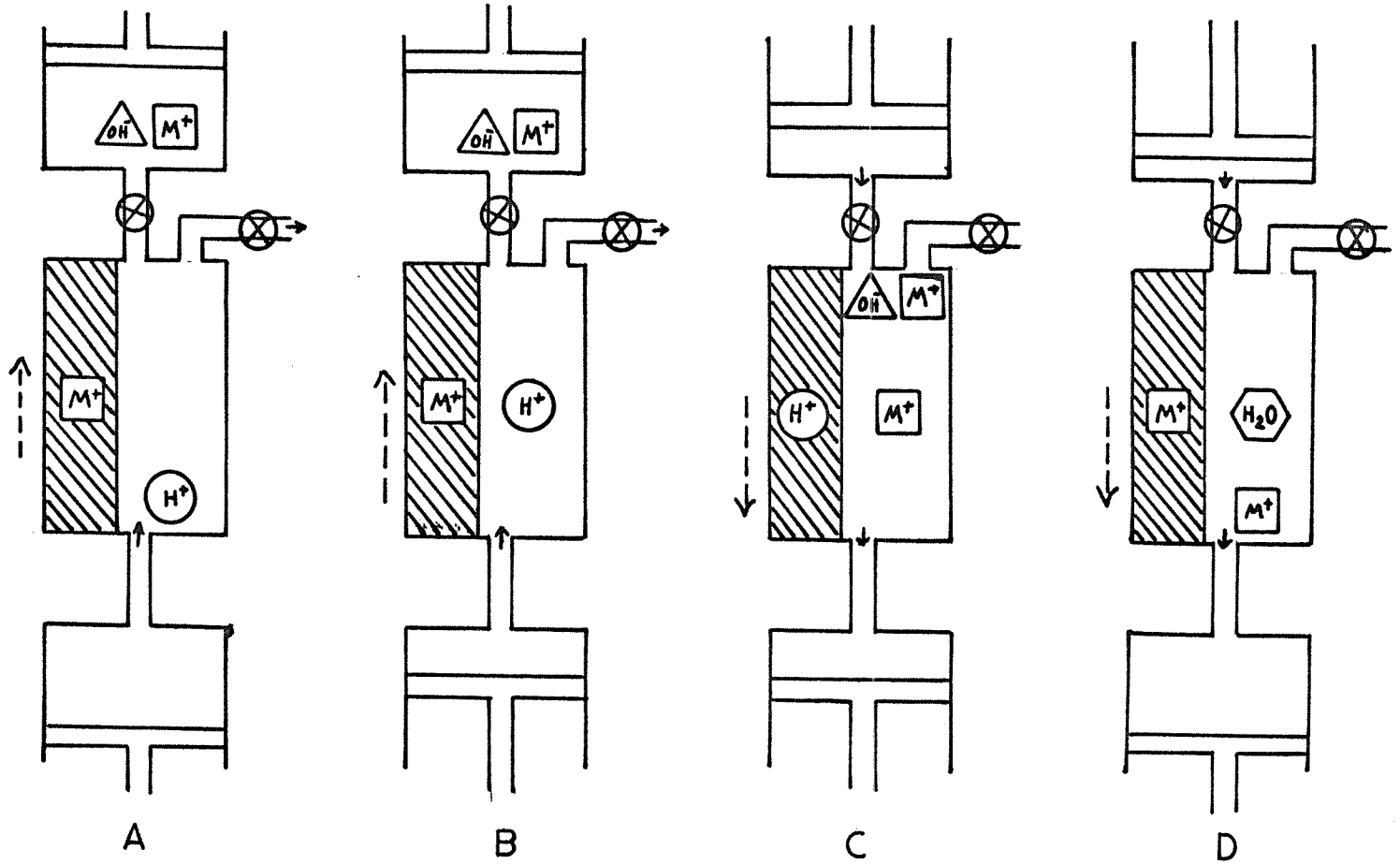
$$RM + H^+ = RH + M^+$$

where R represents the resin phase (column packing), and M^+ represents the exchangeable cations.

Thus, at the end of an upward half cycle, the column matrix is filled with hydrogen ions, and the column voids are filled with aqueous solution rich in metallic cations.

During the downward half cycle, the more concentrated solution moves out of the bed, and feed rich in OH^- comes in contact with the resin packing, displacing H^+ from the matrix via the reaction $H^+ + OH^- \rightarrow H_2O$ and fixing Na^+ and/or K^+ onto the resin again. The net result of many of these semi-batch parametric pumping cycles is to concentrate a feed solution in the bottom, low pH, reservoir, and thus effect a separation of the Na^+ and K^+ from the feed. A diagram of the proposed separation scheme is Figure 2, A, B, C, D. Figure 2, A, shows the packing matrix filled with cations, Na^+ and K^+, during the beginning of an upward half cycle. Figure 2, B, shows movement of fluid, rich in H^+, filling the column interstitial volume at the end of an upward half cycle. Figure 2, C, depicts the ion exchange between the packing solid phase and interstitial fluid, where the solid phase Na^+ and K^+ are replaced by a H^+ ion during the beginning of a downward flow half cycle. In Figure 2, D, the H^+ in the packing solid phase is irreversibly de-absorbed from the column matrix due to reaction with the OH^- present in fluid provided in the downward flow from the top reservoir. This reaction, $H^+ + OH^- \rightarrow H_2O$, creates an available site on the column matrix to which Na^+ and K^+ ions present in the feed from the top reservoir may attach. The de-absorbed Na^+ and K^+ ions previously in the column solid phase are either found absorbed back on the column solid matrix further down the column or present in the fluid phase bottom reservoir.

FIGURE 2, pH PARAMETRIC PUMPING USED TO CONCENTRATE K^+ AND Na^+ IONS



- OH⁻ - Hydroxyl ion in feed
- M⁺ - Na^+ and K^+ initially in feed and packing
- H⁺ - Hydrogen supplied from auxillary titration loop(not drawn), as H^+
- cation exchanger resin
- direction of fluid flow in packed column
- H_2O - water molecule formed by reaction of H^+ in column and OH^- in feed

Results of Sabadell and Sweed confirm the Na^+ and K^+ ions accumulate at the acid end of the packed resin column. Data are presented with respect to the Na^+ and K^+ ions concentration in the upper reservoir. Ratios of sustained bottom reservoir concentration of Na^+ and K^+ to upper reservoir feed Na^+ and K^+ concentration to be between 1.2 - 1.8.

The effect of ionic concentration on the ion exchange equilibria of this process and the mass transfer rates between solid and liquid phase were linked as important factors to the process, however.

With the development of bio-fiber devices, the ability to maintain a pH in the top and bottom reservoir without addition of HCl and subsequent increase of system volume was achieved. This offered more flexibility in operating mode for pH parametric pumping apparatus.

Affinity chromatography and parametric pumping were combined to reduce trypsin concentration in aqueous solution (Shaffer and Hamrin, 1973)[7]. The column packing consisted of trypsin inhibitor, chicken ovomucoid, covalently bonded to Sepharose HB beads. An attempt was also made to separate trypsin from a tertiary mixture of trypsin and chymotrypsin in an aqueous solution.

The system employed for investigation was essentially that of a batch pH parametric pumping apparatus. Samples of

the top and bottom reservoirs were taken at wide intervals and were of small volume. No feed volume was introduced into the system to replace the sample volumes taken.

A schematic of the apparatus used is Figure 3.

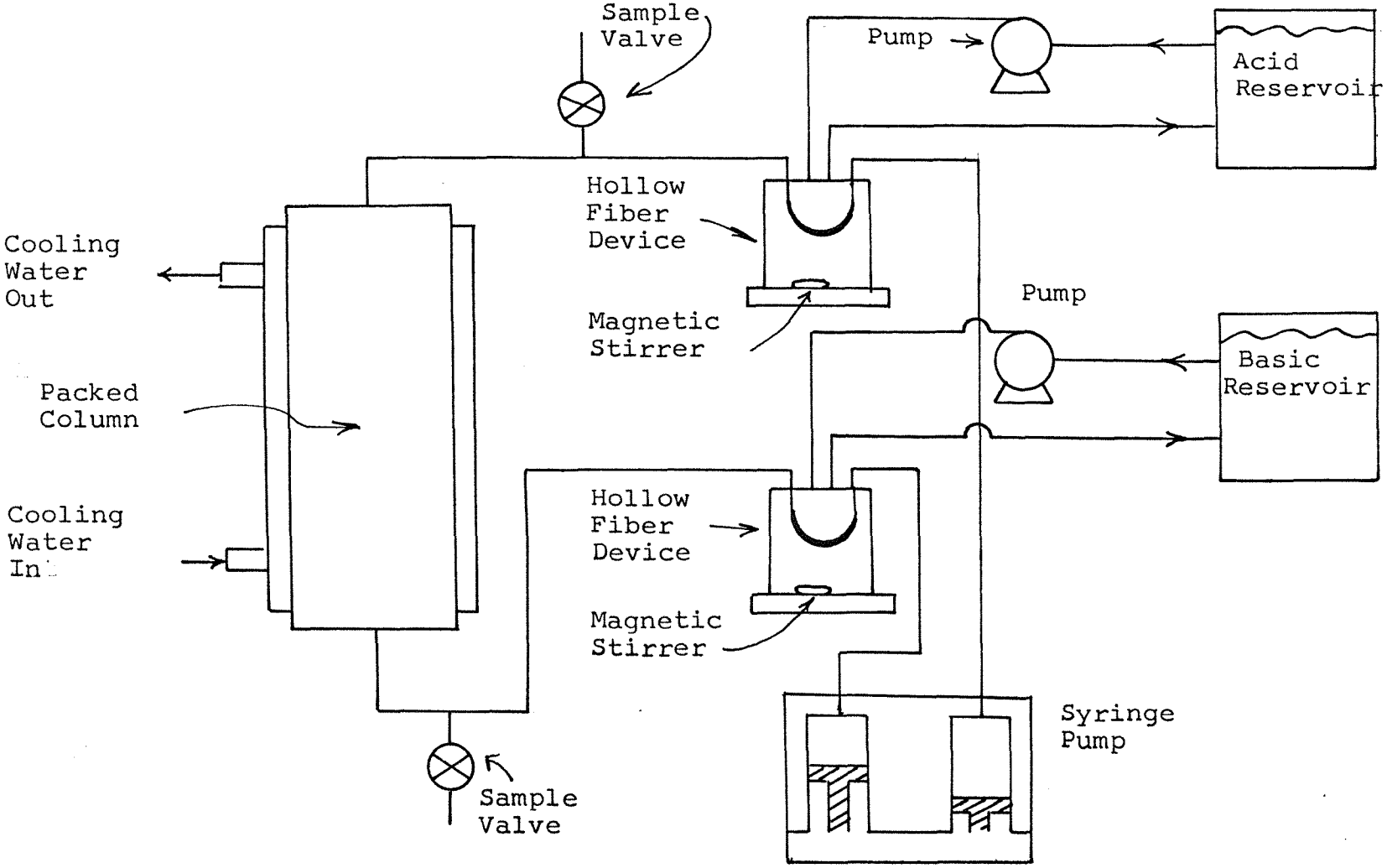
A low pH reservoir was located at the top of the column. The required pH was controlled by dialysis cells of required molecular weight cut off. McIllvaines citrate buffer was circulated outside the dialysis tubes to maintain the required low pH. The high pH reservoir was located at the bottom of the column, with pH controlled similar to the top column reservoir.

Concentrating solutions of water-trypsin binary system and of water-trypsin- α-chymotrypsin tertiary systems depends on the fact that at pH 8.0, aqueous trypsin bonds reversibly to chicken ovomucoid. The bonded trypsin may be recovered by exposing it to aqueous solutions of pH = 4.0 or less. The application of parametric pumping to affinity chromatography was as follows. A specialized packed column is prepared by covalently bonding chicken ovomucoid to inert Sepharose beads, and thus creating a solid phase to which trypsin can reversibly bond. This column was then alternately exposed to high and low pH solutions to effect separation.

Specifically, the technique used by Shaffer and Hamrin is as follows. To start operation of the system, the prepared

FIGURE 3

A Schematic of pH Parametric Pumping Apparatus - Shaffer and Hamrin(1973)[7]



packed column was equilibrated at a pH equal to that used in the top, low pH reservoir. The top reservoir was filled with the same low pH aqueous enzyme mixture in the column matrix voids. The bottom reservoir, also of the same concentration, was filled with high pH aqueous-enzyme mixture. The first half cycle consisted of passing fluid from the top, low pH reservoir, through the packed column and into the bottom reservoir. Fluid was then returned similarly to the top reservoir from the bottom reservoir.

The mechanism of concentrating the binary mixtures of water and enzyme can most easily be understood if first an upward half cycle is discussed, then followed by a downward half cycle. A full cycle is defined by Shaffer and Hamrin as first a downward half cycle followed by an upward half cycle due to the procedure required for initiating a run. On the first upward half cycle, trypsin is bonded to the solid packing matrix. This is due to the favored trypsin retention by chicken ovomucoid in high pH aqueous solutions. During the downward, low pH, half cycle, trypsin is released, and pushed by convection to the high pH reservoir.

In tertiary mixtures of water-trypsin- α chymostrypsin, the α chymotrypsin is not bonded to the chicken ovomucoid matrix, and hence, can be considered to have no effect on separation.

For the water-trypsin system, results show separation is

a function of the number of cycles and of the pH gradient, which is placed across the column. For a pH gradient between the top reservoir, pH = 3.0, and the bottom reservoir, pH = 6.0,

$$\log \left[\frac{Y_B}{Y_{BO}} \right] = -0.01572n$$

For a pH gradient between the top reservoir, pH = 4.0, and bottom reservoir, pH = 6.0, results are

$$\log \left[\frac{Y_B}{Y_{BO}} \right] = -0.00689n$$

where in both cases

Y_B = concentration of trypsin in the bottom acid syringe mg/ml

Y_{BO} = initial concentration of trypsin in bottom syringe mg/ml

n = number of cycles

In the case where an attempt was made to remove trypsin from a tertiary mixture with a pH gradient of 4.0 to 6.0, the results show the trypsin concentration follows quite closely to that of the separation of a trypsin binary alone.

Hence, the advantage of the technique used by Shaffer and Hamrin is centered around the use of hollow fiber devices in substaining a required pH in top and bottom process reservoirs. These hollow fiber devices can be set at any required pH level by simply circulating appropriate buffer through the device. The disadvantages of the process employed, however, include the inability to separate a feed solution with simul-

taneous collection of diluted and concentrated product volumes.

In the previous references, alternating axial flow through a packed column have been discussed. Parametric pumping, however, falls under a broader subclassification of separation processes entitled cycling zone separation. pH parametric pumping deals specifically with alternating axial flow coupled with cotemporaneous alternating pH fields. The cycling pH field through a packed column need not be affected by alternating fluid flow from two reservoir solutions, but also can be achieved by alternating the pH of a feed solution flowing continuously through a packed column in one direction.

Phillip C. Wankat (1975)[8], has extended cycling zone adsorption into the realm of preparative chromatography. Multicomponent mixtures are theoretically separated by utilizing several changes in the cyclic thermodynamic variable entering with a feed solution into a packed column. Studying two equilibrium models, Wankat shows two or more solutes can be recovered individually if the cyclic thermodynamic variable entering the column with the feed varies as a series of steps instead of a simple square wave.

Hence, it is theoretically possible to separate two or more feed solutes, which have different column adsorption distribution coefficients, by changing the pH of the feed solution in several steps to the desired maximum, then directly

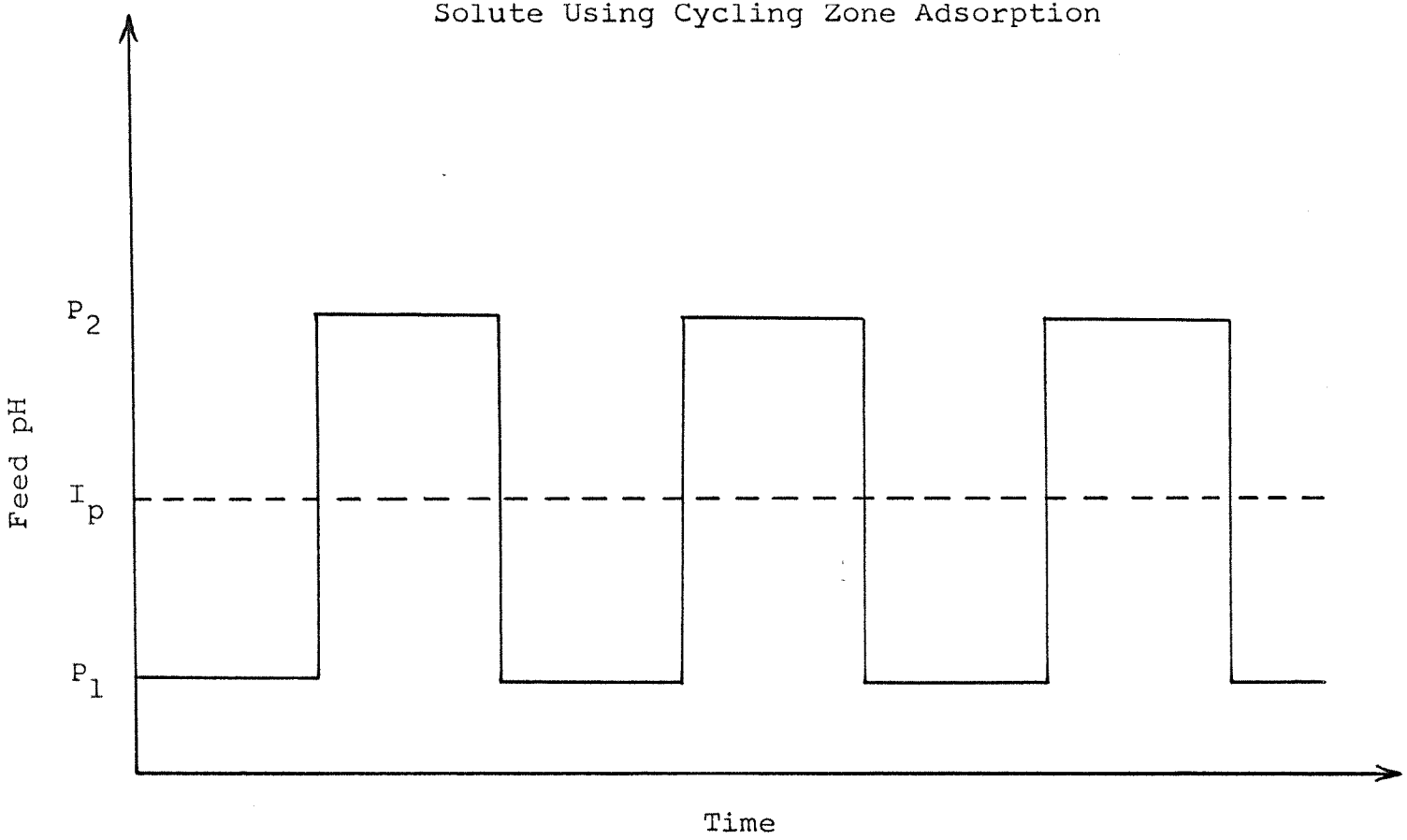
to its minimum, cyclically. In the case of a binary system, where there is one solute, only one cyclic step change in the pH of a feed solution from a minimum to a maximum back to a minimum, would be required to concentrate the solute.

Specifically, this discussed technique is very close to conventional batch chromatography processes used in purification and isolation of proteins. Assuming in high pH solutions ($pH = P_2$), a given protein has a net positive charge, and in low pH solutions ($pH = P_1$) it has a net negative charge, separation from its solvent can be effected if a packed cationic exchanger column is used. When feed solution of high pH is poured through the column, the protein adheres to the column via ion exchange. If the pH of feed solution is brought below the isolectric point of the protein ($pH = I_p$), the protein is exchanged from the packing matrix back into the low pH feed, and elutriation occurs. In conventional batch protein preparitive work, for the same protein system, the feed solution consists of intermittent feed pulses of high pH protein feed solution followed by low pH solvent to effect elutriation when using a cationic exchanger packing. The simple square wave inlet feed pH of the binary process discussed by Wankat is pictured in Figure 4.

The disadvantages of the continuous cycling zone separation process presented by Wankat is the inability to obtain a continuous flow of dilute and concentrated product streams.

FIGURE 4

Feed pH Profile for Separation of Solution with One Protein Solute Using Cycling Zone Adsorption



The advantage of such a process is that it allows a continuous feed stream to be processed.

In this thesis, an experimental study is undertaken to determine the possibility of separating and concentrating a Hemoglobin-water, binary mixture, using batch and semi-continuous mode pH parametric pumping and ion exchange. In the experimental apparatus to be used, a pH in the top reservoir will be sustained at 6.0 and 8.0 in the bottom reservoir. Bio-fiber hollow tube devices at either end of the column will be used to obtain the required pH gradient. A packed column of SP-Sephadex cation exchanger will also be employed. The amphoteric nature of the selected protein was the deciding factor in choosing this macromolecule as a solute.

