Copyright Warning & Restrictions

The copyright law of the United States (Title 17, United States Code) governs the making of photocopies or other reproductions of copyrighted material.

Under certain conditions specified in the law, libraries and archives are authorized to furnish a photocopy or other reproduction. One of these specified conditions is that the photocopy or reproduction is not to be "used for any purpose other than private study, scholarship, or research." If a, user makes a request for, or later uses, a photocopy or reproduction for purposes in excess of "fair use" that user may be liable for copyright infringement,

This institution reserves the right to refuse to accept a copying order if, in its judgment, fulfillment of the order would involve violation of copyright law.

Please Note: The author retains the copyright while the New Jersey Institute of Technology reserves the right to distribute this thesis or dissertation

Printing note: If you do not wish to print this page, then select "Pages from: first page # to: last page #" on the print dialog screen

EFFECT OF CONCENTRATION AND pH ON PARAMETRIC PUMPING SEPARATIONS

BY

TSU-KUN S. HSIEH

A THESIS

PRESENTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE IN CHEMICAL ENGINEERING

AT

NEW JERSEY INSTITUTE OF TECHNOLOGY

Newark, New Jersey

October, 1978

APPROVAL OF THESIS

EFFECT OF CONCENTRATION AND pH ON PARAMETRIC PUMPING SEPARATIONS

BY

TSU-KUN S. HSIEH

FOR

DEPARTMENT OF CHEMICAL ENGINEERING

NEW JERSEY INSTITUTE OF TECHNOLOGY

BY

FACULTY COMMITTEE

APPROVED: _____

_____

_____

NEWARK, NEW JERSEY

OCTOBER, 1978

ABSTRACT

The technique of parametric pumping represents a new and valuable development in the separation science of proteins. Many conventional protein separation are often processed batchwise. However, parametric pumping offers the possibility of continuous processing; the continuous process provides both the advantages of decreasing the processing time and of minimizing degradation.

Preliminary step testing experiments were conducted to study the equilibrium of protein between the stationary and mobile phase of the sephadex ion exchanger.

It was the purpose of this research to study the feasibility of operating a semi-continuous parametric pump with a sephadex ion exchanger for the separation of a man-made protein mixture of albumin and hemaglobin. Factors affecting separation performance, such as concentration and pH were investigated experimentally.

It was found that an increase in the buffer concentration and, hence, in the sodium counter ion concentration results in a shifting of position of equilibrium involving the ion exchanger. This results in less uptake of hemoglobin and high concentration in the top product stream. The albumin concentration is unaffected by the pumping

operation. In both top and bottom product streams, it remains essentially at the feed concentration.

It also been concluded that change of pH towards the isoelectric point of the protein renders it neutral and thus reduces interaction with the ion exchanger, less separation is found at a smaller spread of pH values. A larger spread of pH values will cause significant increase in separation.

ACKNOWLEDGEMENTS

I wish to extend my gratitude to my advisor Professor H. T. Chen for his excellent guidance and patience. I am also indebted so much to both Dr. Bart and Dr. Greenstein for their valuable suggestions. Thanks to many faculty members who give me encourgements.

Some way in particular, I deeply appreciate my parents for their continuous spiritual support, understanding and encourgements.

SCOPE

A. Previous Developments

The late R.H. Wilhelm of Princeton University first introduced the name "Parametric Pumping" to the separation science in 1966. Ever since then, much experimental and theoretical work has been done on thermal and heatless parametric pumps. Included are the contributions of Wilhelm et al. (1966, 1968), Wilhelm and Sweed (1968), Pigford et al. (1969), Aris (1969), Waver and Hamrin (1974) and Chen et al. (1971, 1973, 1974a, 1974b, 1975, 1976a, 1976b). By contrast, very little work has been done on pH parametric pumping. Sabadell and Sweed (1970) used pH changes to concentrate aqueous solutions of K^+ and N_a^+ using ion exchange resins. Shaffer and Hamrin (1975) studied trypsin concentration by affinity chromatography and parametric pumping.

B. Relevance of the Work to the Profession

The feasibility of the application of parametric pumping process to the separation of proteins will provide a bright prospect to the extensive application of proteins, because the parametric pumping process has several advantages over conventional methods:

(a) Parametric pumping offers the possibility of continuous processing, thereby tending to minimize both processing time and degradation. To prevent protein from

degradation is certainly of prime importance.

(b) No regeneration chemicals are needed for the continuous process, and no regenerant will contaminate the product. With regard to the cost consideration, parametric pumping process is certainly preferable when compared to other methods in which the regeneration chemicals are needed.

(c) Control problems for the continuous process may be simpler than those competing batch processes.