THE PROCESS

Batch Mode pH Parametric Pumping Separation of Proteins

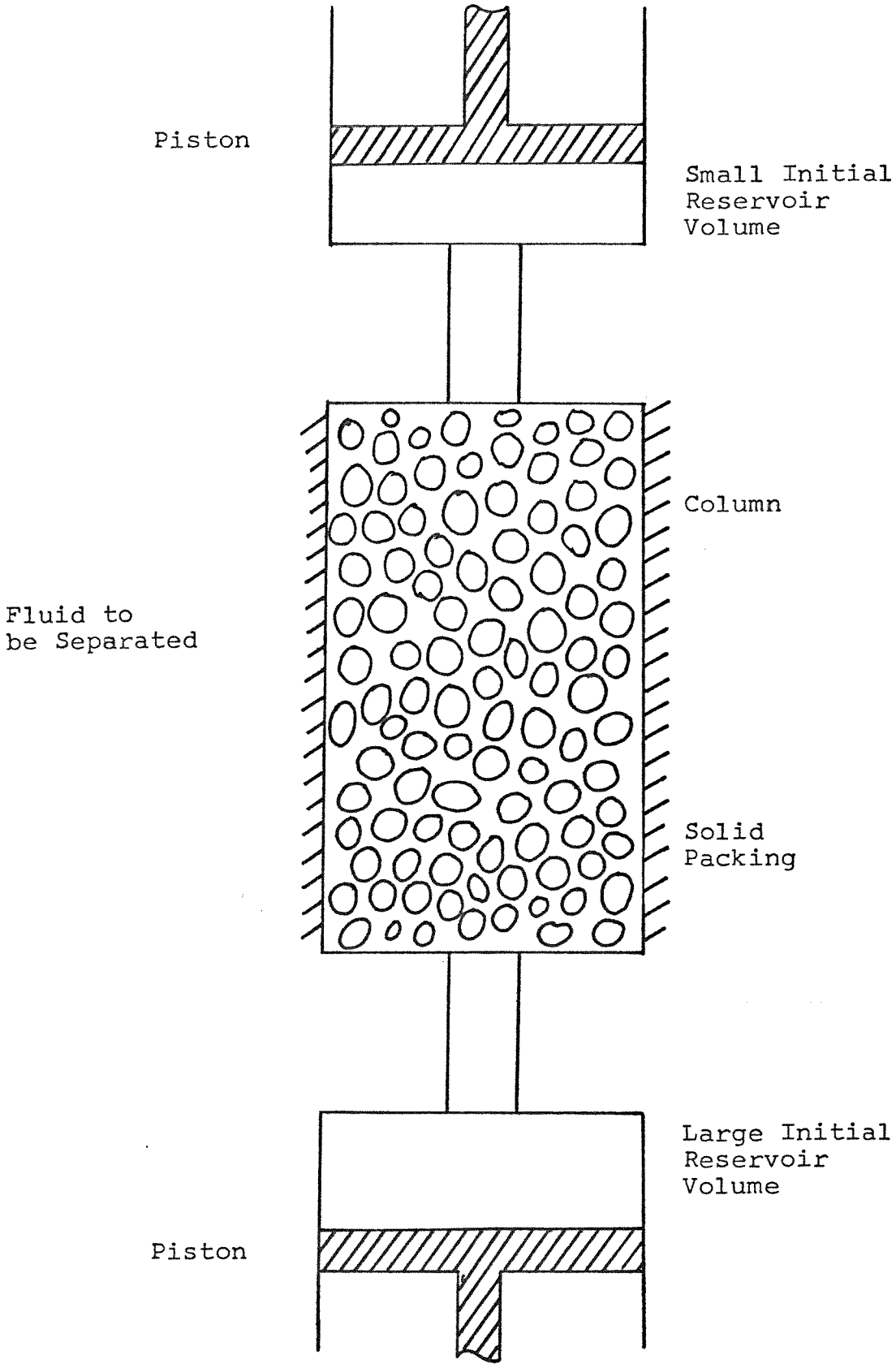
Before discussing the batch mode pH parametric pumping separation of a protein-water binary mixture, a general discussion on parametric pumping fluid flow is provided. From the introductory discussion, the development of the parametric pumping process using cyclic pH change is more easily understood.

Before the start of this generalized full parametric pumping cycle, both reservoirs of the parametric pump apparatus are filled with the fluid to be separated. The volumes in each reservoir are not equal. The column is also initially packed with a specialized solid adsorbent, and the remaining voids are also filled with fluid. See Figure 5 for the parametric pumping systems prepared before a run.

At the start of a batch parametric pump cycle, the fluid in the reservoir with the larger volume is passed through the column into the opposite reservoir. When a specific volume of fluid is displaced into the opposite receiving reservoir, one parametric pump half cycle is completed. At this point, the complementary second parametric pump half cycle is initiated. In the second half cycle, the flow of the fluid in the system is reversed. The original receiving reservoir displaces a specific portion of its volume in the opposite direction through the column into the reservoir, which

FIGURE 5

GENERAL PARAMETRIC PUMPING SYSTEM BEFORE RUN



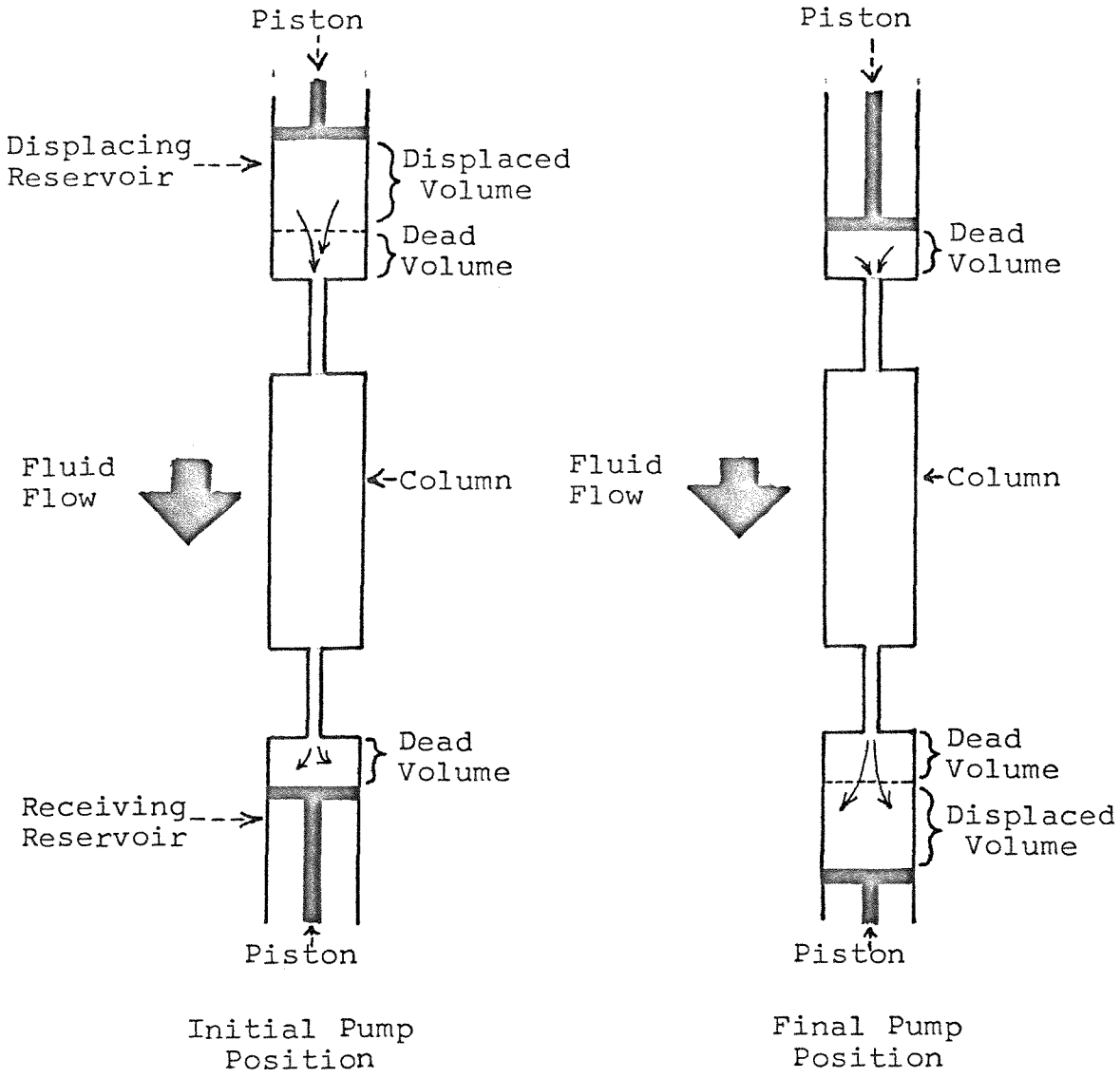
initially had the larger volume at the start of the parametric pumping cycle.

Figure 6 shows the first half cycle, and Figure 7 shows the second half cycle of a full parametric pumping cycle.

In this general parametric pumping cycle, the deal volumes of each reservoir (that portion of the reservoir volume which is not displaced), need not be equal. Also, the total reservoir volumes need not be the same volume. Ideally, either reservoir can be used to initiate a parametric pumping cycle. Certain advantages can be found in practice, however, by initializing the cycles from one reservoir.

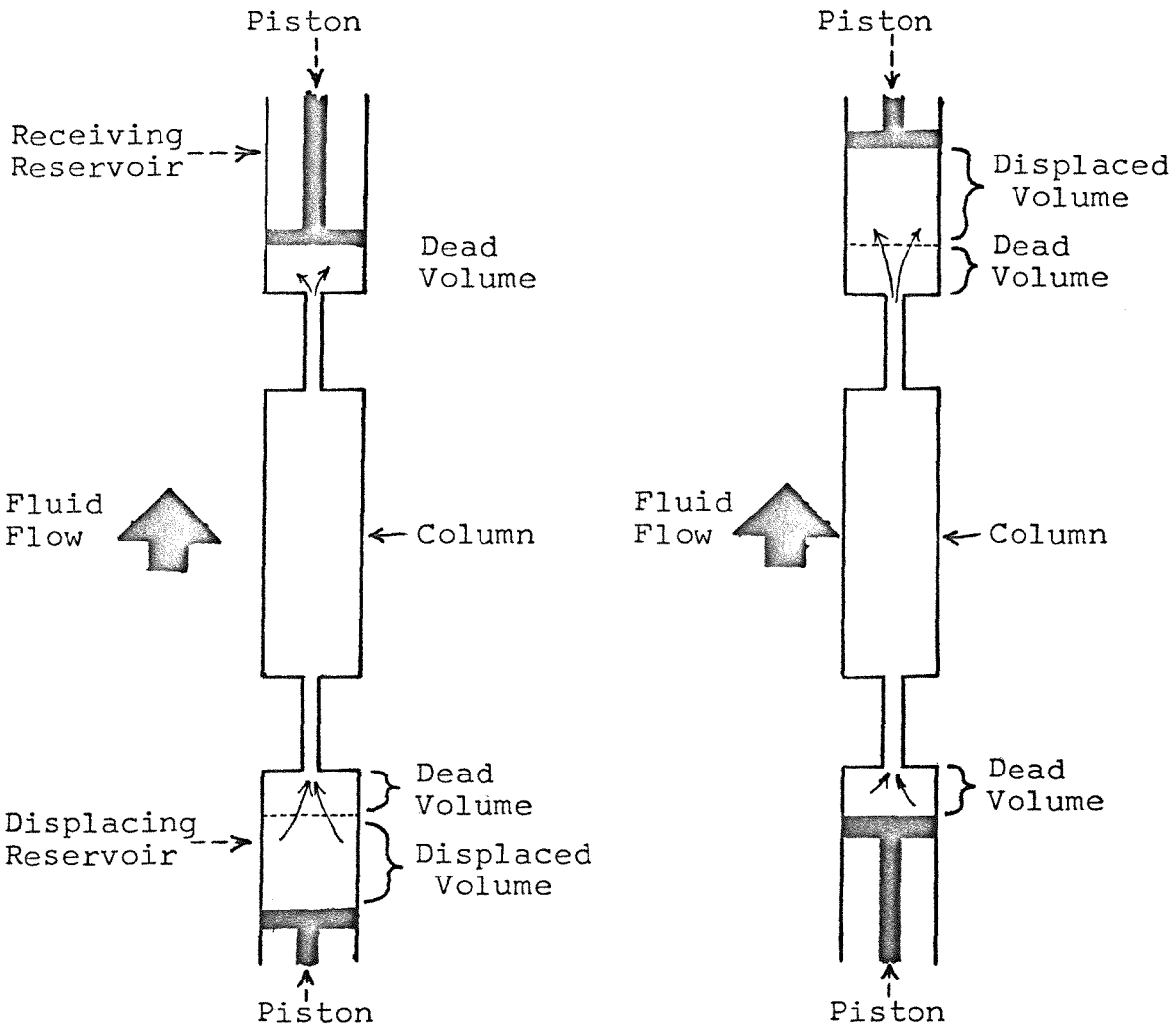
In pH parametric pumping there is synchronous alternating pH change associated with the preciprocating fluid flow previously described to effect separation. The pH change is achieved by the use of bio-fiber, hollow tube, dialysis devices, placed in series between each reservoir and the column. As fluid flows from the column through a hollow fiber device into a receiving reservoir or from a displacing reservoir through a hollow fiber device into the column, its pH is adjusted to that of the bio-fiber device. In this experiment, the bio-fiber devices were set at a high pH equal to P_1 and a low pH equal to P_2. Hence, at a steady state, one reservoir is maintained at a pH of P_1, and the opposite was maintained at a pH of P_2. By convention, due to the physical setup of the experiment, the pH = P_2 reservoir and bio-fiber device

FIGURE 6



First Half Cycle of
A Full Parametric Pumping Cycle

FIGURE 7



Second Half Cycle of
A Full Parametric Pumping Cycle

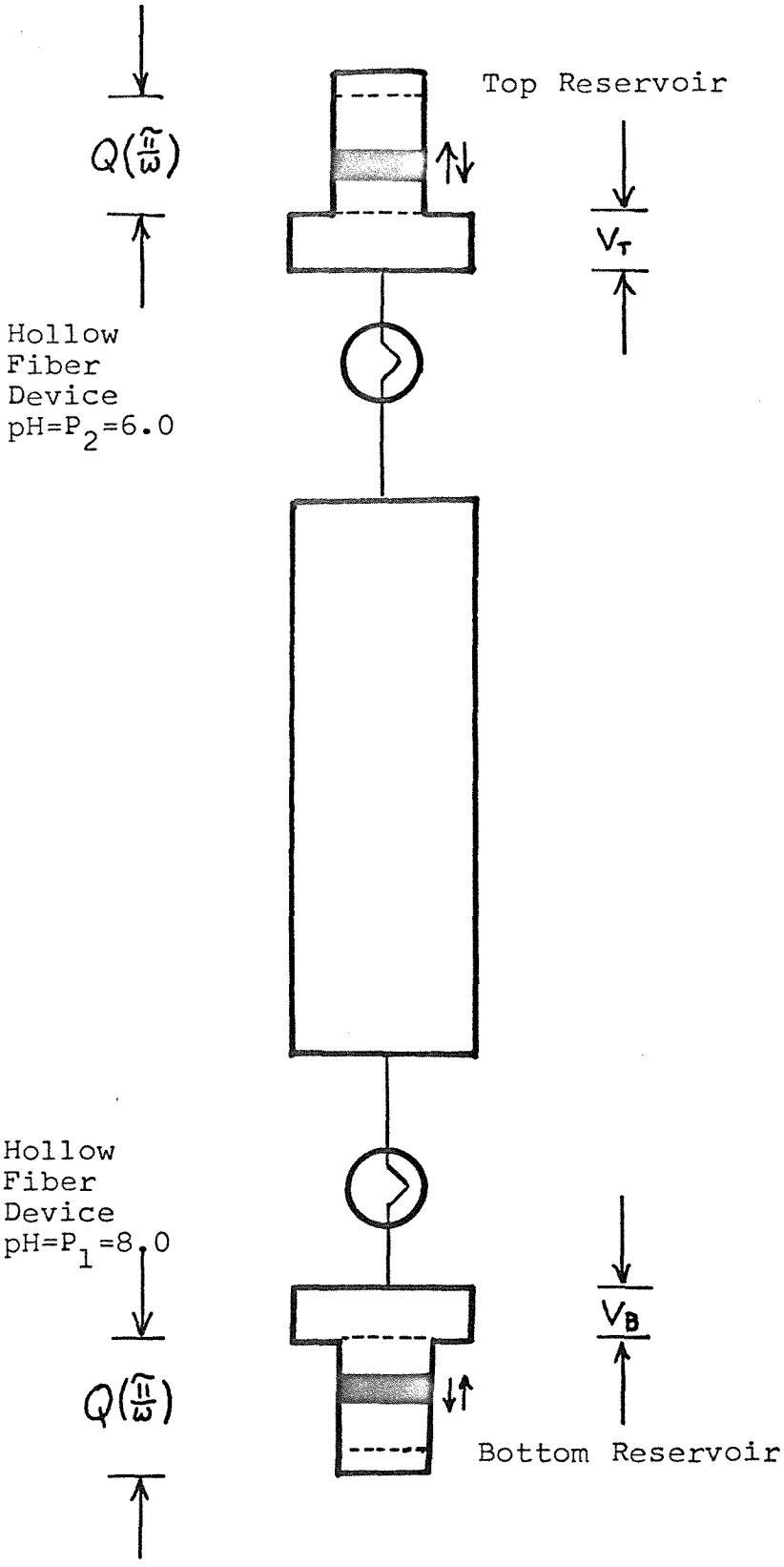
were located on the top of the column, and the pH = P_1 reservoir and bio-fiber device were located at the bottom.

Figure 8 depicts the schematic diagram of the pH parametric pumping process used for separation of a protein-water binary mixture. Flow to and from the reservoirs, within the column and through the dialysis cells, is at the rate Q. At steady state, the mixture within the top reservoir is at a pH equal to the top dialysis, P_2, and the mixture within the bottom reservoir is equal to a pH equal to the bottom dialysis, P_1. The pH of the column contents is equal to P_1 during a downward half cycle and at a pH equal to P_2 during an upward half cycle. The half-cycle time length is $\frac{\pi}{\omega}$, and the displacement volume is $Q(\frac{\pi}{\omega})$. The corresponding top and bottom reservoir dead volume are V_T and V_B.

Initially, the column contents consist of a cationic exchanger gel solid phase and a void spare filled with a solute and buffer of pH depending on the packing preparation technique used. Also, before the start of a batch run, the top reservoir is filled with solute and buffer pH = P_2, and the bottom reservoir is filled with solute and buffer pH = P_1.

In the following discussion the cyclic pH change effecting an alternating adsorption-deadsorption of the solute from the packing material is presented. In this example, a full cycle will consist of an upward fluid displacement from the P_1 reservoir and a complementary equal fluid displacement in the

FIGURE 8



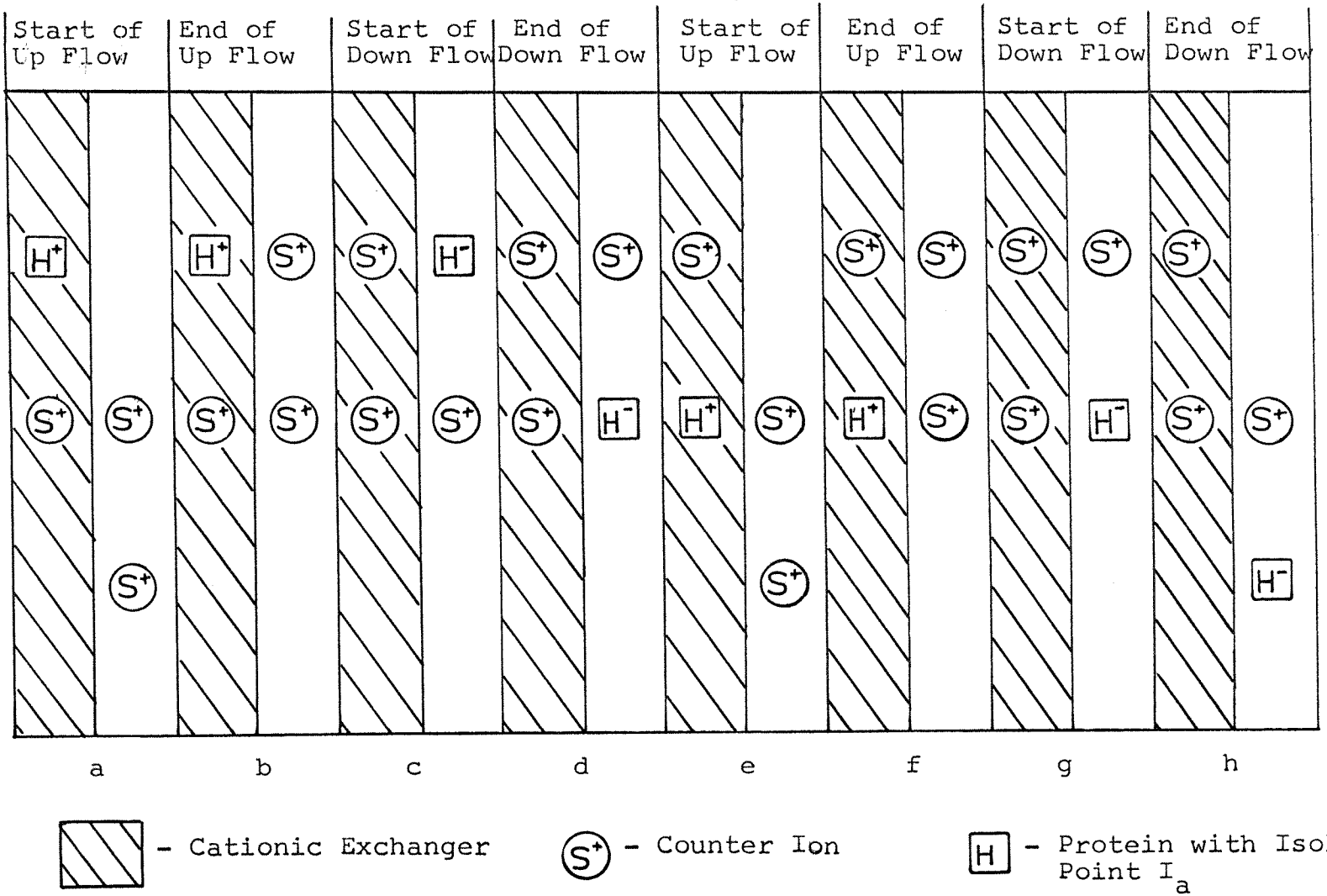
Batch pH Parametric Pumping Process

opposite direction from the P_2 reservoir. The solute is protein with an isolectric point I_A between P_1 and P_2. At pH = P_2 the protein molecule has a net positive charge, and at pH = P_1 it has a net negative charge. The buffers selected are mixtures of Monosodium phosphate salts and Di-sodium phosphate salts. The sodium ions of the buffer (S^+) are used as exchangeable counter ions in the process. The solid phase of the column consists of a negatively charged exchanger matrix saturated with exchangeable positive sodium ions (also S^+) and positive hemoglobin molecules.

During the first up-flow half cycle, the fluid initially in the column voids is displaced into the top reservoir. pH = P_1 buffer and protein from the bottom reservoir simultaneously fill the voids. Because the protein is in a medium of pH higher than its isolectric point, it carries a net negative charge. The protein originally in the packing is displaced from the anionic matrix by ion exchange with the positive sodium ions (S^+) supplied from the buffer. A diagram of this exchange process is in Figure 9, b and c or f and g.

As the down-flow half cycle occurs, the pH = P_1 fluid containing some de-adsorbed protein in the column voids is displaced back into the bottom reservoir. At the same time, pH = P_2 fluid containing positive-charged hemoglobin is introduced into the top of the column voids. The protein from the top reservoir displaces the positive exchangeable counter

FIGURE 9
The Net Movement of a Protein Molecule During One Full pH Parametric Pumping Cycle



ions to resupply the cation exchanger with protein. This adsorbed protein is them de-adsorbed on the second upward half cycle and then moved further down the column or into the bottom reservoir on the second downward cycle. The result of a large number of cycles is to move the protein from the top reservoir to the bottom of the column or bottom reservoir. See Figure 9, c and d or g and h.