C. Objective

The experimental work has been carried out in two parts:

A. Step Testing Experiments

(1) Study of Equilibrium of Hemoglobin between the Mobile and Stationary Phase of Sephadex Ion Exchanger

(2) Study of Equilibrium of Albumin between the Mobile and Stationary phase of Sephadex Ion Exchanger

B. Semi-continuous pH Parametric Pumping Experiments

The data from part A (1) indicated both pH and concentration would influence the equilibrium of protein (hemoglobin) between the mobile and stationary phase of the ion exchanger and thus affect the separation performance.

TABLE CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

(1) Literature Review

A. Parametric Pumping. In 1966, the inventor of the batch pump, the late R.H. Wilhelm of Princeton University firstly introduced the "Parametric Pumping" to the separation science. The principle mainly involved the application of dynamic adsorption particles for separating components of a homogeneous mixture. The central concept of parametric pumping comprises the coupling of alternating variable and composition field of alternating flow direction in some defined fashion. Since then, the process has been of great interest to many research investigators (some references have been cited in scope section). This is due not only to the versatility of the process but also to the feasibility of employing a small set-up to achieve very high separation factors with continuous operation.

B. Equilibrium Theory of Parametric Pumping. Very large separation factors have been obtained by using cycling flow of a binary mixture upward and downward through a column containing a fixed bed of solid adsorbant which is alternately heated and cooled. The theory of such separation is based on the assumption of local equilibrium between the stationary and mobile phase. The origin of the separation is the ability of the stationary phase to store

solute deposits on it by the fluid flow into the column from a reservoir containing enriched mixture. Pigford et al. (1969) originated an important and simple equilibrium theory and derived mathematical expressions for the performance of the batch pump.

C. Semi-continuous Parametric Pump. Chen, Reiss and Hill (1973) developed the mathematical expression for the concentration transients in the semi-continuous pump. In these expressions, each reservoir is assumed to have a dead volume of arbitrary size. Solutions are given for the region of infinite separation factors, that is, those for which the reservoir displacement volume or the flow rate of the dilute product stream is excessive. Experimental data for the concentration transients obtained nominally in the region of infinite separation factors agree reasonably well with the theory.

D. Continuous Parametric Pump. Chen et al. (1972) has investigated experimentally a continuous pump in which the feed and products streams flow steadily both in up-flow and down-flow in the model system toluene-n-hyptane on silica gel adsorbent. Also, comparison is made between the experimental data and the analytical results obtained by equilibrium theory. The work has been emphasized on the operating conditions necessary to achieve high separation factors.

E. pH Parametric Pumping and Applications. The concept of this process essentially involves a chromatographic column of cation exchange resin which is subjected simultaneously, to an alternating axial displacement of solution and to an alternating pH gradient. pH alternations can cause equilibrium solute distribution between the two phases of the ion exchanger to vary and so produce an alternating interphase (e.g., mobile and stationary phase) solute flux, when coupled with an alternating flow direction, result in separation.

Sabadell and Sweed (1970) used pH changes to remove K^+ and N_a^+ from water. This work did prove the feasibility of using the pH parametric pumping process to produce a separation as the theory predicted because of the strong dependence of the solubility of protein in solution on pH. Thus, it is popular to carry out protein separations by monitoring the pH in solution. However, to incorporate the pH dependent nature of protein into a continuous process like parametric pumping is still rare.

The application of this process to separate materials like enzymes and proteins is of increasing concern. Shaffer and Hamrin (1975) investigated the removal of trypsin from α-chymo trypsin-trypsin mixture by employing a technique with a combination of affinity chromatography and parametric pumping. The result did indicate the

viability of parametric pumping as an enzyme separation method.

2. PRINCIPLES OF PROTEIN SEPARATION AND CONVENTIONAL METHODS

A. Principles of Protein Separations

(a) Separation Based on Molecular Size. The most striking characteristic of proteins is their large size. This makes possible the use of simple methods for the separation of proteins from smaller molecules, as well as methods for resolving mixtures of proteins. Dialysis, ultrafiltration, density-gradient centrifugation, and molecular-exclusion chromatography (gel-filtration) are the separation methods based on the molecular sizes. Gel-filtration will be discussed in a later section.

(b) Separation Based on Ligand Specificity. Some proteins can be isolated from a very complex mixture and brought to very high dgree of purification, often in a single step, by affinity chromatography. It is based on some biological properties of proteins, namely, their capacity for specific, noncovalent binding of other molecules, called ligands.

The difference between (a) and (b) is the separation mechanism. Principle (a) functions solely based on the different sizes of the protein molecules. However, in

principle (b), the separation was accomplished because different proteins have different ligands with different biological properties and thus have different affinities to the stationary phase of the chromatography.

(c) Separation Based on Solubility Differences. Proteins in solution show profound changes in solubility as a function of (a) pH, (b) ionic strength, (c) dielectric properties of the solvent, and (d) temperature (Lehninger, A.L. Biochemistry). These variables reflect the fact that proteins are electrolytes of very large molecular weight can be used to separate mixtures of proteins, since each protein has a characteristic amino acid composition, which determines its behavior as an electrolyte.

(d) Separation Based on Electric Charge. The principle depends on their acid-base properties, which are largely determined by the number and types of ionizable groups in their polypeptide chains. Since proteins differ in amino acid composition and sequence, each protein has distinctive acid-base properties. Electrophoretic methods are based on this principle. Also, a second way of utilizing the acid-base behavior of proteins as a basis for separation is ion exchange chromatography.

(e) Separation Based on Selective Adsorption. Proteins can be adsorbed to and selectively eluted from columns of finely divided, relatively inert material with

very large surface area in relation to particle size. The adsorbance include nonpolar substances, eg., charcoal, and nonpolar substances like silica gel or alumina.

Principles (d) and (e) are basically different. Principle (d) is essentially dependent on the proteins' acid-base properties and those, in turn, are largely determined by the number of ionizable groups in their polypeptide chains. In principle (e), the stationary phase is a relatively inert material with a very large surface area in relation to protein particle size.

In addition, as has been mentioned in principle (c), the solubility of protein will be influenced by the temperature (Lehninger, A.L. Biochemistry). Therefore, theoretically, it should be able to serve as an intensive variable to separate proteins. Within a limited temperature range, from 0 to about 40° C, most proteins increase in solubility with increasing temperatures, although there are some exceptions, as there are for simple electrolytes. Above 40 to 50° C, most proteins become increasingly unstable and begin to denature. It is always favorable to carry out protein separations at lower temperatures.

B. Conventional Methods

Techniques commonly used for separation of protein materials which might adopt to semi-continuous parametric pumping include gel-filtration, affinity chromatography, and ion exchange chromatography.

(a) Gel-filtration. Gel-filtration is a chromatographic separation resulting from restricted molecular diffusion through a column of gel particles having suitable porosity and properties. One type of gel used for this purpose is a modified macromolecule of dextran crosslinked to produce a three-dimensional network of polysaccharide chains. When mixed with water or electrolyte solutions, the material swells considerably. Placed in a chromatographic column, it acts as a sieve for molecules of different sizes, since the porosity of the gel is determined by the amount of crosslinkage in the dextran network. A high degree of crosslinkage produces a highly porous structure. Gels of a variety of crosslinkages are commercially available. The liquid imbibed by gel particles is available as solvent to solute molecules of different sizes, to a degree dependent on the porosity of granules.

(b) Affinity Chromatography. The principle of affinity chromatography involving selective separation of certain proteins can be achieved by taking advantage of

their interaction with specific ligands that have been immobilized in suitable a chromatographic matrix. When a mixture of proteins in solution is passed through a column of this type, proteins that do not interact with the immobilized ligands will pass through the column without retardation. Those that do interact will be retarded to varying extents, depending on their affinities for the ligand under the conditions employed. The applicability of this method depends on the availability of a specific ligand for the protein to be separated, which can be covalently attached to the matrix material.

An effective matrix is obtained with beaded derivatives of agarose (a crosslinked polysaccharide); the resulting gel is sufficiently porous to allow noninteracting macromolecules with molecular weights as high as millions to pass through freely. In cases where the desired protein is strongly bound to the immobilized ligand, elution is achieved by a change in solution conditions, (e.g., pH, temperature), addition of a more strongly interacting ligand to the elution solution, or cleavage of matrix-ligand bond.

A related chromatographic technique based on protein-ligand affinities involves specific elution of proteins bound to a nonspecific matrix by addition of a substrate or other ligand of the desired protein to the eluting

solution. Any protein that interacts with the added ligand is likely to undergo a change in its affinity for the matrix, with the result that a selective elution occurs.

(c) Ion Exchange Chromatography. An ion exchange stationary phase consists of a polymer or a material of high molecular weight that is insoluble yet permeable to the solution with which it is in contact and with those ions it will exchange. The polymer matrix (stationary phase) carries charged groups that are fixed. Balancing the charges of the fixed groups are the counter ions, the mobile ions that can exchange places with ions of similar charge in the solution.

In this investigation, the process is the semi-continuous pH parametric pumping which is a combination of ion exchange chromatography and parametric pumping.

2. THEORY

Parametric pumping is a separation process which involves reciprocating flow of the fluid mixture to be separated through a fixed bed of ion exchanger and simultaneously, synchronously cyclic variation of an intensive variable such as pH or temperature.