At steady state, the upward half cycle effects separation of the water-protein mixture by de-adsorbing hemoglobin to be moved towards the bottom of column on the next downward half cycle. The downward half cycle at pH = P_2 has a dual role of displacing the pH = P_1 fluid in the voids towards the bottom of the column and resupplying the column solid anionic exchanger with protein.

Due to the initial transient nature of the pH distribution in the column voids, the previous discussion does not hold for the initial cycles of a run.

Semi-Continuous Mode pH Parametric Pumping Separation of Proteins

The semi-continuous process of protein separation consists of essentially the same alternating pH change associated with the reciprocating fluid flow in a batch process. The added modifications to the batch process include a feed volume delivered to the column over the entire duration of a selected half cycle and the simultaneous collection of product volumes

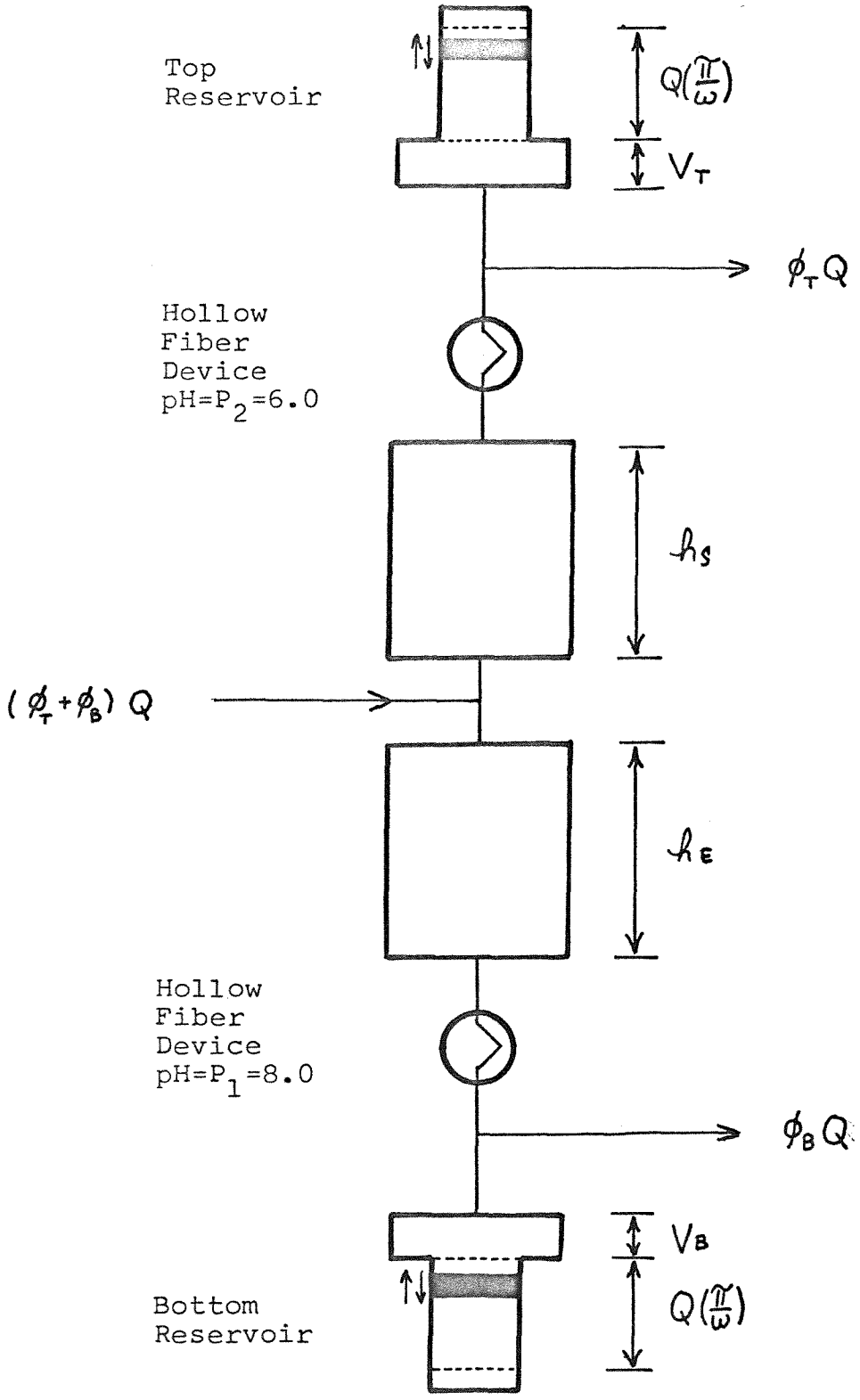
from the top and bottom of the column. The net movement of a protein from one of the reservoir volumes and of the feed, through the column, towards the opposite reservoir, is caused by the same adsorption and de-adsorption scheme discussed in the batch process.

Consider the Figure 10. Feed to the column is at a rate $(\phi_T + \phi_B)$ Q. Sample volumes taken from the top of the column are at the rate of ϕ_T (Q) and sample volumes taken from the bottom of the column are at a rate of ϕ_B (Q). All samples are simultaneously collected during the half cycle, on which the feed is delivered for each full cycle of the process.

At steady state, the mixture within the top reservoir and top sample volume is at a pH equal to the top dialysis, P_2, and the mixture within the bottom reservoir and bottom samples are equal to a pH of the bottom dialysis, P_1.

The half-cycle time length is $\frac{\pi}{\omega}$ and the displacement volume is Q $(\frac{\pi}{\omega})$. The corresponding top and bottom reservoir dead volumes are V_T and V_B. The entire column length consists of two segments. The top segment, h_S, is considered the stripping section. The bottom segment, with height h_E, is considered the enriching portion of the total column. Flow rate through both stripping and enriching section voids is at a rate Q during the half cycle without introduction of a feed volume. During the half cycle with feed, the flow rate through the top stripping segment of the column is $(1-\phi_T)$ Q,

FIGURE 10



Semi Continuous pH Parametric Pumping Process

and the flow rate through the bottom enriching segment of the column is $(1 + \phi_B)$ Q.

Continuous Mode pH Parametric Pumping Separation of Proteins

As in the semi-continuous mode of separation, the continuous process of protein separation consists of essentially the same alternating pH change associated with the reciprocating fluid flow in a batch process. One alteration to the batch mode is a continuous feed volume delivered to the column over the entire duration of a complete parametric cycle, and hence, run. The second modification is the continuous collection of product volumes from the top and bottom of the column during a run. The net movement of a protein from one of the reservoir volumes and the feed through the column towards the opposite reservoir is caused by the same adsorption and de-adsorption scheme discussed in the batch process.

The diagram of the continuous mode process is identical to that for the semi-continuous mode process shown in Figure 10. The flow rate through the top stripping section of the column is $(1 + \phi_T)$ Q , and the flow rate through bottom enriching section of the column is $(1 - \phi_B)$ Q during up-flow. During down-flow, the flow rate in the top stripping section column voids is $(1 - \phi_T)$ Q and the flow rate through the bottom enriching section of the column is $(1 + \phi_T)$ Q.

EXPERIMENTAL

Experimental Apparatus

The experimental apparatus used for both batch and semi-continuous pH parametric pumping runs are similar. Hence, a detailed discription of the semi-continuous apparatus will be presented first. Subsequent modifications to the apparatus for batch runs will then be discussed. The experimental, semi-continuous apparatus is shown schematically in Figure 11. Detailed discussions of each piece of equipment follows.

Packed jacketed column. The column used is a glass-jacketed chromatography column manufactured by Pharmicia, Inc. Its dimensions are 1.6 cm diameter and 40 cm length. Capillary tubing was attached to either end of the column with two variable length flow adaptors also manufactured by Pharmicia, Inc. Jacket ports of the column were attached to a constant temperature refrigeration unit with water used as a coolant.

Top and bottom reservoirs. The reservoirs at opposite ends of the column were two 50 cc glass syringes equipped with removable, Luer Locking needles. Both syringes were operated by a dual infusion-withdrawal pump manufactured by Harvard Apparatus Company. Both syringe plungers were greased with Dow Corning stopcock grease to stop fluid from leaking as the plunger was moved in and out of the syringe. Capillary tubing was connected to each reservoir by simply placing the syringe

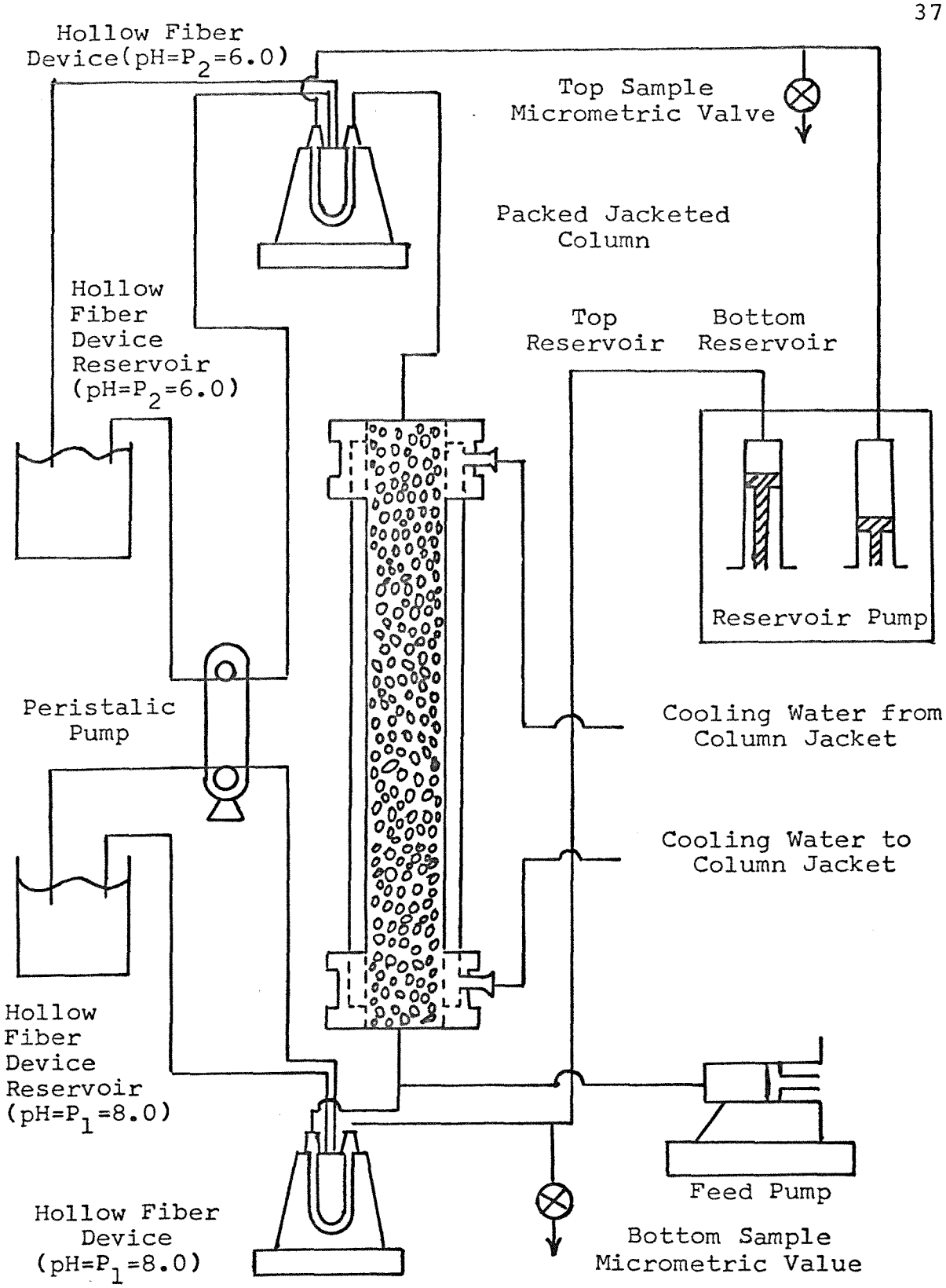


FIGURE 11
SCHEMATIC OF SEMI - CONTINUOUS
pH PARAMETRIC PUMP EQUIPMENT

needle into the open end of the tubing.

Feed pump. A second, similar, infusion-withdrawal pump to the top and bottom reservoirs was used for delivering feed to the system. A 50 cc glass syringe was again incorporated, having a removable, Luer Locking needle. This feed pump was connected to the system by placing the open end of the capillary tubing over the syringe needle.

Hollow fiber devices. The bio-fiber, hollow tube devices at either end of the column, between the reservoirs and ends of the column used for providing the proper pH to the reservoir contents, are Bio-Fiber 50 Beakers. These were chosen because they had a characteristic molecular weight cutoff of 5,000, required to prevent loss of the protein solute. All devices were obtained from Bio-Rad Laboratories, and manufactured by the Dow Chemical Company. The hollow fibers of this device are made of inert cellulose with a fiber surface area of 9,000 square cm and a jacket volume of 100 cc. In installing each hollow fiber device into the system, the following connections were made. Capillary tubing from the reservoir pump was connected to one of the fiber ports on the bio-fiber device by inserting the capillary tubing into a 2-inch length of 1/16 inch inner diameter Tygon tubing. The opposite end of the Tygon tubing length was next inserted into the Beaker O-Ring Connectors, also purchased from Bio-Rad Laboratories. The remaining unconnected portion of the Tygon

tubing was then placed into the fiber port. Connections between the capillary tubing extending from the end of the column and the remaining fiber port were made in a similar fashion. Connections of the hollow fiber device jacket ports to the hollow fiber device reservoirs, containing buffer, will be discussed in the Peristalic pump discussion.

Hollow fiber device reservoirs. The hollow fiber device reservoirs consisted of two 2-liter beakers filled with appropriate buffer solutions. The 1/16 diameter silicone and Tygon tubing lines to and from each hollow fiber device jacket were submerged in each separate 2-liter beaker. These 2-liter reservoirs provided a fresh supply of buffer, which, after being pumped to the hollow fiber device, came in contact with the solution passing through hollow fibers. After contact with the hollow fibers, buffer still remaining in the jacket portion of the hollow bio-fiber tube devices was circulated back into the hollow fiber device reservoirs. The net effect of circulating buffer through the hollow fiber devices from the hollow fiber device reservoirs was to sustain the required pH of the protein solution passing through the bio-fibers.

Peristalic pump. The dual head, peristalic pump, was used in providing two separate buffer circulations, one for each hollow fiber device. Appropriate buffers were circulated from the two hollow fiber device reservoirs, into the hollow fiber device jackets, where they contacted the hollow fibers,

and back into the hollow fiber device reservoirs. Silicone tubing of 1/16 inch inner diameter was used in carrying buffers from the hollow fiber device reservoirs to the hollow fiber devices. A continuous length extended from each hollow fiber device reservoir, through one of the dual heads of the Peristalic pump, to the hollow fiber device. A second continuous length of Tygon tubing, 1/16 inner diameter was used to carry buffer back from the hollow fiber device to the hollow fiber device reservoir. All tubing connections made at hollow fiber device jacket ports were made using connectors purchased from Bio-Rad Laboratories. Tubing entering and exiting the hollow fiber device reservoirs were simply submerged under the buffer levels of the 2-liter beakers. The dual head Peristalic pump was also purchased from Bio-Rad Laboratories.

Micrometric valves. Micrometric capillary valves manufactured by Gilmont, Inc., were used as sample taps at the top and bottom of the column. These provided an adjustable back pressure on the system when open. This type of system tap was used so as to minimize the disturbance to the system when collecting products, and to facilitate obtaining controlled product volumes.

The process lines between each piece of equipment comprising the system had an inside diameter of 0.1 cm. This capillary tubing was a plastic type, inert to water.

The experimental apparatus used in the batch process

consisted of the same equipment of the semi-continuous apparatus minus both the feed pump and top and bottom sample micrometric valves. A schematic diagram of the batch pH parametric pumping apparatus employed is Figure 12.

Experimental Preparation and Technique

General run preparation for both batch and semi-continuous mode operation will first be presented. Different running technique for each experimental mode will later be discussed.

Prior to each run, buffers were prepared for use in the top and bottom hollow tube device reservoirs and for use in preparing feed solutions. Selected amounts of Monosodium phosphate salt solution and Disodium phosphate salt solution were combined to provide a buffer mixture of the required pH. Table 1 presents the relative volumes of each salt solution to be mixed for a desired 100 cc of buffered pH solution. When larger volumes of buffer were required, each quantity in the table was multiplied by the ratio of volume required/ 100 cc.

The concentration of the buffers prepared using Table 1 are 0.2M. When buffers of different concentration were required, solution A and B of Table 1 were prepared by decreasing the amount of Monobasic sodium phosphate and Dibasic sodium phosphate to be dissolved in de-ionized water. The newly prepared solution of A and B were then mixed to obtain

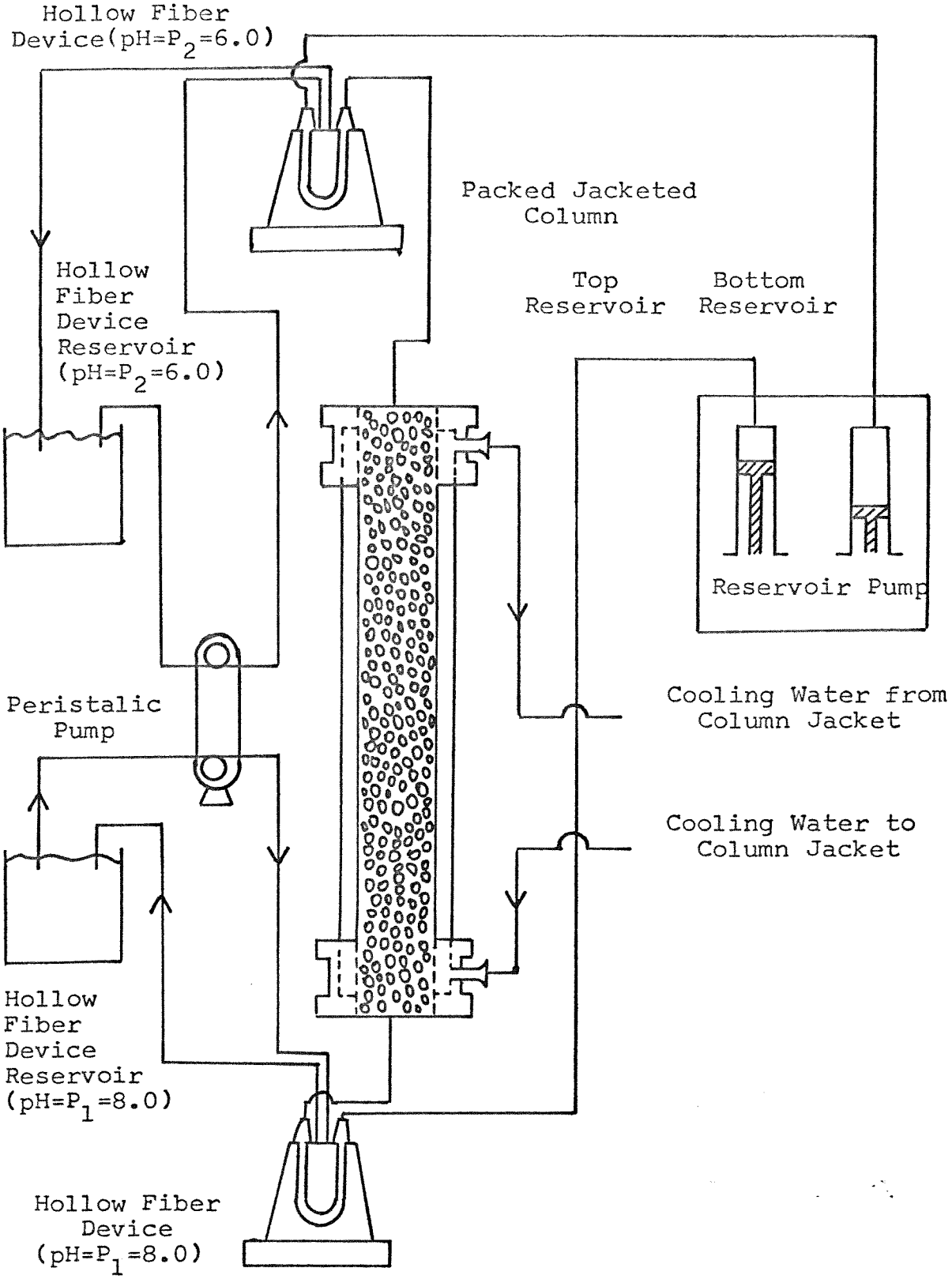


FIGURE 12
SCHEMATIC OF BATCH pH PARAMETRIC PUMP EQUIPMENT

the required pH using the relative volumes in Table 1.

Table 9 shows the concentration of buffer used in each experimental run.

Preparation of the column packing was done prior to each run. Several techniques were used for column packing preparation, but the following technique has been found to be the most advantageous. Initially, 0.8 grams of SP-Sphadex packing is expanded in 40 cc of pH 6.0 sodium phosphate buffer prepared as previously discussed in this section. The packing is allowed to expand for approximately 12 hours at room temperature, after which time, it is assumed to have reached its maximum volume. After full expansion of the packing mixture, 10 cc of the top layer is decanted off, and 100 cc of the desired concentration hemoglobin and buffer solution is stirred into the packing mixture gently. The subsequent packing mixture is then allowed to equilibrate for 12 hours under regrigeration. After the equilibration period, the top layer of the hemoglobin packing mixture is decanted, and the bottom portion is poured into the process column.

Preparation of the packed column is as follows. The jacketed column was filled with the prepared packing and sealed off, avoiding air entrapment. The technique used will be outlined. One of the variable length flow adaptors was fitted into the bottom of the column, which was mounted with its axis vertical. Next, the O-ring in the flow adaptor was

tightened to seal the bottom of the packing chamber. The capillary tubing exiting the bottom of the column was sealed with a line plug. The bottoms of the decanted packing mixture was slowly poured into the column using a continuous motion, down a glass rod to avoid air entrapment.

The bottom capillary line plug was next removed. As the interstitial column liquid exited the bottom capillary tube, the packing began to settle. Three distinct regions in the column packing could be seen in the settling process. The bottom layer was the orderly dense solid packing material, the middle contained the solid portion, the packing solids in solution moving randomly in a downward direction, and the third layer consisted of only the fluid originally equilibriated with the packing. When the interface of the first and second layers met the interface of the second and third layers, the bottom capillary line was plugged again. At this point, the second middle layer finally settled, and two distinct regions were in the column. The packed solid and equilibriated liquid made up the bottom layer of the column, while the upper layer consisted of equilibriated liquid only.

Next, the second variable length flow adaptor was fitted into the top of the column, the tip of which was allowed only to touch the upper fluid layer of the packing. The O-ring, at the tip of the adaptor, was slightly tightened upon to marginally contact the sidewalls of the column. The top adaptor was next pushed down to meet the interface between

the lower solid packing region and the upper fluid region of the poured column. As the top adaptor was pushed down, fluid originally in the top of the column was displaced into the capillary tubing exiting the top flow adaptor. When the tip of the top flow adaptor met the top of the solid packing layer, all the fluid in the upper region was displaced out of the column, and the top capillary line was completely filled and could be plugged. The top flow adaptor was then tightened securely. This procedure rendered a completely packed column, ready to be connected into the process lines extending from the top and bottom reservoirs.

Initial top and bottom reservoir solutions and feed solutions for batch and semi-continuous runs were made by weighing a known amount of hemoglobin and mixing it with known volumes of pH 6.0 or pH 8.0 phosphate buffer.

All lines between the top of the column and the top reservoir, as well as between the bottom of the column and the bottom reservoir, were next connected in the required fashion.

In batch mode pH parametric pumping single hollow fiber devices of appropriate pH were connected between top of the packed column and top reservoir and between the bottom of the packed column and the bottom reservoir.

In semi-continuous mode pH parametric pumping, the same

hollow fiber device connections were made as in the batch mode. Auxillary lines were added between the top hollow fiber device and bottom reservoir to accommodate the micrometric valves. Connections were made with 3-way plastic capillary tubing connectors. An additional auxillary line was added between the column and hollow fiber device or between the hollow fiber device and micrometric valve, either at the top or bottom of the column to accommodate the feed pump. Several pH semi-continuous feed locations were examined. Table 10 shows the different feed locations attempted. Again, plastic 3-way connectors were used in accommodating capillary feed lines from the feed pump.

The hollow fiber devices were next filled with appropriate buffer as well as the hollow fiber device reservoirs. All line connections required for circulation of the buffer were made as discussed in the Experimental Apparatus section.

In initializing both batch and semi-continuous runs, the following techniques were used. All lines and the hollow fiber device at the top of the column were flushed and filled with pH 6.0 hemoglobin solution. The jacketed column, filled with the prepared packing and sealed off, was connected to the top and bottom process lines, simultaneously avoiding air entrapment.

Next, the jacket of the column was connected to a cool

water source of approximately 10° C and water circulation through the column was turned on. This circulation of cool water through the column jacket during the entire experiment was used to eliminate denaturing of the protein solute.

After the dead volume and displacing volume of the reservoir pumps were checked, the top reservoir syringe was filled with hemoglobin solution at pH 6.0 and the bottom reservoir syringe was filled with pH 8.0 hemoglobin solution. The feed syringe, if any, was filled with appropriate hemoglobin solution. All syringes were then mounted in appropriate positions on the infusion-withdrawal pumps.

Buffer circulation through the hollow fiber devices was turned on and set at approximately 20 cc/minute. Magnetic stirrers placed below the hollow fiber devices were also turned on to insure buffer circulation and contact with the bio-fibers. Magnetic stirrers also placed in the top and bottom reservoir syringes of the process to insure homogenous concentration were started. These last procedures rendered a complete run set up for hemoglobin-water separation.

To start a batch run, the reservoir pump was switched on. The initial displacing reservoir pushed fluid into the column, and the opposite reservoir received the fluid from the column. The half cycle time was $\frac{\pi}{\omega}$ and the displaced volume through the column was (Q) $\frac{\pi}{\omega}$. At the end of this first half cycle, one full batch parametric pumping cycle was completed. A

total of n successive batch cycles made a batch run. Table 6, Remarks section, tabulates which reservoir was the initial displacing reservoir for each batch run.

To start a semi-continuous run, the reservoir switch was turned on. The initial displacing reservoir pushed fluid into the column, and the opposite reservoir received the fluid from the column. At the end of this first half cycle, the second half cycle was initiated. During this half cycle, the feed is switched on, and sample volumes are taken from top and bottom of the column. The original receiving reservoir displaced fluid in a reverse direction into the column and opposite reservoir, thus completing one pH semi-continuous pumping cycle. Table 8, Remarks section, tabulates which reservoir was the initial displacing reservoir for each semi-continuous run. Table 10 depicts feed location, and Table 7, Feed Concentration section, depicts feed pH for each run.

A total of n successive semi-continuous cycles made a pH semi-continuous run.

Experimental Analyzation

The procedure for measuring the top and bottom reservoirs for batch and also top and bottom reservoirs and feed samples for semi-continuous runs is as follows. Samples of known concentration of hemoglobin in dilute buffer of three pH's were prepared. Each sample was then measured by visible light

spectrometry on a Beckman Model DU Spectrophotometer. The measurements were made at a wave length, λ, of 403 mμ against a standard of pure de-ionized water. This standard was chosen because the absorbance of water and dilute buffer at any pH was identical. Calibration curve data is in Tables 2 through 4.

The data show absorbance, l, versus solute concentration, was linear. Figure 13 presents this data. The slope of the line decreased with increasing pH, as seen in Figure 14. Thus, knowing the pH and absorbance of a given sample, its concentration could be found from either Figure 13 or Figure 14, using the following development:

$$l = \log_{10} \frac{100}{T}$$

where T = sample transmission

and l = sample absorbance

$$c = \frac{\alpha}{l}$$

where α = slope of calibration curve at sample pH (see Figure 14)

and c = concentration of protein solute in sample.

In run H-12, where all samples were diluted before measurement, the concentration obtained from the calibration curve was multiplied by the dilution factor to get the proper sample concentration.

Final analysis for the five binary batch runs is in

FIGURE 13

Absorbance of Hemoglobin in 0.05 Molar Phosphate Buffer at 403 m , Visible Light, verses Concentration

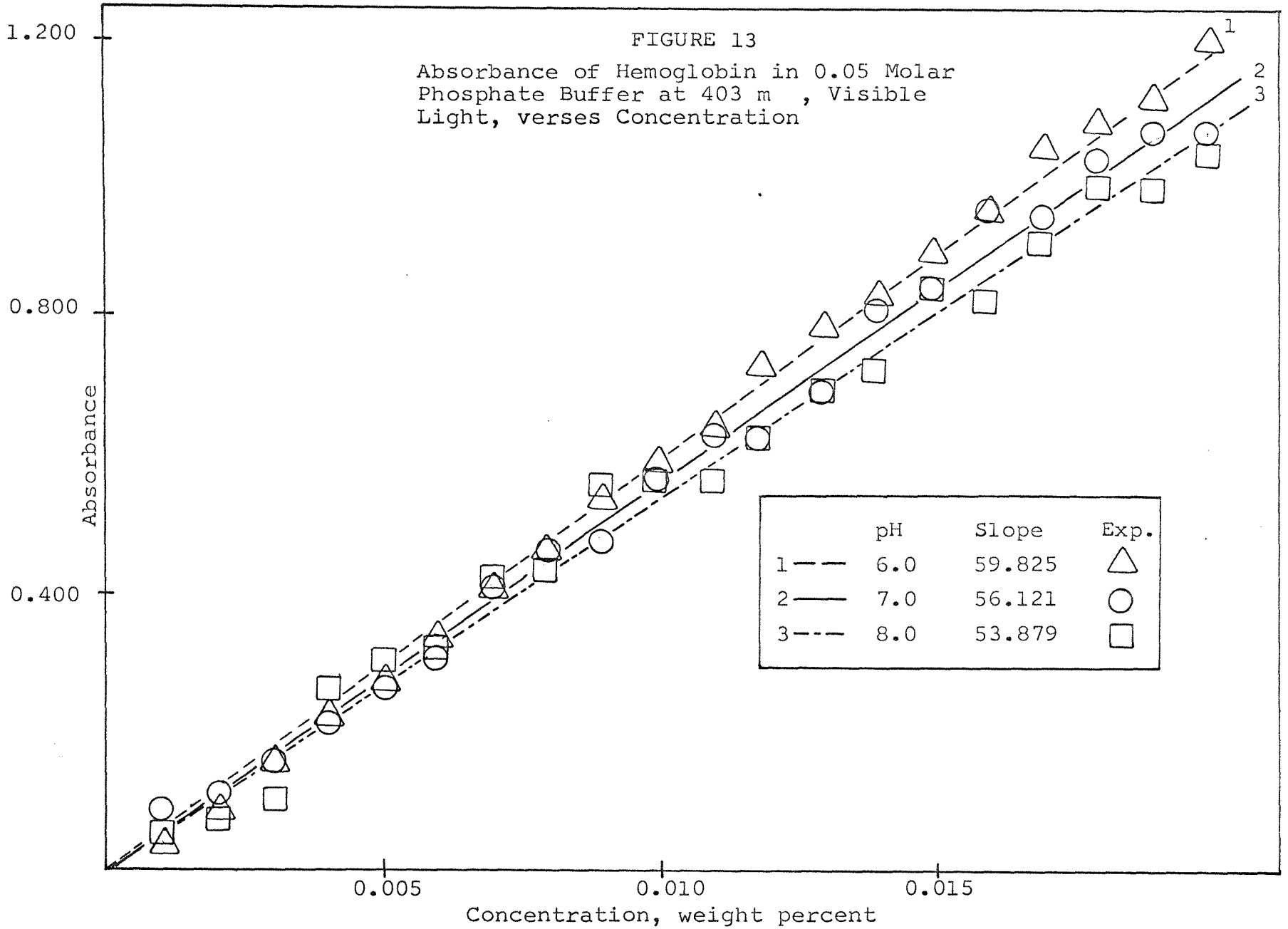


FIGURE 14

Hemoglobin Calibration Curve Slope at 403 mμ, Visible Light, verses pH of Hemoglobin Solution

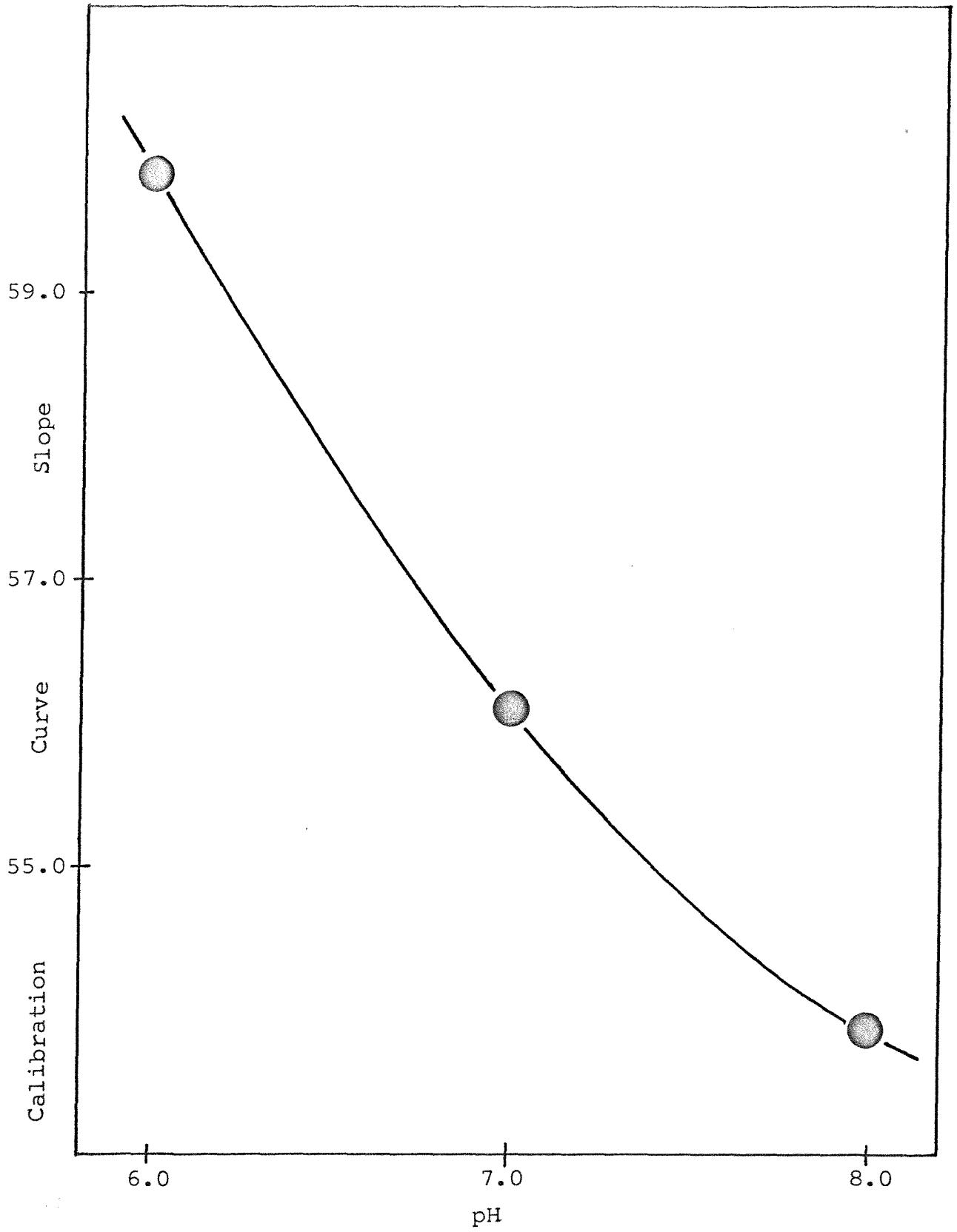


Table 11. Final analysis for the nine semi-continuous hemoglobin-water binary runs are Tables 12 to 20.

An example sample concentration calculation is as follows:

Take Sample 4B, Semi-Continuous Run H-9

pH = 7.90 sample transmission = 34.60

using the expression:

$$l = \log_{10} \frac{100}{T}$$

letting T = 34.60

$$l = \log_{10} \frac{100}{34.60}$$

$$l = 0.461$$

using the second expression:

$$c = \frac{\alpha}{l}$$

where $l = 0.461$

and $\alpha = 54.1$ at pH 7.90 from Figure 14,

$$c = \frac{0.461}{54.1}$$

$c = 0.0085$ weight percent hemoglobin

An example sample concentration calculation using a dilution factor is as follows:

Take sample 2T, Semi-Continuous Run H-12

An initial volume of 3 cc of sample was diluted to a total volume of 5 cc with de-ionized water. Hence, the sample concentration must be $\frac{5}{3}$, or 1.67 times greater than the observed sample concentration.

For given sample

pH = 7.00 sample transmission = 53.9

using the expression:

$$l = \log_{10} \frac{100}{T}$$

letting T = 53.9

$$l = \log_{10} \frac{100}{53.9}$$

$$l = 0.268$$

using the second expression:

$$c = \frac{\alpha}{l}$$

where $l = 0.268$

and $\alpha = 56.1$ at pH 7.00, from Figure 14,

$$c = \frac{0.268}{56.1}$$

$c = 0.0048$ weight percent hemoglobin for the diluted sample.

To obtain the actual sample concentration, the diluted sample concentration is multiplied by the dilution factor. Hence,

$$c = 1.67\ (0.0048)$$

$c = 0.0080$ weight percent hemoglobin

DISCUSSION AND RESULTS

A total of five batch pH parametric pumping runs and nine semi-continuous pH parametric pumping runs were made. In an attempt to normalize all runs for comparison, due to the non-reproductibility in preparing feed concentration, all actual sample and reservoir concentrations obtained from the calibration curve were divided by the feed concentration characteristic of that run. This yielded a normalized ratio of sample to feed, with which samples from different runs could be compared. The above development is as follows:

let C_F = concentration of protein solute in feed solution of any pH

C_S = concentration of protein solute in sample solution (sample may either be top or bottom reservoir)

n = normalized ratio of sample concentration to feed concentration

$$N = \frac{C_S}{C_F}$$

The normalized ratios of sample to feed from initial displacing reservoir, for the five batch runs, are in Table 11. For the nine semi-continuous runs, the normalized ratio of sample to feed are in Tables 12 to 20. Also, for each semi-continuous run, plots of normalized ratio of sample to feed were made versus n number of cycles. These plots are

Figures 15 through 23.

In surveying the batch runs, some presentation of experimental conditions is required. The approximate height of packing for each run was 10 cm. The concentration of all buffers and feed solution was kept uniform. The displacement volume, $(Q)\frac{\pi}{\omega}$, displacement rate, Q, bottom and top reservoir dead volume, V_B and V_T, were all kept constant. See Table 5.

The bulk of the batch pH parametric pumping effort was to obtain an idea of optimum feed location (top or bottom) with packing of different containing liquid of different pH in the voids. The position and pH of the initial displacing reservoir in the batch process was felt to give some indication of the results to be expected from introducing the feed at that same position in a semi-continuous run of the same packing pH and operating conditions. Runs H-1, H-2, H-3, and H-5 show all combinations effected separation except an initial displacement or half cycle from the bottom, pH 8.0, reservoir into a packing of pH 6.0 liquid voids. As a result, all four feed locations, as in Figure 26, were later attempted in the semi-continuous runs.

In an attempt to determine the approximate number of cycles required to reach steady state, one run of twelve cycles was made. The top and bottom reservoir concentrations were measured every four cycles for hemoglobin concentratino. See Figure 24. Results show steady state was not achieved.

From data of nine semi-continuous experiments performed, a separation of hemoglobin protein was effected. Hemoglobin accumulates more in the basic end of column packing fluid than in the opposite acid end. Figures 15 through 23 show these results. During most runs, the concentration trend of the bottom is maintained around that of the feed, while that of the top samples remain approximately 50 percent of the feed. In some runs, particularly H-12 through H-14, a transient region occurs where the bottom product contains 1.5 - 2.0 times the initial feed concentration. This is attributed to the column packing preparation procedure and not the pH parametric pump concentrating effect found past the transient portion of the process.

In terms of time required in achieving steady state, the column packing preparation technique has been found to be important. An example of this can be seen by comparing run H-12 with H-14. All parameters were kept the equal in each run, except the method of preparing the column packing. The effect of the packing preparation procedure was to move top and bottom sample curves higher on the y axis.

The final recommended column packing procedure is discussed in Experimental section, and was used for the last two runs, H-13 and H-14. Techniques used in preparing packing in earlier runs is in Tables 6 and 8, Remarks section.

The importance of the distance of reservoir fluid displacement into the column can be seen in Figure 17 for run H-8. In this experiment samples for top and bottom reservoirs could only be obtained during scattered cycles. As a result, there is a non-uniform reservoir volume penetration into the column voids during all cycles of the run. The net result is small, if not any separation of the feed solution. In comparison, during Run H-9, with duplicate conditions of Run H-8, almost all samples were collected from the top and bottom reservoirs during all cycles of the run.

Other expected operating parameters controlling the separation are half cycle time $\frac{\pi}{\omega}$, buffer concentration of system, pH range between the hollow fiber devices, column height, and relative size of sample volumes taken from top and bottom of the column. Explanation of why these parameters are considered important is presented next.

The half cycle time, $\frac{\pi}{\omega}$, is considered an important parameter controlling this separation process, because it is the period of time during which a given segment of packing is exposed to a given pH field. The concentration of exchangeable counter ions from the packing and buffers used in the system is important due to inter-molecular attractions between solute and solvent, or between packing and solute. Table 9 shows the buffer concentrations used in all semi-continuous runs. The pH range of the hollow fiber devices must be considered because

it determines the maximum numbers of molecules which will change charge around a given isolectric point. This range is limited by the ability of the solute protein molecule to withstand pH variation without denaturation. The column height of the separation process, $h_s + h_e$, as displacement volume, effects the penetration of the pH field into the packing voids. For this reason an optimal ratio of column volume to reservoir displacement volume could be investigated. Top and bottom sample volumes, or $(\frac{\pi}{\omega})\phi_T$ and $(\frac{\pi}{\omega})\phi_B$, are important because they also set pH field penetration into the column. Ratios of ϕ_T and ϕ_B determine top reservoir pH field penetration into the column packing at uneven volumes. In analyzation of product concentrations a separation factor is defined:

$$\text{Separation factor} = \text{S.F.} = \frac{\text{bottom of column sample hemoglobin concentration for cycle n}}{\text{top of column sample hemoglobin concentration for cycle n}}$$

The defined separation factor yields relative magnitude of top and bottom sample concentrations for cycle or cycles of a run. As such, it is suggested for analyzing data from future runs. It presents a relative magnitude of the process product concentrations, and hence, may be used in process optimization.

Separation factors for Run H-14 are tabulated in Table 21. Also, a plot of separation factor versus the number of cycles

is in Figure 25 for Run H-14. This is considered one of the most important runs, because data were obtained using experienced lab technique. The data fit a smooth curve, from which reasonably accurate separation factors could be calculated.

Figure 25 shows the high separation factors for the initial cycles of the run due to the movement of the hemoglobin from the initial packing procedure.

More important, however, is the relatively constant separation factor trend around 2 during the last cycles of the run. This plot shows separation can be effected and sustained by pH parametric pumping. Table 7 presents some of the parameters for the semi-continuous pH parametric pumping runs H-6 through H-14. Also, Table 8 included remarks on each run in general. The best separation results were achieved using the process parameters for Run H-14. From the experimental data, it is demonstrated that the use of pH parametric pumping for the separation of a protein solute in water is feasible. No complete explanation of the single or combined effects of the process parameters can be made, however.

With the apparatus employed, the feed location in each of the semi-continuous runs attempted has been limited to either the top or bottom of the column (h_s or $h_e = 0$). Table 10 and Figure 26 indicate the locations specifically used in each run. From the separation data presented, feed from the

bottom of the column clearly seems to be advantageous. In future optimization of the feed location, however, a two-column system could be substituted for the single column presently employed. If each column is fitted with variable flow adaptors, feed can be easily introduced at any location along the column axis.

In optimizing this protein separation process, one particularly important parameter should be isolated. In future studies, the effect of buffer concentration should and can be easily studied. Runs can be made holding all other parameters constant, and varying the buffer concentration circulating through the hollow fiber devices. The buffer concentration used in preparing feed and initial reservoir volumes must also be changed appropriately. This investigation is specifically suggested because pH parametric pumping process with ion exchange greatly depends on the ability of a protein to exchange onto and from the packing matrix with counter ions supplied not only with the packing material, but also in all preparations where buffers are used.