Proteins carry both positively and negatively charged groups and can be bound to either cationic or anionic ion exchangers. The net charge of the protein is dependent upon the isoelectric point of the protein. The isoelectric point of hemoglobin is pH 6.7 and pH 4.7 for albumin, I_H and I_A respectively. In these experiments, the pH has been carefully selected so that $I_A < P_2 < I_H < P_1$ (P_1 for high pH reservoir, P_2 for low pH reservoir). As a result of a change in the pH within the column, hemoglobin experiences a change in net charge and migrates towards the bottom reservoir. At low pH for example, the net charge is positive, and hemoglobin will be taken up by a sephadex ion exchanger (cationic). However, for albumin the net charge is always negative during the up-flow and down-flow and in the ion exchanger, so the partition of albumin between the mobile and stationary phase does not change to any appreciable extent. (Notice that albumin can establish equilibrium very rapidly).

The preceding discussion signifies the feasibility of this process, because for a binary protein mixture of hemoglobin and albumin, during the down-flow (i.e., passing through the ion exchanger in a low pH medium), albumin in the mixture can pass through the sephadex ion exchanger without major retardation, while the other component, hemoglobin, is abundantly adsorbed by the sephadex ion exchanger. During the up-flow (i.e., eluting the ion exchanger with a medium of higher pH, the higher pH solution tends to render the adsorbed hemoglobin to bear a negative charge so that it will be desorbed from the ion exchanger, and the separation is thus achieved.

3. EXPERIMENTAL

A. Step Testing Experiments

(1) Equilibrium of Hemoglobin Between the Mobile and Stationary Phase in Sephadex Ion Exchanger

(a) Description of the Equipment. The column diagram of step testing experiments is shown in Figure 1. The operation involved a jacketed pyrex column 0.4 m in length and 0.016 m inside diameter which was packed with sephadex adsorbent particles (Pharmacia Fine Chemicals). The feed reservoir, which was a 50 cc glass syringe, was operated by an infusion pump. The syringe was sealed with stopcock grease. The product samples were collected with a 10 cc graduated cylinder. All the flow lines were made of 0.031 inch diameter teflon tubing.

(b) Packing the Column. The amount of sephadex ion exchanger needed depends on the concentration of the buffer, that is, 0.85, 0.70 and 0.65 gm, respectively, per 30 cc of buffer solution for the buffer concentration of 0.20, 0.15, and 0.10 M. The gel for packing was made by introducing the required amount of SP C-50 sephadex powder to 30 cc of pH 6 buffer solution and was then allowed to swell for at least 24 hours. The buffer solution used was essentially a mixture of monobasic and diabasic sodium phosphates. The swollen gel was then mixed with

16 cc of the feed (protein mixture of interest). According to experience, if the slurry is too thick, air bubbles will be trapped in the gel when it is poured, so the column was always packed from a thin suspension. This however introduced problems of convection current during the packing. The Column was set up and checked if it was mounted vertically. Ensured that the bed support was covered by about one cm of elutant, that no air remained in or under the bed support and the column outlet was closed. The ion exchanger (i.e., mixture of the swollen gel and 16 cc of feed) was poured into the column down the glass rod thus avoiding air bubble formation. The gel was allowed to stabilize for 5 to 10 minutes. The outlet was opened to allow the ion exchanger to pack under a constant pressure head. Until only about one inch high of the elutant was above the bed, the outlet was closed and the ion exchanger was pressed from the top. Simultaneously the inlet tubing would be filled with the elutant and thus connected to the feed reservoir.

(c) Procedures. At time zero, the feed reservoir was filled with approximately 0.01% hemoglobin buffer solution. The void volume in the column was assumed to be taken by the previously mixed 16 cc of feed solution. The feed flow rate was controlled at 0.5 cc/min. Once started, for every 3 cc, the products were collected from

the outlet for spectrophotometric measurements. The collecting procedures were repeated until the product concentrations were constant i.e., equilibrium was established by hemoglobin between the mobile and stationary phase of the ion exchanger.

(2) Equilibrium of Albumin Between the Mobile and Stationary Phase in Sephadex Ion Exchanger

For Run 7 through Run 10, the experimental section including (a) Description of the Equipment, (b) Packing the Column, and (c) Procedures, are essentially the same as that described in the preceding section, except that the feed is now a 0.05% albumin buffer solution.

B. Semi-continuous pH Parametric Pumping Experiments

This section discusses the manner in which all the parametric pumping experiments were conducted. It consists of five parts: (a) System Selection, (b) Preliminary Work, (c) Apparatus and Procedures, (d) Measurements, and (e) Sample Calculation.

(a) System Selection. A two-component protein mixture was selected to experimentally examine the feasibility of this parametric pumping separation scheme:

Component	Protein	Molecular Weight	Isoelectric Point
A	Hemoglobin	63,000	pH 6.7
B	Albumin	69,000	pH 4.7

Worthington human hemoglobin and human serum albumin were used.