NOTATION

INTRODUCTION

R - resin phase (column packing), Sabadell and Sweed (1970)

M^+ - exchangeable cations, Sabadell and Sweed (1970)

Y_B - concentration of trypsin in bottom acid syringe, mg/ml, Shaffer and Hamrin (1973)

Y_{BO} - initial concentration of trypsin in bottom acid syringe, mg/ml, Shaffer and Hamrin (1973)

n - number of cycles in pH parametric pumping run, Shaffer and Hamrin (1973)

I_p - isolectric point of protein, used in discussion of work by Wankat (1975)

P_2 - pH of protein solution or solvent greater than I_p, used in discussion of work by Wankat (1975)

P_1 - pH of protein solution or solvent less than I_p, used in discussion of work by Wankat (1975)

THE PROCESS

P_1 - pH greater than protein isolectric point, I_A

P_2 - pH less than protein isolectric point, I_A

I_A - protein isolectric point

$\frac{\tilde{\tau}}{\omega}$ - half cycle time in pH parametric pumping, sec

$Q\frac{\tilde{\tau}}{\omega}$ - displacement volume from either top or bottom process reservoir, cc

V_T - top reservoir dead volume in pH parametric pumping, cc

THE PROCESS (cont'd.)

V_B - bottom reservoir dead volume in pH parametric pumping, cc

S^+ - exchangeable counter ions used in pH parametric pumping

ϕ_T - product volumetric flow rate from top reservoir, cc/sec

ϕ_B - product volumetric flow rate from bottom reservoir cc/sec

Q - reservoir displacement rate, cc/sec

EXPERIMENTAL

λ - wavelength of visible light used in spectroscopic analysis of product volumes, 403 M

l - sample absorbance in spectroscopic analysis

T - sample transmission in spectroscopic analysis

$\propto$ - slope of calibration curve

c - concentration of protein solute in product sample volume as determined by spectroscopic analysis

TABLES

cc - cubic centimeter

sec - seconds

Prod. - product volume collected during half cycle

Slope - slope of calibration curve at sample pH

Trans.- transmission of sample in spectroscopic analysis

Absb. - absorbance of sample in spectroscopic analysis

Mμ - millimicrons (10^{-9} meters)

TABLES (cont'd.)

Conc. - concentration of protein in sample as determined by spectroscopic analysis

wt. % - weight percent, ratio of weight of protein solute to total weight of solution

T - top

B - bottom

FOOTNOTES AND REFERENCES

1 Wilhelm, R. H., Rice, A. W., Bendelius, A. R., "Parametric Pumping: A Dynamic Principle for Separating Fluid Mixtures;" Industrial Engineering Chemistry Fundamentals, Volume 5, Page 141 (1966).

2 Wilhelm, R. H., Sweed, N. H., "Parametric Pumping: Separation of Mixtures of Toluene and n-Hepatne;" Science, Volume 159, Page 522 (1968).

3 Chen, H. T., Rak, J. L., Stokes, J. D., Hill, F. B., "Separations via Continuous Parametric Pumping;" AIChE Journal, Volume 18, Page 356 (1972).

4 Chen, H. T., Reiss, E. H., Stokes, J. D., Hill, F. B., "Separations via Semi-Continuous Parametric Pumping;" AIChE Journal, Volume 19, Page 589 (1973).

5 Chen, H. T., Lin, W. W., Stokes, J. D., Fabisiak, W. R., "Separation of Multicomponent Mixtures via Thermal Parametric Pumping;" AIChE Journal, Volume 20, Page 306 (1974).

6 Sabadell, J. E., Sweek, N. H., "Parametric Pumping with pH;" Separation Science, 5(3), Page 171 (1970).

7 Shaffer, A. G., Hamrin, C. E., "Enzyme Separation by Parametric Pumping;" Department of Chemical Engineering, University of Kentucky, Lexington, Kentucky 40506.

FOOTNOTES AND REFERENCES (cont'd.)

8 Wankat, P. C., "Multicomponent Cycling Zone Separation;" Industrial Engineering Chemistry Fundamentals, 14(2) (1975).

APPENDIX A - FIGURES

FIGURE 15

Plot of Normalized Top and Bottom Product Concentrations verses Number of Cycles for Semi Continuous Run H-6

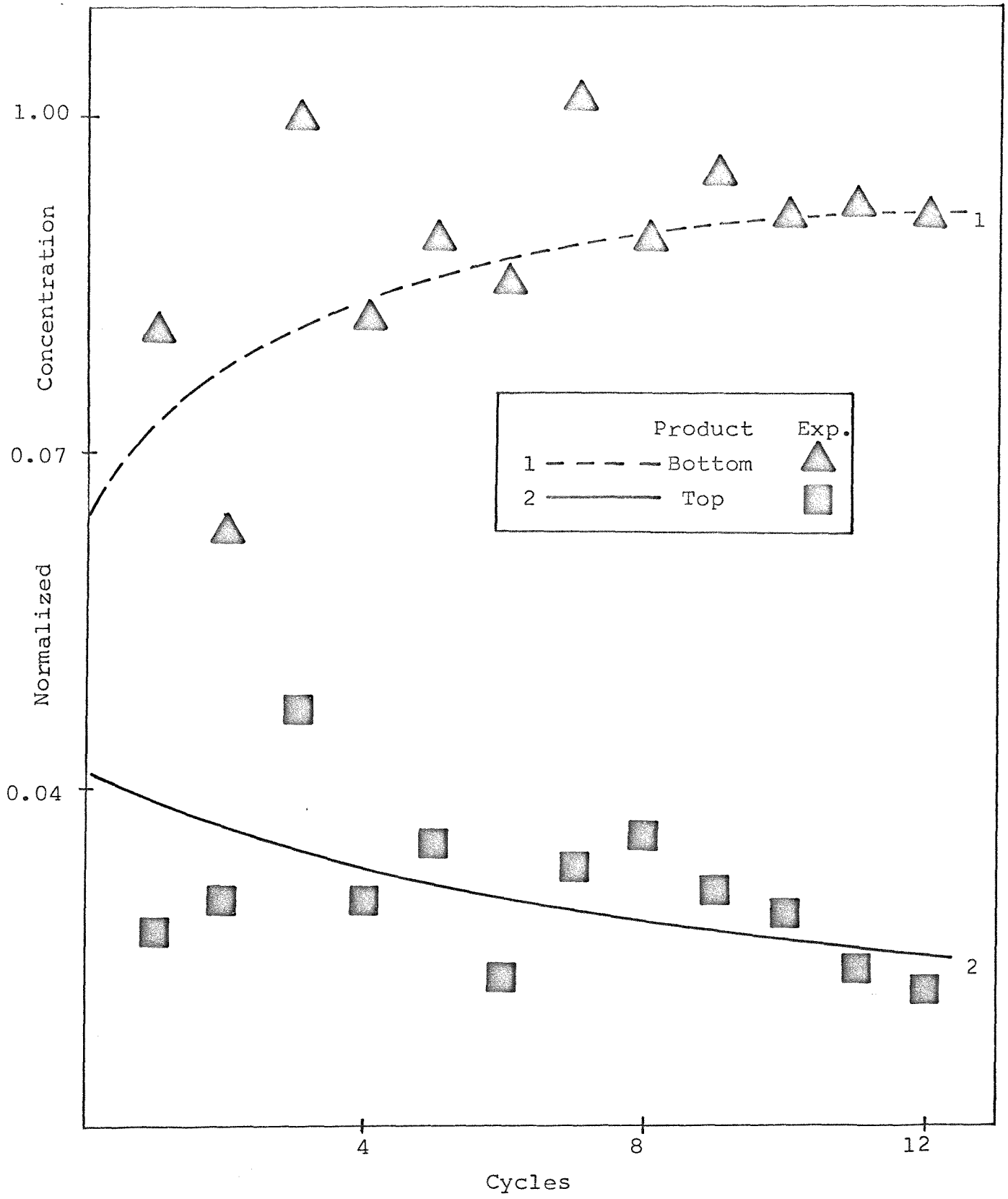


FIGURE 16

Plot of Normalized Top and Bottom Product Concentrations verses Number of Cycles for Semi Continuous Run H-7

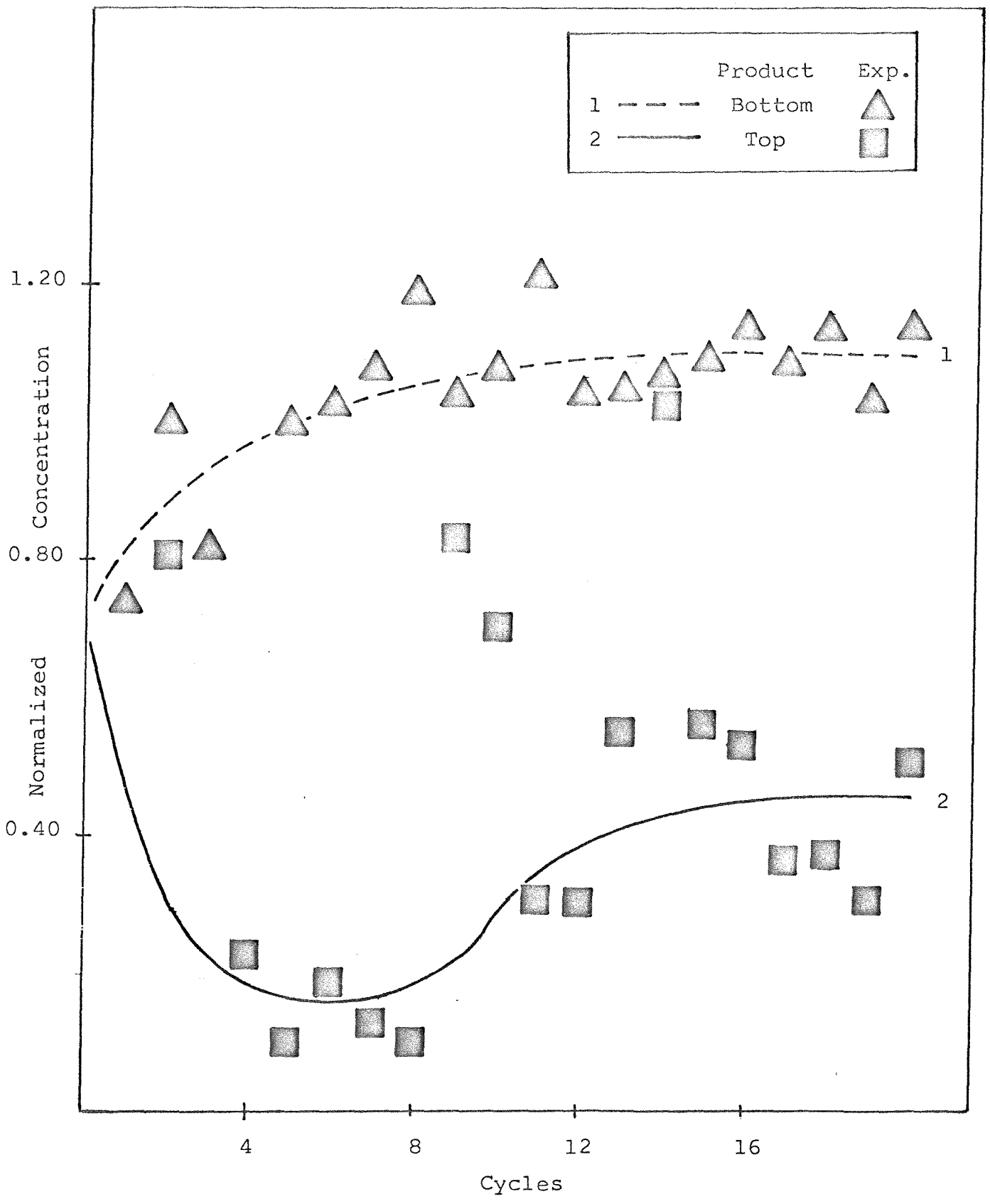


FIGURE 17

Plot of Normalized Top and Bottom Product Concentrations verses Number of Cycles for Semi Continuous Run H-8

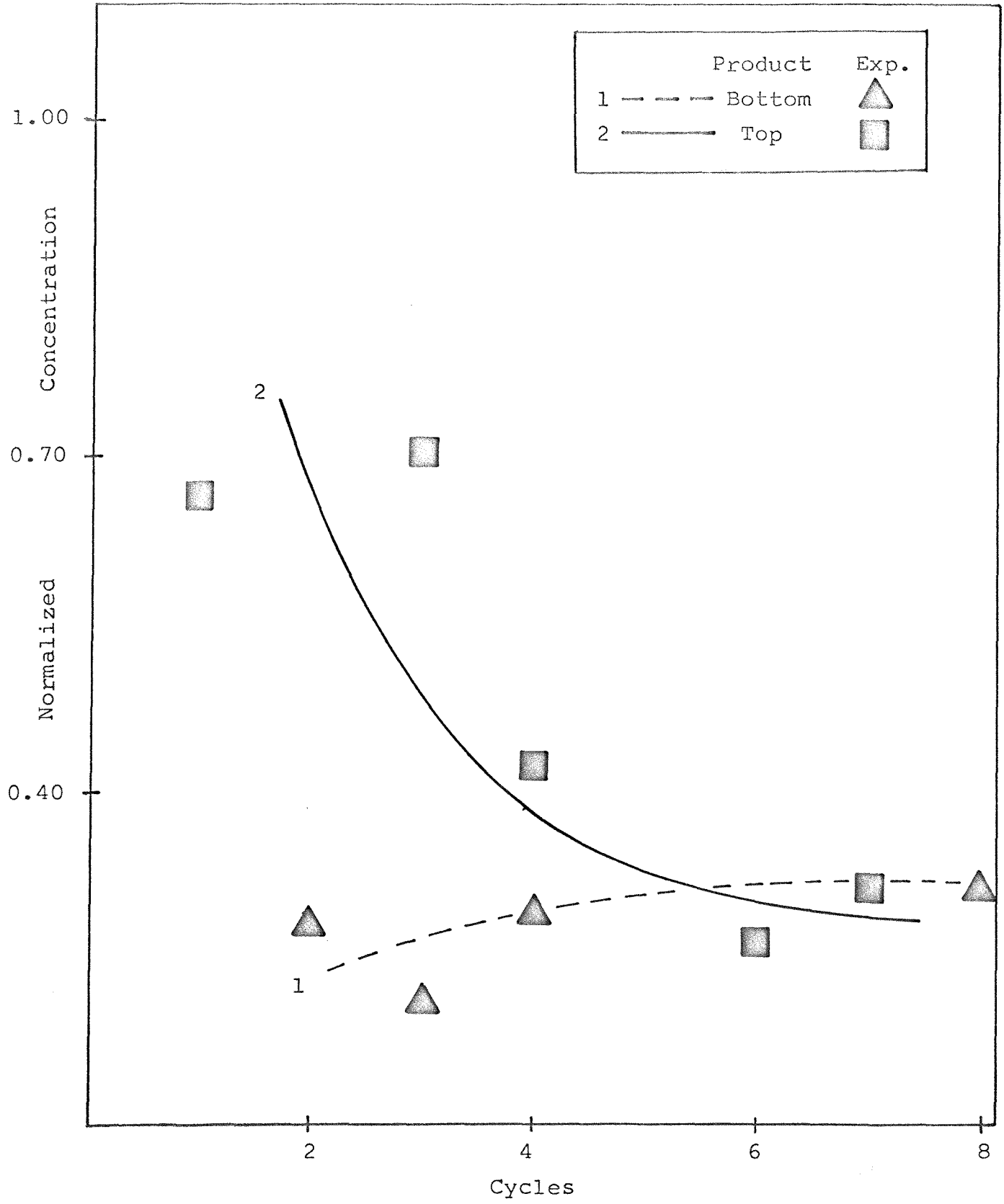


FIGURE 18

Plot of Normalized Top and Bottom Product Concentrations verses Number of Cycles for Semi Continuous Run H-9

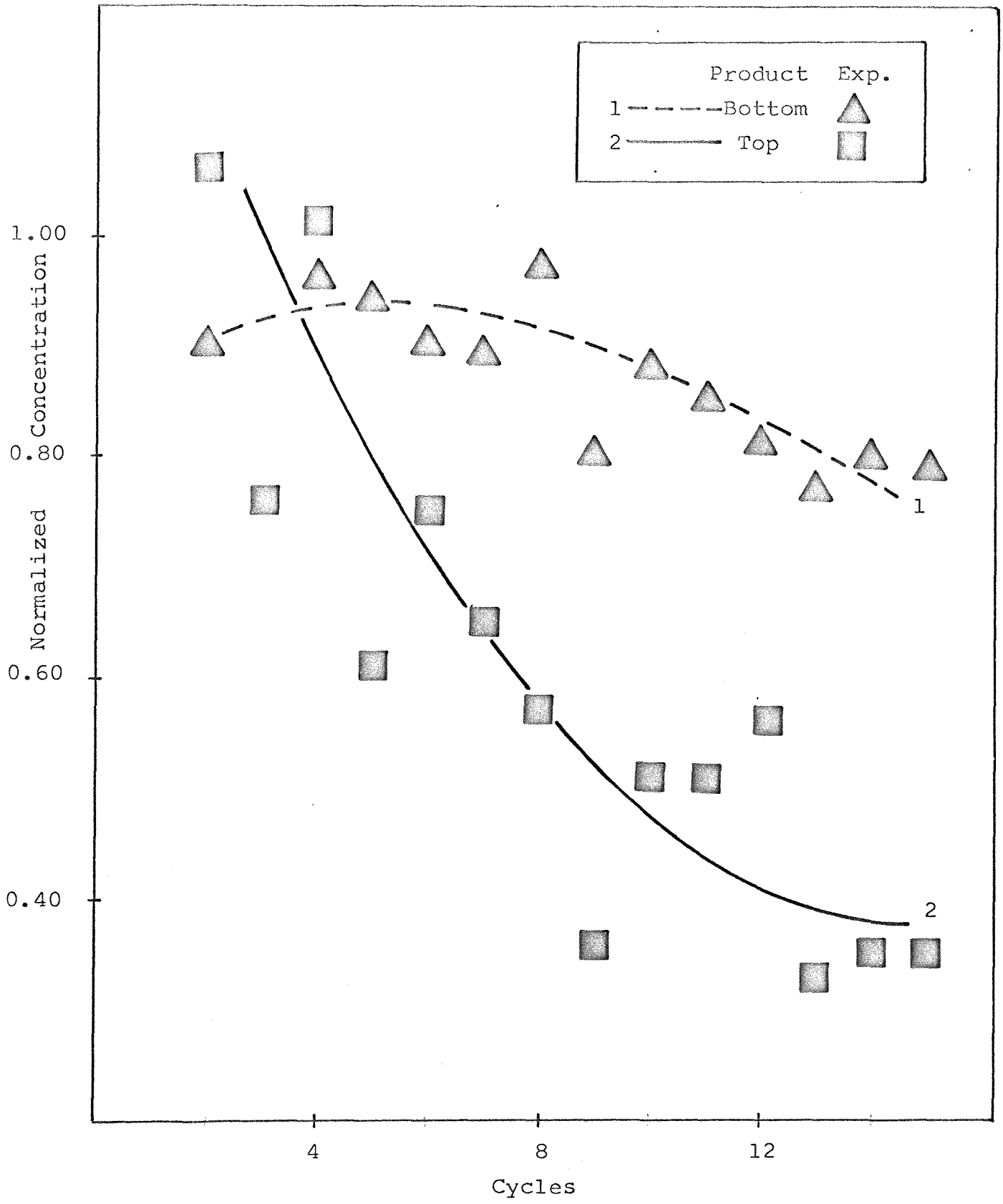


FIGURE 19

Plot of Normalized Top and Bottom Concentration of Products verses Number of Cycles for Semi Continuous Run H-10

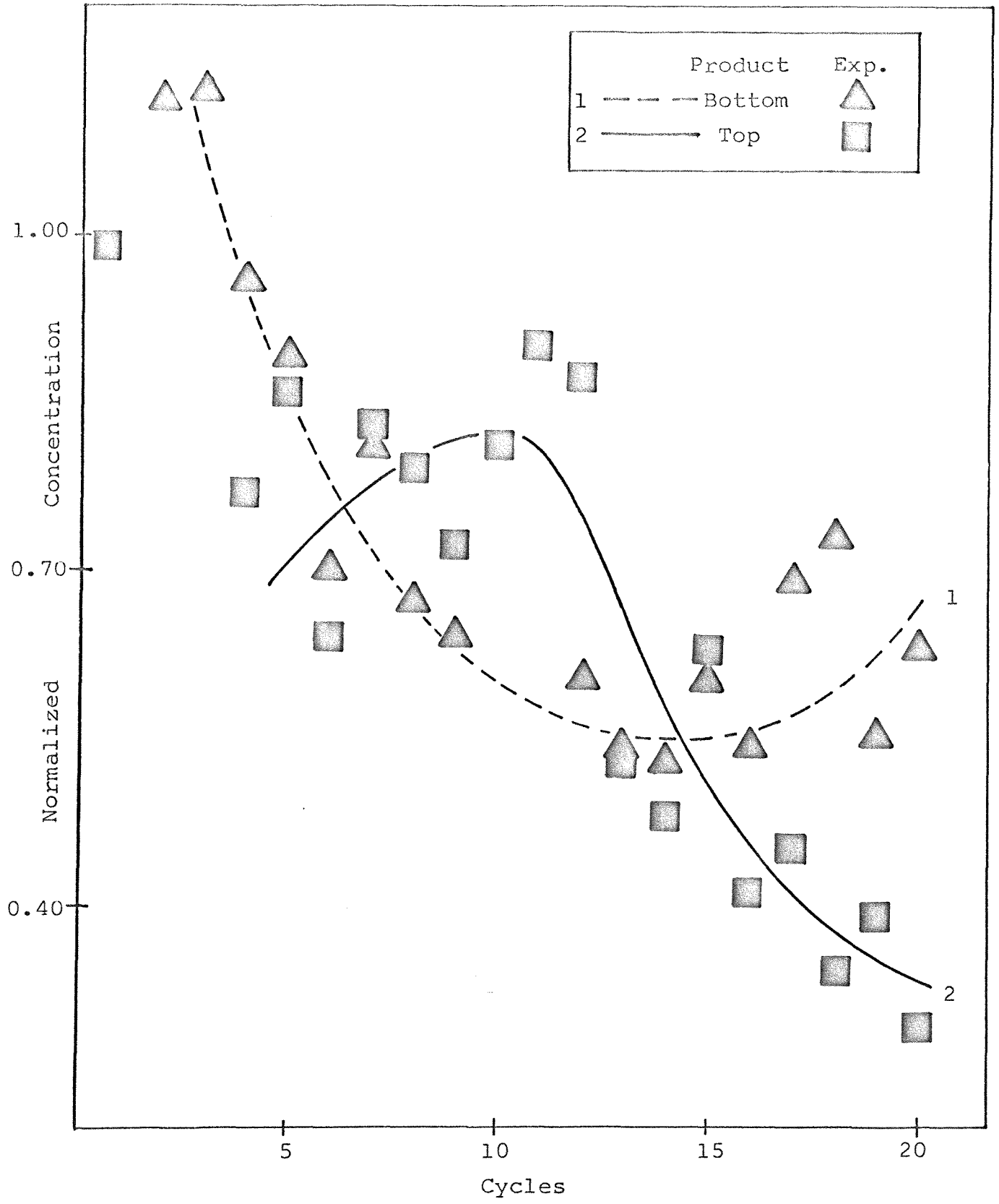


FIGURE 20

Plot of Normalized Top and Bottom Concentrations of Products verses Number of Cycles for Semi Continuous Run H-11