For the stationary phase of the column, the sephadex ion exchanger media, manufactured by Pharmacia Fine Chemicals, was chosen (SP-sephadex C-50). This is the sodium form of a relatively high porosity, strongly acidic, cation exchanger. The porosity is suitable for the molecular weight range 30,000 to 200,000. The particle size ranges from 40 um to 120 um. The ion exchanger capacity is high at high ionic strength and is insensitive to pH over the range of pH 3 to pH 11. It is thus suitable for protein separations, since low ionic strength, where aggregation and protein instability may occur, is avoided.

(b) Preliminary Work. Prior to each parametric pumping experiment, the buffer solution is again prepared by mixing the monobasic and dibasic sodium phosphates with certain ratios. The feed was a man-made protein mixture of 0.01% hemoglobin and 0.05% albumin in pH 6 buffer. The sephadex ion exchanger were made by introducing 0.85, 0.70, and 0.65 gm respectively per 30 cc of buffer solution for the buffer concentrations of 0.20, 0.15, and 0.10 M. The details concerning the packing of the column are essentially the same as those described in the section of Experimental (1) (b) Packing the Column.

The entire system, including all the connecting tubings, Bio-Fiber beaker and micrometer capillary valves tube bundles, were all filled with feed to insure that only negligible amounts of air remained in the system, thus avoiding the difficulty in product take-off procedures.

Both feed and top reservoir were filled with feed. The bottom reservoir was filled with pH 8 feed solution. (The formulation of this solution is the same as that of feed except with a higher pH.)

(c) Apparatus and Procedures. The apparatus set-up for the parametric pumping experiments is shown schematically in Figure 2. The column was made of two sections, one for stripping and the other for enriching, and consisted of two jacketed chromatographic columns (0.016 m inside diameter and 0.4 m length, manufactured by Pharmacia Fine Chemicals). The columns were packed with SP sephadex C-50. The columns were maintained at a constant temperature of 288^{o}K by the use of a refrigeration unit which circulates cooling water in the jacket. Reservoirs, each having a dead volume of 6 cc, were located at two opposite ends of the columns and consisted of two 50 cc glass syringes. Reciprocating flow within the columns was obtained by coupling the syringe plungers to a dual infusion-withdrawal pump manufactured by Harvard Apparatus Company. The feed was introduced between the stripping and enriching columns by a second pump with a

50 cc syringe. After every six cycles, operation was interrupted and the feed syringe refilled. To insure perfect mixing in the reservoirs, small magnetic stirrers were placed in the syringes. The product take-off valves were micrometer capillary valves used both to regulate flow and impose a small back pressure on the system.

The two pH levels were produced by the two Bio-Fiber breakers manufactured by Bio-Red Laboratory, one for high pH and the other for low pH. Both were magnetically stirred. The protein solution was allowed to pass through the tube bundles of these beakers, while buffer solutions were circulating the tubes by a Bio-Fiber pump module.

During the first half circle, the fluid in the bottom reservoir was pumped through the high pH beaker and into the bottom of column B (Figure 2). At the same time, solution that emerged from Column A flowed through the low pH beaker and filled the top reservoir. On the next half circle, the solution in the top reservoir flowed back through the low pH beaker, passed through A and B, and then through the high pH beaker to the bottom reservoir. Simultaneously, the feed pump was activated and product take-off valves were opened and adjusted for the desired product flow rate. The procedures were repeated for each subsequent cycle.

(d) Measurements. After the products have been taken off, all the samples are analyzed spectrophotometrically. Hemoglobin will absorb both at visible light range 403 mμ and ultra-violet light range 280 mμ. The transmittance obtained from spectrophotometer measurements can be related to the protein concentrations.

(e) Sample Calculation. Since sample transmittance obtained from the spectrophotometer would be related to the concentration of protein, for single protein systems like hemoglobin-water or albumin-water, the conversion of transmittance into concentration is straightforward because the concentration of protein is linearly proportional to the absorbance. Albumin will absorb at wavelength of 280 m ; hemoglobin will absorb at both 280 m and 403 m that is:

For pH 6

$$(1_{280})_A = (\eta_{280})_A \beta_A \quad (1)$$

$$(1_{280})_H = (\eta_{280})_H \beta_H \quad (2)$$

$$(1_{403})_H = (\eta_{403})_H \beta_H \quad (3)$$

Where 1 represents the absorbance, is a function of pH.