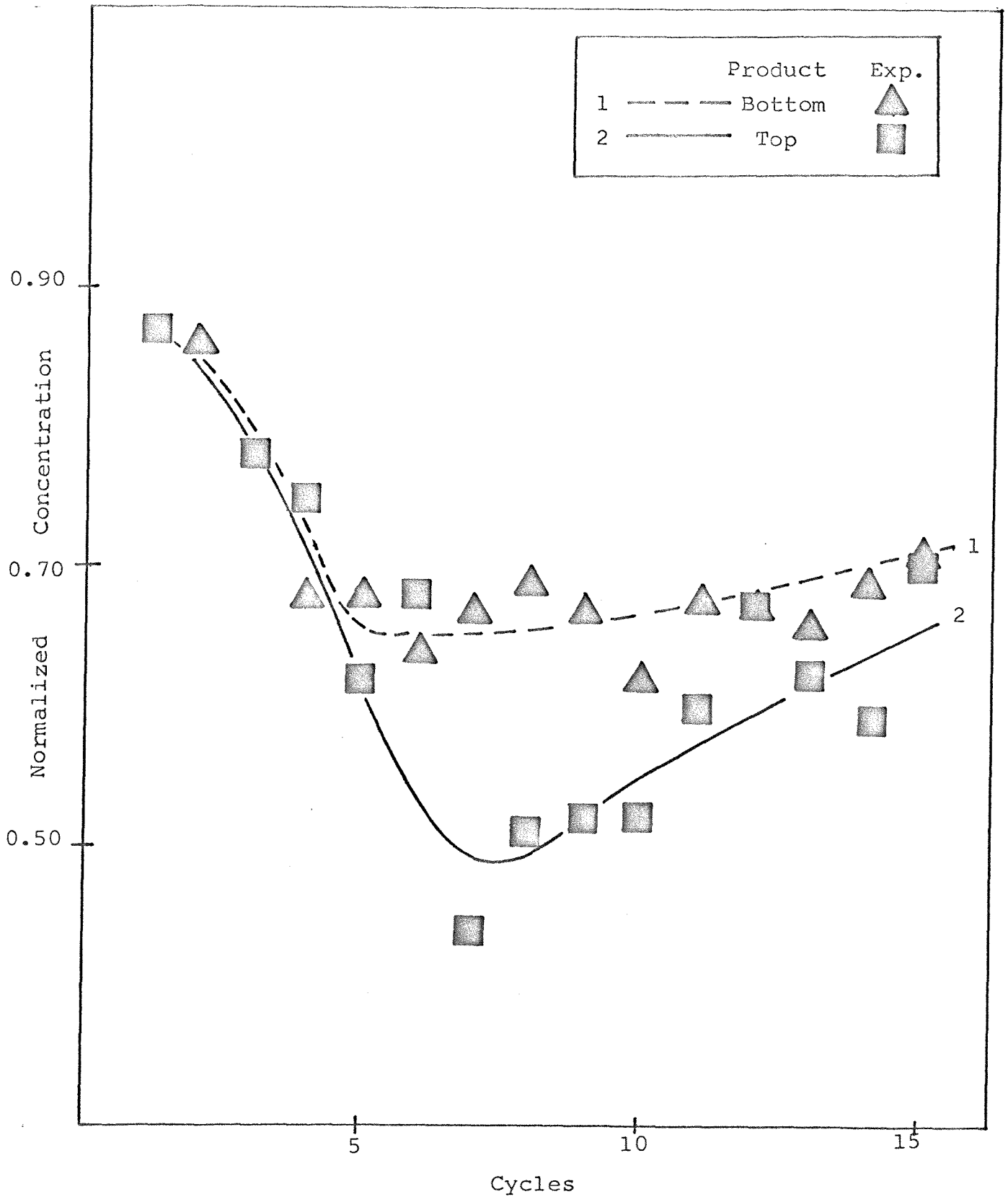


FIGURE 21

Plot of Normalized Top and Bottom Product Concentrations verses Number of Cycles for Semi Continuous Run H-12

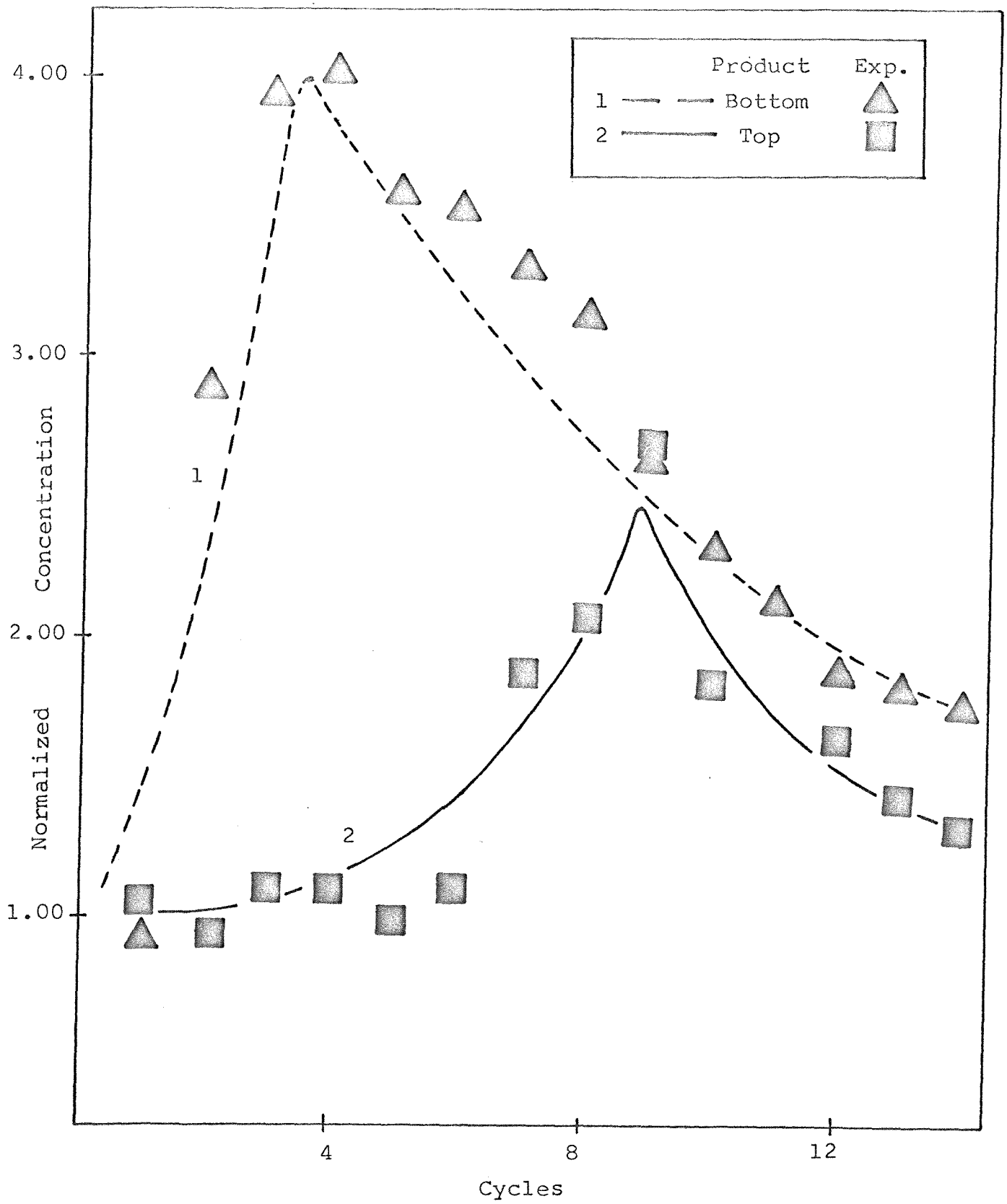


FIGURE 22

Plot of Normalized Top and Bottom Product Concentrations verses Number of Cycles for Semi Continuous Run H-13

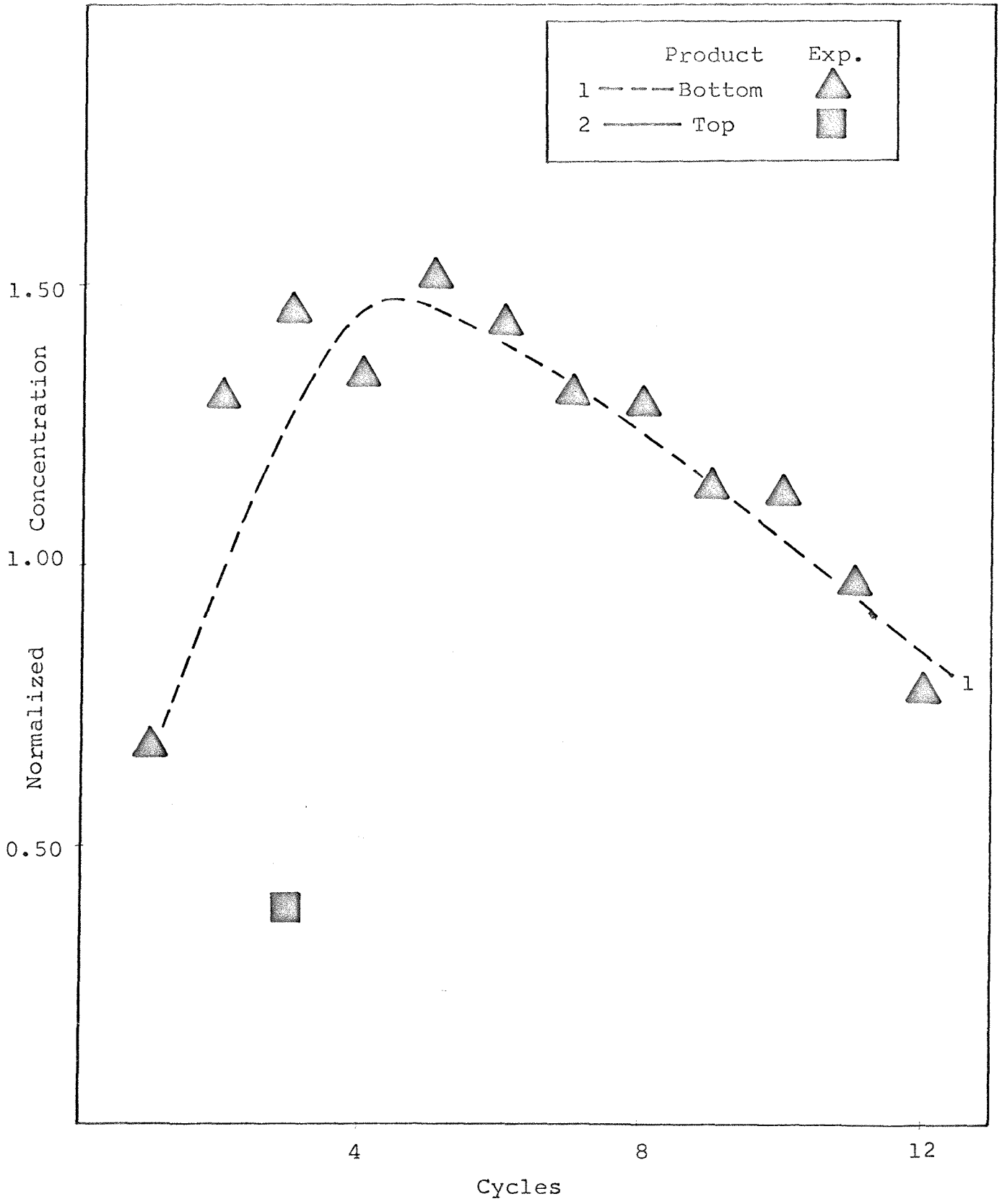


FIGURE 23

Plot of Normalized Top and Bottom Product Concentrations verses Number of Cycles for Semi Continuous Run H-14

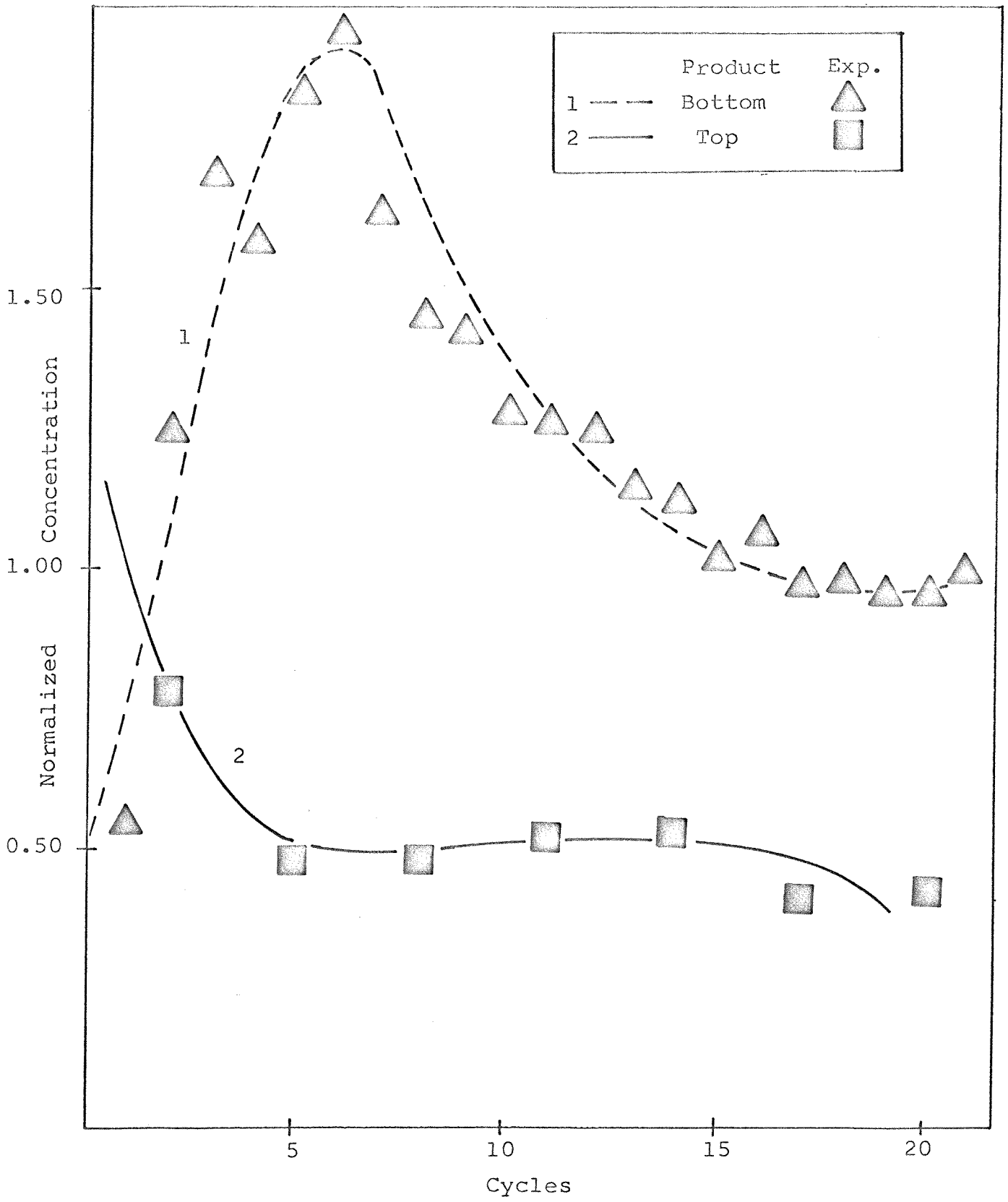


FIGURE 24

Plot of Normalized Top and Bottom Reservoir Concentrations verses Number of Cycles for Batch Parametric Pumping Run H-5

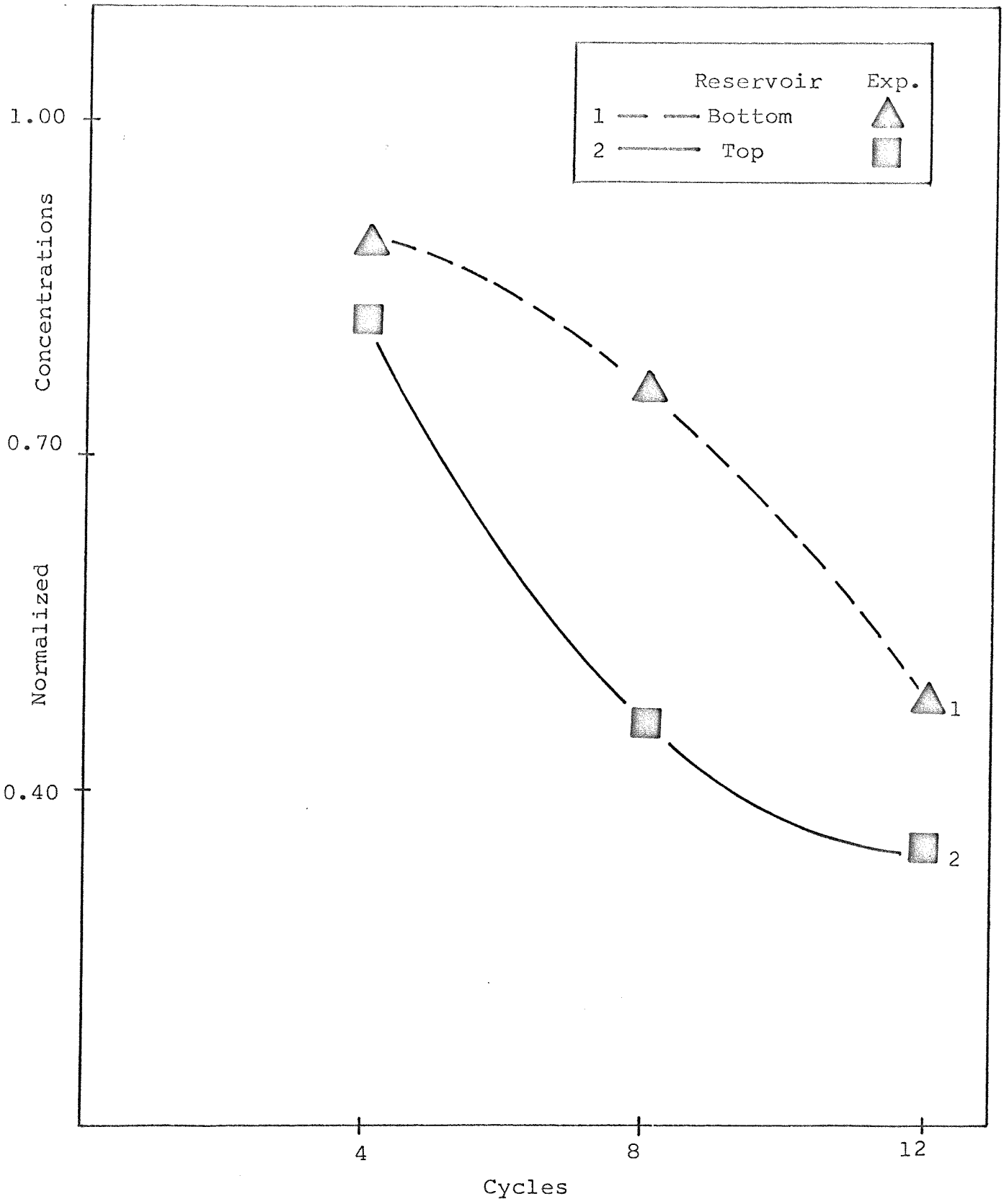


FIGURE 25

Plot of Separation Factor verses Number of Cycles for Semi Continuous pH Parametric Pumping Run H-14