For pH 6

$$(\eta_{280})_A = 5.29$$

$$(\eta_{280})_H = 21.69 \quad (4)$$

$$(\eta_{403})_H = 60$$

For pH 8

$$(\eta_{280})_A = 6.06$$
$$(\eta_{280})_H = 16.05 \qquad (5)$$
$$(\eta_{403})_H = 45.19$$

A - albumin

H - hemoglobin

η - slope of log transmittance vs concentration

β - weight % of concentration of proteins

Since the system being examined was a binary mixture, the conversion of transmittance into concentration of the protein is somewhat more complicated. This is due to the fact that absorbance at 280 mμ was contributed from both albumin and hemoglobin. Therefore, for a product sample, the concentration of hemoglobin was determined directly from the absorbance of the sample at 403 mμ (equation (3)). Once the concentration of hemoglobin was known, the absorbance of the sample at 280 mμ contributed from albumin can be determined. The absorbance contribution from hemoglobin at 280 mμ can be determined by equation (2). Subtraction of the contribution from hemoglobin at 280 mμ will enable us to figure out the absorbance contribution from albumin and thus the concentration of albumin in the product sample (equation (1)).

For the cycling zone experiment, calculation of the concentration of albumin samples were based on the pH of the samples. For a given sample with a pH larger than pH 6, the calculation of the albumin concentration was based on the slope of 6.06 for pH 8 albumin at 280 m . The fact that the sample's pH was larger than pH 6, implied that the pH 8 albumin feed was functioning to change the pH of the column.

CYCLING ZONE

A. Background

Pigford et al., (1969) proposed that cyclic changes in concentration could be produced in a fluid which flowed through a fixed bed of solid adsorbent owing to temperature cycling the bed. The product stream was collected separately during the periods of positive and negative deviations from the feed composition. In 1975, Wankat proposed that from his study of sugar separation, adsorption of sugar was stronger at higher pH but almost negligible at acidic conditions. The idea behind this cycling zone supplement experiment was inspired by Wankat's work. The experiment was conducted in a manner parallel to Wankat's work.

The purpose of this experiment was to supplement the part of experimental section A., Step Testing Experiments (2) Study of Equilibrium of Albumin between the Mobile

and Stationary Phase of Sephadex Ion Exchanger. In light of the fact that the isoelectric point of albumin is pH 4.7, periodic alteration of albumin feed pH (Busbice and Wankat, 1975), will cause a change of environment in the ion exchanger and thus cause a change of distribution of albumin between the mobile and stationary phase of the ion exchanger.

Cycling zone technique involves that the fluid mixture to be separated be pumped in one direction through one or more columns. The columns are cooled and heated periodically, or in the traveling wave mode, where the entering streams are heated and cooled periodically. Variables, like temperature and pH, can be monitored to achieve separation.

B. Experimental

(a) Packing the Column. This portion was essentially the same as that described in section 3, Experimental A., (1) (b) except without adding 16 cc of albumin feed to the prepared gel.

(b) Procedures. (1) 0.2 M pH 6 0.0157% albumin feed (totally 22 cc) was introduced into the column packed with gel (prepared by introducing 0.85 gm of sephadex powder to 30 cc of pH 6 sodium phosphates buffer solution) at a rate of 0.5 cc per minute. In the meantime the eluted samples were collected every two cc.

(2) After the first 22 cc were introduced into the column, the system switched to another albumin feed with pH 8, 0.0137% in concentration for another 22 cc at the same time. The samples were collected as usual.

(3) Repeated the procedures in 1 and 2 for three cycles alternatively.

(4) The sample concentration were determined by spectrophotometric measurements.

RESULTS AND DISCUSSION

This section consists of two phases:

A. Step Testing

(1) Equilibrium of Hemoglobin Between Mobile and Stationary Phases of Sephadex Ion Exchanger. This part consists of six experiments from Run 1 through Run 6 (tabulated in Table 1) to demonstrate the effect of concentration and pH on the equilibrium of hemoglobin between the mobile and stationary phases of the ion exchanger.

(2) Equilibrium of Albumin Between Mobile and Stationary Phases of Sephadex Ion Exchanger. This portion consists of five experiments including Run 7 through Run 10 and the cycling zone experiment to demonstrate the equilibrium of albumin within the ion exchanger.

B. Semi-continuous pH Parametric Pumping

Ten experiments were conducted to demonstrate the effect of concentration and pH spread on the semi-continuous pH parametric pump separation performance. Experimental parameters were tabulated in Table 2. Feed concentrations for parametric pumping experiments were tabulated in Table 2a. Table 2b tabulated all the initial conditions of the ion exchanger for both (A) Step testing and (B) Semi-continuous pH Parametric Pumping.

TABLE 1

PARAMETERS FOR RUN 1 TO RUN 10

Run	Conc	Feed Trans.	Log Trans.	% Feed	pH Feed	pH Gel, Swell
1	0.15 M	35.8	0.430	0.00971 H	8	6
2	0.15 M	39.8	0.369	0.00616 H	6	6
3	0.15 M	27.0	0.539	0.00899 H	6	8
4.	0.20 M	33.0	0.519	0.01148 H	8	6
5	0.20 M	37.0	0.428	0.00714 H	6	6
6	0.20 M	37.0	0.428	0.00714 H	6	8
7	0.20 M	50.5	0.276	0.0522 A	8	8
8	0.20 M	53.0	0.253	0.0479 A	8	6
9	0.20 M	53.0	0.253	0.0479 A	8	4.9
10	0.20 M	51.5	0.279	0.0528 A	6	6

H-Hemoglobin

A-Albumin

Trans.- Transmittance

TABLE 2

PARAMETERS FOR PARAMETRIC PUMPING EXPERIMENTS

		pH										CC		
			Reservoir		Dialysis		Product			Q			Product	
Exp	Conc,M	Feed	Top	Bottom	High	Low	Top	Bottom	Q	$\frac{Q}{W}$	U,D	Feed	Top	Bottom
1	0.035	6	6	8	8	6	6.1	7.3	18	72	D	8	3	3
2	0.035	6	6	8	8	6	6.2	7.5	18	72	D	8	4	4
3	0.100	6	6	8.5	8.5	6	6.0	8.0	26	104	D	8	4	4
4	0.035	6	6	8	8	6	6.1	7.4	18	72	U	8	3	3
5	0.100	6	6	6	8.9	6	6.1	7.2	20	80	U	8	3.8	3.2
6	0.200	6	6	8.9	8.9	6	6.0	7.9	20	80	U	8	3.3	4
7	0.035	8	6	8.9	8.9	6	6.2	8.1	20	80	U	8	3.9	4
8	0.035	6	6	8.9	8.9	6	6.0	7.8	20	80	U	8	3.8	3.8
9	0.150	6	4.9	8.9	8.9	4.9	5.5	7.8	20	80	U	8	3.9	3.9
10	0.150	6	6	8.9	8.9	6	6.1	7.7	20	80	U	8	3	3

Conc-buffer concentration, pH reservoir- initial pH in reservoir, U,D- Up or down at 1st pH product- Average pH for product, CC product- Average value Half cycle

Q- Displacement in cc, $\frac{Q}{W}$ - cycle time in min., pH dialysis- initial pH in dialysis

TABLE 2a

FEED CONCENTRATIONS FOR PARAMETRIC PUMPING EXPERIMENTS

Exp	Trans.H	Log Trans.	Y_{HO} '%	Trans.A	Log Trans.	Y_{AO} '%
1	27.0	0.545	0.00910	26.0	0.541	0.0660
2	26.8	0.543	0.00906	30.0	0.472	0.0522
3	24.2	0.594	0.00999	31.0	0.496	0.0532
4	28.8	0.522	0.00870	36.0	0.412	0.0422
5	27.3	0.546	0.00910	28.9	0.512	0.0596
6	31.6	0.480	0.00800	32.7	0.465	0.0552
7	32.0	0.466	0.01030	20.3	0.670	0.0833
8	35.4	0.458	0.00764	37.8	0.430	0.0497
9	37.4	0.424	0.00705	41.3	0.383	0.0436
10	30.2	0.494	0.00823	33.8	0.500	0.0608

H-Hemoglobin

A-Albumin

Trans. - Transmittance

TABLE 2b

INITIAL CONDITIONS OF ION EXCHANGERS

Conc	G Sephadex	pH Buffer	CC Buffer	pH Feed	CC Feed	Approx. Height	Exp
0.035	0.65	6	30	6	16	8	1,2,4,8
0.035	0.65	6	30	8	16	8	7
0.100	0.80	6	30	6	16	8	3,5
0.150	0.85	6	30	6	16	8	9.10
0.200	0.85	6	30	6	16	8	6

Conc	G Sephadex	pH Buffer	CC Buffer	pH Feed	CC Feed	Approx. Height	Run
0.15	0.85	6	30	8	16	8	1
0.15	0.85	6	30	6	16	8	2
0.15	0.85	8	30	6	16	8	3
0.20	0.85	6	30	8	16	8	4
0.20	0.85	6	30	6	16	8	5
0.20	0.85	8	30	6	16	8	6
0.20	0.85	8	30	8	16	8	7
0.20	0.85	6	30	8	16	8	8
0.20	0.85	4.9	30	8	16	8	9
0.20	0.85	6	30	6	16	8	10

Conc in molarity M

Approx. Height in Centimeter

TABLE 3

CORRECTION FACTORS FOR RUN 1 TO RUN 10

Run	mμ	Cell 1	Cell 2	Cell 3	Cell 4
1	403	100.0	98.4	101.0	107.0
2	403	100.0	92.0	90.8	93.4
3	403	100.0	93.5	98.2	96.0
4	403	100.0	109.0	106.2	107.4
5	403	100.0	99.2	99.5	99.5
6	403	100.0	99.2	99.5	99.5
7	280	100.0	95.4	87.0	84.0
8	280	100.0	95.0	88.0	90.0
9	280	100.0	95.0	88.0	90.0
10	280	100.0	98.0	101.0	95.6

Note: Table 3 - Table 7 are Correction Factors for the four different cells which were used for taking spectrophotometric measurements at different pH's and different wavelengths. Because different cells have different cell characteristics, these have to be corrected.