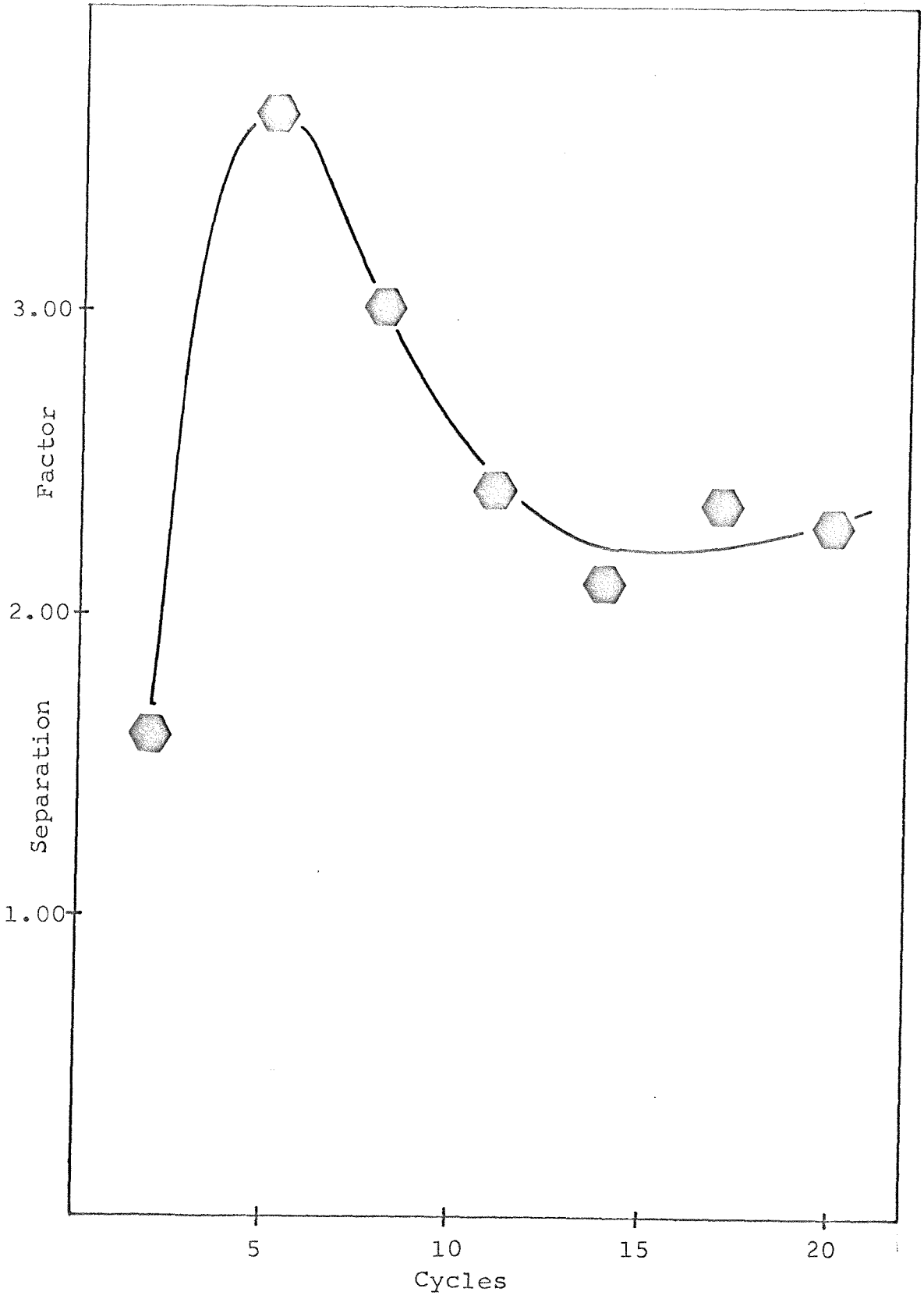
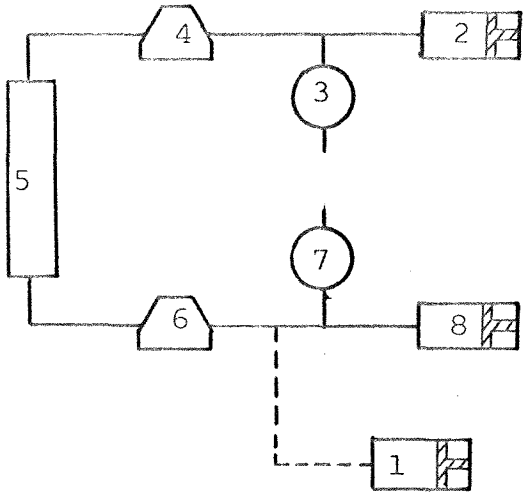


FIGURE 26
Types of Feed Location Used in pH Parametric Pumping,
Semi Continuous Runs H-6 through H-14

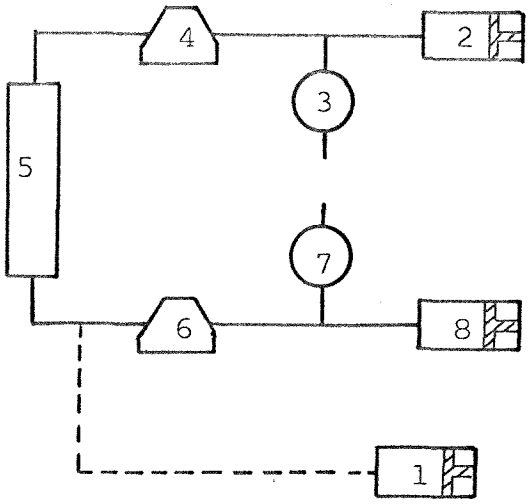
Type 1

Feed delivered to process between Bottom Hollow Fiber Device(pH=8.0) and Bottom Micrometric Sample Valve



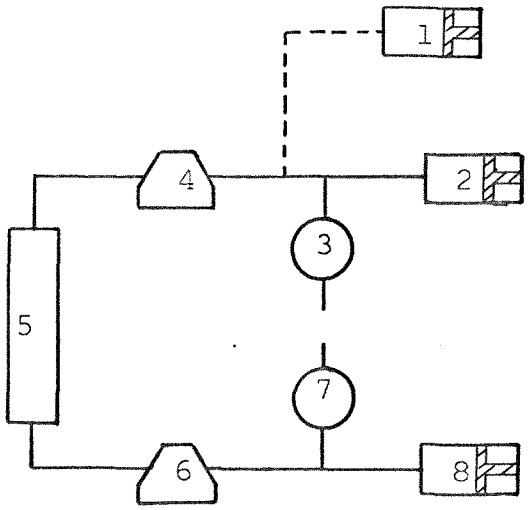
Type 2

Feed delivered to process between Bottom Hollow Fiber Device(pH=8.0) and bottom of Packed Jacketed Column



Type 3

Feed delivered to process between Top Hollow Fiber Device(pH=6.0) and Top Micrometric Sample Valve

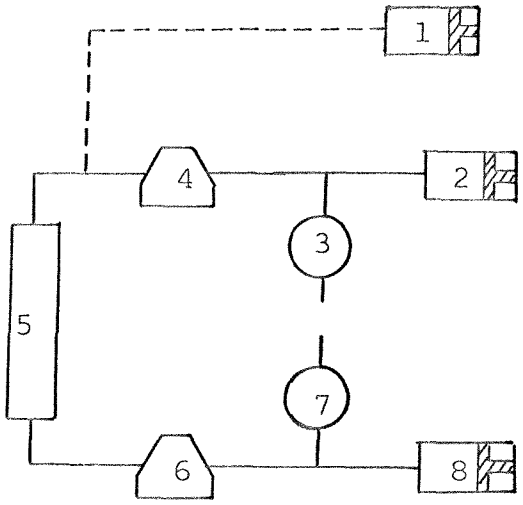


(Continued Next Page)

FIGURE 26, Continued

Type 4

Feed delivered to process between Top Hollow Fiber Device(pH=6.0) and top of Packed Jacketed Column



1 Feed Pump and Syringe
2 Top Reservoir Pump and Syringe(pH=6.0)
3 Top Micrometric Sample Valve
4 Top Hollow Fiber Device(pH=6.0)
5 Packed Jacketed Column
6 Bottom Hollow Fiber Device(pH=8.0)
7 Bottom Micrometric Sample Valve
8 Bottom Reservoir Pump and Syringe(pH=8.0)
-- Feed Line
—— Process Line

APPENDIX B - TABLES

TABLE 1

Sodium Phosphate Buffer Preparation
at Selected pH Values

A: 0.2 Molar solution of Monobasic Sodium Phosphate; prepared by dissolving 27.5 grams of NaH_2PO_4 in 1000 ml of De-Ionized Water.

B: 0.2 Molar solution of Dibasic Sodium Phosphate; prepared by dissolving 53.7 grams of $Na_2HPO_4 \cdot 7H_2O$ in 100 ml of De-Ionized H_2O, or 71.7 grams of $Na_2HPO_4 \cdot 1H_2O$ in 1000 ml of De-Ionized H_2O, or 28.0 grams of Na_2HPO_4 in 1000 ml of De-Ionized H_2O

pH	A (ml)	B (ml)
5.5	100.0	0.0
5.7	93.5	6.5
5.8	92.0	8.0
5.9	90.0	10.0
6.0	87.7	12.3
6.1	85.0	15.0
6.2	81.5	18.5
6.3	77.5	22.5
6.4	73.5	26.5
6.5	68.5	31.5
6.6	62.5	37.5
6.7	56.5	43.5
6.8	51.0	49.0
6.9	45.0	55.0
7.0	39.0	61.0
7.1	33.0	67.0
7.2	28.0	72.0
7.3	23.0	77.0
7.4	19.0	81.0
7.5	16.0	84.0
7.6	13.0	87.0
7.7	10.5	90.5
7.8	8.5	91.5
7.9	7.0	93.0
8.0	5.3	94.7
8.2	0.0	100.0

TABLE 2

CALIBRATION CURVE DATA
HEMOGLOBIN IN pH 8.0, (0.05 MOLAR)
PHOSPHATE BUFFER AT WAVELENGTH
403 MILLIMICRONS (VISIBLE SPECTRUM)

Concentration Wt. Percent Hemoglobin	Transmission	Absorbance
0.001	88.0	0.056
0.002	84.0	0.076
0.003	78.1	0.107
0.004	54.7	0.262
0.005	49.9	0.302
0.006	47.2	0.326
0.007	37.3	0.428
0.008	36.3	0.440
0.009	27.5	0.561
0.010	26.4	0.578
0.011	26.8	0.572
0.012	23.5	0.629
0.013	20.1	0.697
0.014	18.7	0.728
0.015	14.2	0.848
0.016	14.9	0.827
0.017	12.2	0.914
0.018	10.1	0.996
0.019	10.2	0.991
0.020	9.0	1.046

TABLE 3

EXPERIMENTAL CALIBRATION DATA
HEMOGLOBIN IN pH 7.0, (0.05 MOLAR)
PHOSPHATE BUFFER AT WAVELENGTH
403 MILLIMICRONS (VISIBLE SPECTRUM)

Concentration Wt. Percent Hemoglobin	Transmission	Absorbance
0.001	81.1	0.091
0.002	79.3	0.101
0.003	68.9	0.162
0.004	60.7	0.217
0.005	54.8	0.261
0.006	49.8	0.303
0.007	38.9	0.410
0.008	34.1	0.467
0.009	33.2	0.479
0.010	26.7	0.574
0.011	22.9	0.640
0.012	23.5	0.629
0.013	20.0	0.699
0.014	15.6	0.807
0.015	14.2	0.848
0.016	11.1	0.955
0.017	11.2	0.951
0.018	9.3	1.032
0.019	8.4	1.076
0.020	8.4	1.076

TABLE 4

CALIBRATION CURVE DATA
HEMOGLOBIN IN pH 6.0, (0.05 MOLAR)
PHOSPHATE BUFFER AT WAVELENGTH
403 MILLIMICRONS (VISIBLE SPECTRUM)

Concentration Wt. Percent Hemoglobin	Transmission	Absorbance
0.001	89.7	0.047
0.002	81.2	0.090
0.003	69.0	0.161
0.004	59.3	0.227
0.005	53.0	0.276
0.006	45.5	0.342
0.007	39.0	0.409
0.008	34.4	0.465
0.009	29.0	0.538
0.010	25.7	0.590
0.011	22.3	0.652
0.012	18.6	0.731
0.013	16.5	0.783
0.014	14.8	0.830
0.015	12.8	0.893
0.016	11.1	0.955
0.017	8.9	1.051
0.018	8.2	1.086
0.019	7.5	1.125
0.020	6.2	1.208

Table 5

Summary of Several Batch Process Parameters Used

Run	Q cc	Q cc/sec	Column Height cm	V_T cc	V_B cc	Total No. of Cycles
H-1	10.0	0.00833	10.5	5.0	5.0	8
H-2	10.0	0.00833	9.3	5.0	5.0	8
H-3	10.0	0.00833	9.6	5.0	5.0	7
H-4	10.0	0.00833	9.4	5.0	5.0	12
H-5	10.0	0.00833	11.0	5.0	5.0	8

Table 6 -

Summary of Remarks for Batch pH Parametric Pumping Runs

Run	Remarks
H-1	Run started with upward displacement from Bottom Reservoir. Top and Bottom Reservoirs were measured for Hemoglobin at end of 8th cycle. Packing prepared by expansion and equilibration in pH 8.0 phosphate buffer and Hemoglobin solution initially in Bottom Reservoir.
H-2	Run started with downward displacement from Top Reservoir. Top and Bottom Reservoirs were measured for Hemoglobin at end of 8th cycle. Packing prepared by expansion and equilibration in pH 6.0 phosphate buffer and Hemoglobin solution initially in Top Reservoir.
H-3	Run started with downward displacement from Top Reservoir. Top and Bottom Reservoirs were measured for Hemoglobin at end of 8th cycle. Packing prepared by expansion and equilibration in pH 8.0 phosphate buffer and Hemoglobin solution initially in Bottom Reservoir.
H-4	Run started with upward displacement from Bottom Reservoir. Top and Bottom Reservoris were measured for Hemoglobin at end of 4th, 8th and 12th cycles. Packing prepared by expansion and equilibration in pH 8.0 phosphate buffer and Hemoglobin solution initially in Bottom Reservoir.

(Continued Next Page)

Table 6 (Continued)

Run	Remarks
H-5	Run started with upward displacement from Bottom Reservoir. Top and Bottom Reservoirs were measured for Hemoglobin at end of 8th cycle. Packing prepared by expansion and equilibration in pH 6.0 buffer(phosphate) and Hemoglobin solution initially in Top Reservoir.

Table 7

Summary of Several Semi Continuous Process Parameters Used

Run	Figure Number	Q cc	Q cc/sec	Column Height cm	V_T cc	V_B cc	Total No. of Cycles	Feed Concentration	Feed Flow Rate cc/sec
H-6	15	10.0	0.00833	9.6	5.0	5.0	12	0.01 wt. % Hemoglobin in pH 6.0 buffer	0.00667
H-7	16	10.0	0.00833	11.4	5.0	5.0	20	0.01 wt. % Hemoglobin in pH 6.0 buffer	0.00667
H-8	17	12.0	0.00833	10.1	5.0	5.0	8	0.01 wt. % Hemoglobin in pH 6.0 buffer	0.00483
H-9	18	12.0	0.00833	11.1	5.0	5.0	15	0.01 wt. % Hemoglobin in pH 8.0 buffer	0.00483
H-10	19	15.0	0.00833	10.7	5.0	5.0	15	0.01 wt. % Hemoglobin in pH 8.0 buffer	0.00389
H-11	20	15.0	0.00833	10.1	5.0	5.0	20	0.01 wt. % Hemoglobin in pH 8.0 buffer	0.00389

Table 7 Continued

Summary of Several Semi Continuous Process Parameters Used

Run	Figure Number	Q cc	Q cc/sec	Column Height cm	V_T cc	V_B cc	Total No. of Cycles	Feed Concentration	Feed Flow Rate cc/sec
H-12	21	12.0	0.00833	8.0	5.0	5.0	14	0.01 wt. % Hemoglobin in pH 8.0 buffer	0.00278
H-13	22	12.0	0.00833	9.0	5.0	5.0	12	0.01 wt. % Hemoglobin in pH 8.0 buffer	0.00278
H-14	23	12.0	0.00833	8.5	5.0	5.0	21	0.01 wt. % Hemoglobin in pH 8.0 buffer	0.00278

Table 8

Summary of Several Semi Continuous Process Parameters Used

Run	Remarks
H-6	Top and bottom samples of approximately 4.0 cc are collected on up flow. Samples are analyzed without dilution. Feed is during up flow. Run started on down flow. Packing prepared by expansion and equilibriation in pH 6.0 phosphate buffer - Hemoglobin solution used for feed.
H-7	Top and bottom samples of approximately 4.0 cc are collected on up flow. Samples are analyzed without dilution. Feed is during up flow. Run started on down flow. Packing prepared by expansion and equilibriation in pH 6.0 phosphate buffer - Hemoglobin solution used for feed.
H-8	Top and bottom samples of approximately 3.5 cc are collected on down flow. Samples are analyzed without dilution. Feed is on down flow. Run started on up flow. Packing prepared by expansion and equilibriation in pH 6.0, 0.02 wt.% hemoglobin - phosphate buffer solution. After the packing was placed in the column and sealed off, 40 cc of pH 8.0 hemoglobin - phosphate buffer solution used for feed was pushed up from the bottom, through the column, at a rate of 0.69 cc/min.
H-9	Top and bottom samples of approximately 3.5 cc are collected on up flow.

(Continued Next Page)

Table 8 (Continued)

Run	Remarks
H-9	Samples are analyzed without dilution. Feed is on up flow. Run started on down flow. Packing prepared by expansion and equilibriation in pH 6.0 phosphate buffer. After the packing was sealed off in the column, 40 ml of pH 8.0 phosphate buffer - Hemoglobin solution, used for feed, was pushed from the bottom, through the column. The feed solution was fed in at a rate of 0.75 cc/min.
H-10	Top and bottom samples of approximately 3.5 cc are collected on up flow. Samples are analyzed without dilution. Feed is during up flow. Run started on down flow. Packing prepared as in H-9.
H-11	Top and bottom samples of approximately 3.5 cc are collected on up flow. Samples are analyzed without dilution. Feed is during up flow. Run started on down flow. Packing prepared as in H-6.
H-12	Top samples of approximately 1.0 cc and bottom samples of approximately 3.0 cc are collected on down flow. Top samples are analyzed with dilution. Bottom samples are analyzed without dilution. Feed is during down flow. Run started on up flow. Packing prepared by expansion and equilibriation in pH 6.0, 0.01 wt.% Hemoglobin - phosphate buffer solution. Approximately 3 hours later, after packing settled while in refrigerator,

(Continued Next Page)

Table 8 (Continued)

Run	Remarks
H-12	the top layer was poured off. 50 ml of pH 6.0, 0.01 wt.% Hemoglobin - phosphate buffer solution was added to the settled packing. The above procedure of decanting off the top layer and adding new solution after the packing settled was done three times. The final decanted packing was poured into the column, sealed off, and used in the run.
H-13	Top samples of approximately 1.0 cc and bottom samples of 3.0 cc are collected on up flow. Top sample analyzed is cumulative over a five cycle increment. Bottom sample analyzed is not cumulative or diluted. Feed is during up flow. Run started on down flow. Packing prepared as explained in Experimental section.
H-14	Top samples of approximately 1.0 cc and bottom samples of approximately 3.0 cc are collected on up flow. Top samples analyzed are cumulative over three cycle increments and not diluted. Bottom samples are not cumulative or diluted. Feed is during up flow. Run started on down flow. Packing prepared as explained in Experimental section.

Table 9

Concentration of Monobasic Sodium Phosphate and Dibasic Sodium Phosphate Solutions Used in Preparing Buffers Utilized in Each pH Parametric Pumping Run

Run	Concentration (Molarity)
Batch	
H-1	0.05
H-2	0.05
H-3	0.05
H-4	0.05
H-5	0.05
Semi Continuous	
H-6	0.05
H-7	0.05
H-8	0.05
H-9	0.05
H-10	0.05
H-11	0.05
H-12	0.10
H-13	0.10
H-14	0.10

Table 10

Feed Location Used in pH Parametric Pumping, Semi Continuous Runs H-6 through H-14

Run	Feed Location Type (See FIGURE 26 for explaination of Type number)
H-6	1
H-7	3
H-8	4
H-9	2
H-10	2
H-11	2
H-12	2
H-13	2
H-14	2

Table 11

Analyzation for pH Batch Parametric Pumping Runs

Run	Sample	pH	Trans. at 403 M	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
H-1	Reservoirs					
	B initial	8.0	34.4	0.463	0.0086	1.00
	T 8th cycle	6.0	45.2	0.345	0.0058	0.67
	B 8th cycle	8.0	28.6	0.544	0.0101	1.17
H-2	Reservoirs					
	T initial	6.0	24.3	0.614	0.0103	1.00
	T 8th cycle	6.0	92.6	0.033	0.0006	0.05
	B 8th cycle	8.0	47.8	0.321	0.0060	0.58
H-3	Reservoirs					
	T initial	8.0	27.3	0.564	0.0105	1.00
	T 8th cycle	6.0	36.5	0.438	0.0073	0.70
	B 8th cycle	8.0	29.0	0.536	0.0099	0.95

Continued Next Page

Table 11 Continued

Run	Sample	pH	Trans. at 403 M	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
H-4	Reservoirs					
	B initial	8.0	29.4	0.532	0.0099	1.00
	T 4th cycle	6.0	32.6	0.487	0.0081	0.82
	B 4th cycle	8.0	33.5	0.475	0.0088	0.89
	T 8th cycle	6.0	53.2	0.274	0.0046	0.46
	B 8th cycle	8.0	39.5	0.403	0.0075	0.76
	T 12th cycle	6.0	62.1	0.207	0.0035	0.35
	B 12th cycle	8.0	55.7	0.254	0.0047	0.48
H-5	Reservoirs					
	B initial	6.0	29.4	0.532	0.0089	1.00
	T 8th cycle	6.0	43.4	0.363	0.0061	0.69
	B 8th cycle	8.0	100.0	0.000	0.0000	0.00

Table 12

Analyzation For Semi-Continuous Run H-6

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	6.0	-	59.83	31.2	0.506	0.0090	1.00
Reservoirs							
T(12th cycle)	6.0	-	59.83	48.9	0.311	0.0052	0.58
B(12th cycle)	8.0	-	53.88	36.6	0.437	0.0081	0.90
Samples							
1B	8.0	3.5	53.88	40.2	0.305	0.0073	0.81
1T	6.0	3.5	59.83	81.3	0.090	0.0015	0.17
2B	8.0	4.0	53.88	49.7	0.304	0.0056	0.63
2T	6.0	3.8	59.83	80.2	0.096	0.0018	0.20
3B	8.0	3.9	53.88	32.7	0.486	0.0090	1.00
3T	6.0	3.8	59.83	59.3	0.227	0.0042	0.47
4B	8.0	3.9	53.88	40.0	0.398	0.0074	0.82
4T	6.0	3.9	59.83	78.0	0.108	0.0018	0.20
5B	8.0	3.7	53.88	37.1	0.430	0.0080	0.89
5T	6.0	3.7	59.83	73.0	0.137	0.0023	0.25
6B	8.0	3.8	53.88	38.6	0.413	0.0077	0.85

Table 12 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
6T	6.0	3.4	59.83	85.4	0.068	0.0011	0.13
7B	8.0	3.8	53.88	32.2	0.490	0.0091	1.01
7T	6.0	3.6	59.83	75.4	0.123	0.0021	0.23
8B	8.0	3.7	53.88	36.9	0.433	0.0080	0.89
8T	6.0	3.2	59.83	72.7	0.139	0.0023	0.26
9B	8.0	3.8	53.88	34.7	0.460	0.0085	0.95
9T	6.0	3.9	59.83	76.8	0.114	0.0019	0.21
10B	8.0	3.8	53.88	36.3	0.440	0.0082	0.91
10T	6.0	3.8	59.83	79.6	0.100	0.0017	0.19
11B	8.0	3.7	53.88	36.0	0.444	0.0082	0.92
11T	6.0	3.3	59.83	84.2	0.075	0.0013	0.14
12B	8.0	3.4	53.88	36.4	0.439	0.0081	0.91
12T	6.0	3.3	59,83	85.8	0.066	0.0011	0.12

Table 13

Analyzation For Semi-Continuous Run H-7

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	6.0	-	59.83	26.8	0.572	0.0096	1.00
Reservoirs							
T(20th cycle)	6.0	-	59.83	25.1	0.600	0.0100	1.05
B(20th cycle)	8.0	-	53.88	24.6	0.609	0.0113	1.18
Samples							
1B	8.0	3.7	53.88	41.5	0.382	0.0071	0.74
1T	-	-	-	-	-	-	-
2B	8.0	4.0	53.88	30.7	0.513	0.0095	1.00
2T	6.0	3.7	59.83	34.4	0.463	0.0077	0.81
3B	8.0	3.8	53.88	38.0	0.420	0.0078	0.82
3T	-	-	-	-	-	-	-
4B	-	-	-	-	-	-	-
4T	6.0	3.8	59.83	73.4	0.134	0.0022	0.23
5B	8.0	7.5	53.88	30.1	0.512	0.0095	0.99
5T	6.0	3.3	59.83	87.0	0.060	0.0010	0.10
6B	8.0	3.8	53.88	29.3	0.533	0.0099	1.03
6T	6.0	3.9	59.83	78.4	0.106	0.0018	0.19

Table 13 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
7B	8.0	4.0	53.88	27.8	0.556	0.0103	1.08
7T	6.0	3.7	59.83	84.2	0.075	0.0013	0.13
8B	8.0	3.7	53.88	24.4	0.613	0.0114	1.19
8T	6.0	3.4	59.83	87.2	0.059	0.0010	0.10
9B	8.0	3.9	53.88	29.2	0.535	0.0099	1.04
9T	6.0	3.9	59.83	32.9	0.483	0.0081	0.84
10B	8.0	3.9	53.88	27.9	0.554	0.0103	1.08
10T	6.0	3.9	59.83	39.2	0.407	0.0068	0.71
11B	8.0	3.4	53.88	23.7	0.625	0.0116	1.21
11T	6.0	3.9	59.83	66.9	0.175	0.0029	0.31
12B	8.0	3.8	53.88	29.2	0.535	0.0099	1.04
12T	6.0	3.5	59.83	66.3	0.178	0.0030	0.31
13B	8.0	3.5	53.88	28.7	0.542	0.0101	1.05
13T	6.0	3.5	59.83	47.6	0.322	0.0054	0.56
14B	8.0	3.4	53.88	28.0	0.553	0.0103	1.07
14T	6.0	3.2	59.83	25.8	0.588	0.0098	1.03
15B	8.0	4.2	53.88	27.6	0.559	0.0104	1.09
15T	6.0	3.5	59.83	47.1	0.327	0.0055	0.57

Table 13 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
16B	8.0	4.5	53.88	25.8	0.588	0.0109	1.14
16T	6.0	3.8	59.83	48.9	0.311	0.0052	0.54
17B	8.0	3.5	53.88	27.6	0.559	0.0104	1.09
17T	6.0	3.3	59.83	61.1	0.214	0.0036	0.37
18B	8.0	3.4	53.88	25.8	0.588	0.0109	1.14
18T	6.0	3.0	59.83	60.6	0.218	0.0036	0.38
19B	8.0	3.5	53.88	29.5	0.530	0.0098	1.03
19T	6.0	3.2	59.83	66.2	0.179	0.0030	0.31
20B	8.0	3.7	53.88	26.0	0.585	0.0109	1.14
20T	6.0	3.8	59.83	51.4	0.289	0.0048	0.51

Table 14

Analyzation For Semi-Continuous Run H-8

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	6.05	-	59.45	27.1	0.567	0.0095	1.00
Reservoirs							
T(8th cycle)	6.35	-	58.05	48.5	0.316	0.0054	0.57
B(8th cycle)	7.65	-	54.65	68.2	0.166	0.0030	0.32
Samples							
1B	-	-	-	-	-	-	-
1T	6.50	3.4	57.55	43.5	0.362	0.0063	0.66
2B	7.30	3.2	55.40	71.5	0.146	0.0026	0.28
2T	-	-	-	-	-	-	-
3B	7.50	3.4	54.95	88.0	0.056	0.0010	0.11
3T	6.55	3.3	57.37	41.6	0.381	0.0060	0.70
4B	7.45	3.2	55.07	70.8	0.150	0.0027	0.29
4T	6.10	3.3	59.15	57.7	0.239	0.0040	0.42
5B	7.55	0.9	54.85	-	-	-	-
5T	6.05	2.0	59.45	-	-	-	-
6B	-	-	-	-	-	-	-
6T	6.10	3.2	59.15	71.3	0.147	0.0025	0.26

Table 14 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
7B	-	-	-	-	-	-	-
7T	6.10	3.4	59.15	66.9	0.175	0.0030	0.31
8B	7.60	3.2	54.75	69.1	0.161	0.0029	0.31
8T	-	-	-	-	-	-	-

Table 15

Analyzation For Semi-Continuous Run H-9

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	8.10	-	53.70	33.3	0.478	0.0089	1.00
Reservoirs							
T(15th cycle)	6.40	-	57.82	54.5	0.264	0.0046	0.51
B(15th cycle)	7.95	-	54.00	39.5	0.403	0.0075	0.66
Samples							
1B	-	-	-	-	-	-	-
1T	-	-	-	-	-	-	-
2B	7.90	3.2	54.10	37.1	0.431	0.0080	0.90
2T	7.50	3.3	54.96	30.3	0.519	0.0094	1.06
3B	-	0.6	-	-	-	-	-
3T	6.80	3.6	56.63	41.5	0.382	0.0067	0.76
4B	7.90	4.0	54.10	34.6	0.461	0.0085	0.96
4T	6.75	3.3	56.76	30.9	0.510	0.0090	1.01
5B	8.00	3.4	53.90	35.5	0.450	0.0083	0.94
5T	6.50	3.8	57.52	48.5	0.314	0.0055	0.61
6B	7.95	3.4	54.00	36.9	0.433	0.0080	0.90
6T	6.40	3.2	57.82	41.2	0.385	0.0067	0.75
7B	7.95	3.2	54.00	37.3	0.428	0.0079	0.89

Table 15 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
7T	6.15	3.3	58.87	45.4	0.343	0.0058	0.65
8B	7.95	3.6	54.00	34.3	0.465	0.0086	0.97
8T	6.35	3.3	58.05	50.8	0.294	0.0051	0.57
9B	8.00	3.8	53.90	41.1	0.386	0.0072	0.80
9T	6.50	3.3	57.52	65.4	0.184	0.0032	0.36
10B	7.95	3.3	54.00	37.7	0.424	0.0079	0.88
10T	6.45	3.4	57.68	54.4	0.264	0.0046	0.51
11B	8.00	2.9	53.90	38.9	0.410	0.0076	0.85
11T	6.40	3.8	57.82	54.6	0.263	0.0045	0.51
12B	7.95	3.0	54.00	41.0	0.387	0.0072	0.81
12T	6.45	3.4	57.68	51.9	0.285	0.0049	0.56
13B	7.90	2.7	54.10	42.4	0.373	0.0069	0.77
13T	6.40	3.2	57.82	60.4	0.219	0.0038	0.43
14B	7.95	3.0	54.00	41.4	0.303	0.0071	0.80
14T	6.35	3.0	58.05	65.6	0.183	0.0032	0.35
15B	7.95	2.8	54.00	41.9	0.378	0.0070	0.79
15T	6.35	3.1	58.05	65.7	0.182	0.0031	0.35

Table 16

Analyzation For Semi-Continuous Run H-10

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	8.05	-	53.80	33.3	0.478	0.0089	1.00
Reservoirs							
T(15th cycle)	8.05	-	53.80	46.2	0.335	0.0062	0.70
B(15th cycle)	6.45	-	57.68	29.5	0.530	0.0092	1.03
Samples							
1B	-	-	-	-	-	-	-
1T	7.40	4.6	55.17	37.3	0.428	0.0078	0.87
2B	7.65	3.4	54.63	38.3	0.417	0.0076	0.86
2T	-	-	-	-	-	-	-
3B	-	-	-	-	-	-	-
3T	7.10	3.5	55.87	40.8	0.389	0.0070	0.78
4B	7.80	3.3	54.32	47.2	0.326	0.0060	0.68
4T	6.60	4.5	57.42	41.2	0.385	0.0067	0.75
5B	7.82	4.1	54.27	47.0	0.328	0.0060	0.68
5T	6.60	3.2	57.42	48.1	0.318	0.0055	0.62
6B	7.75	3.5	54.42	49.1	0.309	0.0057	0.64
6T	6.45	3.3	57.70	44.6	0.351	0.0061	0.68
7B	8.20	3.7	53.55	48.2	0.317	0.0057	0.67

Table 16 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
7T	6.50	3.6	57.52	59.6	0.225	0.0039	0.44
8B	7.80	3.8	54.32	46.6	0.332	0.0061	0.69
8T	6.40	3.2	57.87	54.7	0.262	0.0045	0.51
9B	7.90	3.8	54.10	47.9	0.320	0.0059	0.67
9T	6.35	4.0	58.05	53.9	0.268	0.0046	0.52
10B	7.80	2.8	54.32	50.3	0.298	0.0055	0.62
10T	6.45	3.2	57.70	53.8	0.269	0.0047	0.52
11B	7.80	3.4	45.32	47.2	0.326	0.0060	0.68
11T	6.45	3.4	57.70	49.1	0.309	0.0054	0.60
12B	7.80	3.4	54.32	47.6	0.322	0.0059	0.67
12T	6.45	3.3	57.70	45.1	0.346	0.0060	0.67
13B	7.90	3.4	54.10	48.0	0.319	0.0059	0.66
13T	6.45	3.1	57.70	47.9	0.320	0.0055	0.62
14B	7.95	3.6	54.00	46.8	0.330	0.0061	0.69
14T	6.45	3.2	57.70	49.7	0.304	0.0053	0.59
15B	8.00	3.2	53.90	45.9	0.338	0.0063	0.71
15T	6.45	3.5	57.70	43.7	0.360	0.0062	0.70

Table 17

Analyzation For Semi-Continuous Run H-11

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	8.10	-	53.70	34.1	0.467	0.0087	1.00
Reservoirs T(20th cycle)	-	-	-	-	-	-	-
B(20th cycle)	-	-	-	-	-	-	-
Samples 1B	-	-	-	-	-	-	-
1T	7.95	3.4	54.00	34.1	0.467	0.0086	0.99
2B	8.00	3.5	53.90	29.7	0.527	0.0098	1.12
2T	-	-	-	-	-	-	-
3B	7.95	3.3	54.00	29.6	0.529	0.0098	1.13
3T	6.35	3.2	58.05	69.3	0.159	0.0027	0.31
4B	7.95	3.2	54.00	35.2	0.453	0.0084	0.96
4T	6.45	2.9	57.70	41.3	0.384	0.0067	0.77
5B	8.00	3.4	53.90	38.4	0.416	0.0077	0.89
5T	6.40	3.6	57.87	37.1	0.431	0.0074	0.86
6B	7.95	3.5	54.00	46.7	0.331	0.0061	0.70
6T	6.35	3.4	58.05	47.4	0.324	0.0056	0.64

Table 17 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
7B	8.00	3.6	53.90	41.5	0.382	0.0071	0.81
7T	6.35	3.2	58.05	37.9	0.421	0.0073	0.83
8B	7.90	3.6	54.10	48.3	0.316	0.0058	0.67
8T	6.35	3.4	58.05	40.1	0.397	0.0068	0.79
9B	7.95	3.2	54.00	50.0	0.301	0.0056	0.64
9T	6.30	4.0	58.23	43.3	0.364	0.0063	0.72
10B	7.95	2.2	-	-	-	-	-
10T	6.35	3.6	58.05	38.8	0.411	0.0071	0.81
11B	-	-	-	-	-	-	-
11T	6.35	3.5	58.05	35.1	0.455	0.0078	0.90
12B	7.90	3.0	54.10	52.2	0.282	0.0052	0.60
12T	6.50	3.6	57.52	36.7	0.435	0.0076	0.87
13B	7.90	3.0	54.10	55.4	0.256	0.0047	0.54
13T	6.25	3.1	58.43	53.8	0.269	0.0046	0.53
14B	8.00	3.4	53.90	56.2	0.250	0.0046	0.53
14T	6.35	3.2	58.05	57.1	0.243	0.0042	0.48
15B	7.95	2.9	54.00	52.0	0.284	0.0053	0.60
15T	6.35	4.3	58.05	48.1	0.318	0.0055	0.63

Table 17 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
16B	7.90	3.4	54.10	55.8	0.253	0.0047	0.54
16T	6.40	3.1	57.87	62.3	0.206	0.0036	0.41
17B	8.00	3.2	53.90	47.3	0.325	0.0060	0.69
17T	6.30	3.2	58.23	59.0	0.229	0.0039	0.45
18B	8.00	3.2	53.90	45.7	0.340	0.0063	0.73
18T	6.35	3.1	58.05	67.2	0.173	0.0030	0.34
19B	7.95	3.1	54.00	54.9	0.260	0.0048	0.55
19T	6.30	3.2	58.23	64.6	0.190	0.0033	0.38
20B	7.95	3.4	54.00	50.6	0.296	0.0055	0.63
20T	6.30	2.9	58.23	71.5	0.146	0.0025	0.29

Table 18

Analyzation For Semi-Continuous Run H-12

Sample	pH	Prod. cc.	Dillution Factor	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	8.00	-	-	53.90	34.7	0.460	0.0085	1.00
Reservoirs								
T(14th cycle)	6.10	-	-	59.13	23.0	0.638	0.0108	1.26
B(14th cycle)	7.90	-	-	54.10	15.4	0.812	0.0150	1.76
Samples								
1B	7.05	3.1	-	56.00	36.2	0.441	0.0079	0.92
1T	7.00	0.9	3.33	56.12	70.4	0.152	0.0090	1.06
2B	7.15	3.1	-	55.75	4.2	1.377	0.0247	2.89
2T	7.00	1.8	1.67	56.12	53.9	0.268	0.0080	0.93
3B	7.40	3.6	-	55.18	1.4	1.854	0.0336	3.94
3T	7.00	0.2	15.00	56.12	92.2	0.035	0.0094	1.10
4B	7.55	3.0	-	54.85	1.3	1.886	0.0344	4.03
4T	7.00	0.4	7.50	56.12	85.0	0.071	0.0095	1.11
5B	7.60	2.7	-	54.74	2.1	1.678	0.0307	3.59
5T	7.00	0.5	6.00	56.12	83.5	0.078	0.0083	0.98
6B	7.80	2.9	-	54.32	2.3	1.638	0.0302	3.53
6T	7.00	1.0	3.00	56.12	66.8	0.175	0.0094	1.10

Table 18 Continued

Sample	pH	Prod. cc.	Dillution Factor	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
7B	7.80	3.1	-	54.32	2.9	1.538	0.0283	3.32
7T	7.00	0.9	3.33	56.12	53.8	0.269	0.0160	1.87
8B	7.90	2.9	-	54.10	3.5	1.456	0.0269	3.15
8T	7.00	1.1	2.73	56.12	43.4	0.363	0.0177	2.07
9B	7.90	2.9	-	54.10	5.7	1.217	0.0225	2.64
9T	7.00	1.0	3.00	56.12	37.6	0.425	0.0227	2.66
10B	8.00	3.1	-	53.90	8.8	1.056	0.0196	2.30
10T	7.00	0.3	10.00	56.12	81.6	0.088	0.0157	1.84
11B	7.85	4.2	-	54.21	10.3	0.987	0.0182	2.13
11T	-	-	-	-	-	-	-	-
12B	7.85	3.0	-	54.21	13.6	0.866	0.0160	1.87
12T	7.00	0.7	4.29	56.12	65.8	0.182	0.0139	1.63
13B	7.90	2.8	-	54.10	14.5	0.839	0.0155	1.82
13T	7.00	1.0	3.00	56.12	59.0	0.229	0.0122	1.43
14B	7.90	3.0	-	54.10	15.5	0.810	0.0150	1.75
14T	7.00	0.7	4.29	56.12	71.5	0.146	0.0112	1.31

Table 19

Analyzation For Semi-Continuous Run H-13

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	7.95	-	54.00	30.9	0.510	0.0094	1.00
Reservoirs							
T(12th cycle)	6.00	-	59.82	50.5	0.279	0.0047	0.49
B(12th cycle)	7.60	-	54.74	37.6	0.425	0.0078	0.82
Samples							
1B	7.00	3.0	56.12	43.9	0.358	0.0064	0.68
1T	*	0.4	*	*	*	*	*
2B	7.20	2.9	55.63	20.7	0.684	0.0123	1.30
2T	*	0.9	*	*	*	*	*
3B	7.40	2.7	55.18	17.3	0.762	0.0138	1.46
3T	6.05	7.2	59.42	60.6	0.218	0.0037	0.39
4B	7.60	2.7	54.74	20.2	0.692	0.0126	1.34
4T	*	2.0	*	*	*	*	*
5B	7.60	2.5	54.74	16.3	0.788	0.0144	1.52
5T	*	2.5	*	*	*	*	*

* Top samples of cycles 1 through 5 are combined for concentration and pH analysis. See <3T> for average concentration and average pH values.

Table 19 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
6B	7.60	2.8	54.74	18.2	0.740	0.0135	1.43
6T	-	-	-	-	-	-	-
7B	7.75	3.0	54.42	21.3	0.672	0.0123	1.31
7T	-	-	-	-	-	-	-
8B	7.80	3.5	54.32	21.9	0.660	0.0122	1.29
8T	-	-	-	-	-	-	-
9B	7.80	3.0	54.32	28.8	0.541	0.0100	1.05
9T	-	-	-	-	-	-	-
10B	7.75	3.3	54.42	30.4	0.517	0.0095	1.01
10T	-	-	-	-	-	-	-
11B	7.60	3.9	54.74	31.3	0.504	0.0092	0.97
11T	-	-	-	-	-	-	-
12B	7.80	3.3	54.32	40.0	0.398	0.0073	0.78
12T	-	-	-	-	-	-	-

Table 20

Analyzation For Semi-Continuous Run H-14

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
Feed	8.00	-	53.90	38.7	0.412	0.0076	1.00
Reservoirs							
T(21th cycle)	6.05	-	59.42	54.5	0.264	0.0044	0.58
B(21th cycle)	7.90	-	54.10	40.0	0.398	0.0074	0.96
Sample							
1B	6.95	2.8	56.25	57.8	0.238	0.0042	0.55
1T	*	0.9	*	*	*	*	*
2B	7.05	2.9	55.98	29.1	0.536	0.0096	1.25
<2T>	6.00	1.2	59.82	43.9	0.358	0.0060	0.78
3B	7.30	2.9	55.40	19.1	0.719	0.0130	1.70
3T	*	0.8	*	*	*	*	*
4B	7.50	2.9	54.97	21.7	0.664	0.0121	1.58
4T	*	1.0	*	*	*	*	*
5B	7.60	2.8	54.75	16.8	0.775	0.0142	1.85
<5T>	6.00	1.1	59.82	60.5	0.218	0.0036	0.48
6B	7.65	2.9	54.64	15.2	0.818	0.0150	1.96

Samples 1T through 3T and 4T through 6T are combined for concentration analysis. See <2T> and <5T> for average concentration and pH values.

Table 20 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
6T	*	0.9	*	*	*	*	*
7B	7.75	2.8	54.42	21.1	0.676	0.0124	1.63
7T	*	0.9	*	*	*	*	*
8B	7.65	2.8	54.64	24.9	0.604	0.0111	1.45
<8T>	6.00	1.1	59.82	60.4	0.219	0.0037	0.48
9B	7.85	2.9	54.21	25.9	0.587	0.0108	1.42
9T	*	0.8	*	*	*	*	*
10B	7.90	2.8	54.10	29.5	0.530	0.0098	1.28
10T	*	1.0	*	*	*	*	*
11B	7.90	2.7	54.10	30.0	0.523	0.0097	1.26
<11T>	6.10	1.0	59.13	58.3	0.234	0.0040	0.52
12B	7.90	2.8	54.10	30.8	0.512	0.0095	1.24
12T	*	1.3	*	*	*	*	*
13B	7.90	2.9	54.10	33.6	0.473	0.0087	1.14
13T	*	1.0	*	*	*	*	*
14B	7.90	2.8	54.10	34.4	0.464	0.0086	1.12

Samples 4T through 6T, 7T through 9T, 10T through 12T, and 13T through 15T are combined for concentration analysis and pH analysis. See <5T>, <8T>,<11T>, and <14T> respectively for average concentration and pH values.

Table 20 Continued

Sample	pH	Prod. cc.	Slope	Trans. at 403 Mμ	Absb.	Sample Conc. wt. %	Sample Conc. / Feed Conc.
<14T>	6.05	1.0	59.42	57.6	0.240	0.0040	0.53
15B	7.95	2.9	54.00	38.1	0.419	0.0078	1.02
15T	*	1.0	*	*	*	*	*
16B	7.90	3.0	54,10	36.6	0.437	0.0081	1.06
16T	*	1.1	*	*	*	*	*
17B	7.90	2.8	54.10	39.6	0.402	0.0074	0.97
<17T>	6.00	1.4	59.82	65.0	0.187	0.0031	0.41
18B	7.95	3.0	54.00	39.4	0.405	0.0075	0.98
18T	*	1.1	*	*	*	*	*
19B	7.90	3.0	54.10	40.1	0.397	0.0073	0.96
19T	*	1.0	*	*	*	*	*
20B	7.90	3.2	54.10	40.0	0.398	0.0074	0.96
<20T>	6.00	1.3	59.82	64.0	0.194	0.0032	0.42
21B	7.95	3.2	54.00	39.1	0.408	0.0076	0.99
21T	*	0.8	*	*	*	*	*

* Samples 13T through 15T, 16T through 18T, 19T through 21T are combined for concentration analysis and pH analysis. See<14T>,<17T>,<20T>, respectively for average concentration and pH values.

Table 21

Separation Facotrs for
Semi Continuous Run H-14

Cycle	Top Sample Average Concentration wt. %	Bottom Sample Average Concentration wt. %	Separation Factor
1	0.0078	0.0125	1.60
5	0.0048	0.0185	3.85
8	0.0048	0.0145	3.02
11	0.0052	0.0126	2.42
14	0.0053	0.0112	2.11
17	0.0041	0.0097	2.37
20	0.0042	0.0096	2.29