TABLE 4

CORRECTION FACTORS FOR pH 6 IN 403 mμ

Exp.	Cell 1	Cell 2	Cell 3	Cell 4
1	100.0	90.3	102.2	97.1
2	100.0	89.0	100.0	101.7
3	100.0	97.1	98.5	98.8
4	100.0	93.0	97.0	102.4
5	100.0	94.0	97.8	95.8
6	100.0	92.8	97.4	96.6
7	100.0	95.7	97.8	100.6
8	100.0	101.6	102.4	107.0
9	100.0	100.0	99.0	99.8
10	100.0	106.8	97.8	97.0

TABLE 5

CORRECTION FACTORS FOR pH 6 IN 280 mμ

Exp.	Cell 1	Cell 2	Cell 3	Cell 4
1	100.0	94.9	96.2	94.5
2	100.0	93.7	97.2	98.4
3	100.0	95.0	97.0	96.8
4	100.0	97.1	98.5	98.8
5	100.0	96.0	98.8	97.0
6	100.0	95.5	98.6	98.4
7	100.0	100.0	96.0	100.6
8	100.0	102.2	100.4	99.0
9	100.0	99.2	99.2	99.4
10	100.0	94.2	96.2	95.1

TABLE 6

CORRECTION FACTORS FOR pH 8 IN 403 mμ

Exp.	Cell 1	Cell 2	Cell 3	Cell 4
1	100.0	97.7	98.0	97.2
2	100.0	91.7	97.7	99.1
3	100.0	106.8	115.0	110.0
4	100.0	97.7	98.0	97.2
5	100.0	100.2	102.2	102.8
6	100.0	96.0	98.2	97.4
7	100.0	93.5	98.7	102.8
8	100.0	97.6	99.8	103.0
9	100.0	96.2	101.2	100.0
10	100.0	104.0	93.8	94.0

TABLE 7

CORRECTION FACTORS FOR pH 8 IN 280 mμ

Exp.	Cell 1	Cell 2	Cell 3	Cell 4
1	100.0	99.2	102.3	95.6
2	100.0	93.2	99.6	96.9
3	100.0	97.5	102.2	97.1
4	100.0	94.8	95.3	100.0
5	100.0	91.6	97.8	98.0
6	100.0	91.0	98.8	91.6
7	100.0	95.0	103.8	104.8
8	100.0	98.8	99.7	103.0
9	100.0	94.2	101.8	98.2
10	100.0	98.0	95.0	93.0

(1) Study of Equilibrium of Hemoglobin Between Mobile and Stationary Phases of Sephadex Ion Exchanger

a. Effect of Concentration. The addition and binding of hemoglobin to the ion exchanger will largely depend on the capacity of the ion exchanger, i.e., how well the sephadex particles swell in the buffer solution. The capacity of the ion exchanger will, in turn, be determined by the concentration of the buffer. Because ions in the vicinity of charged groups on the ion exchanger compete for these groups, an increase in the concentration, therefore, increases the competition for the binding sites on the ion exchanger, thereby decreasing interaction between the charged groups and hemoglobin, and leading to a decrease in the capacity of the ion exchanger. Experimental results from Runs 2 and 5 for 0.15 M and 0.20 M, respectively, do confirm the preceding argument. Figure 3 clearly indicates that the capacity of an ion exchanger in a 0.20 M buffer is poor as compared with that for 0.15 M. The Y_H/Y_{HO} ratio refers to the product to feed concentration ratio. If the ratio is equal to one, this indicates that hemoglobin in the stationary and mobile phases are in equilibrium. This also suggests that hemoglobin is saturated in the ion exchanger so that the product concentration is equal to the feed concentration. Figure 3 indicates that for o.20 M system, hemoglobin will

be saturated in the ion exchanger with great ease, while there is still a remote possibility for the 0.15 M system. Based on the results for 0.15 M, it can be inferred that the capacity of an ion exchanger in a 0.035 M buffer solution will be very large.

The foregoing discussion has been confined to the system with pH 6 feed into pH 6 ion exchanger. Figure 6 demonstrates the situation for a system with pH 6 feed into pH 8 ion exchanger and again for 0.15 M and 0.20 M buffer concentration. Again the Figure shows that for a 0.20 M system, due to the smaller capacity of the ion exchanger, the product to feed concentration ratio Y_H/Y_{HO} can reach a higher value as compared to that for a 0.15 M system.

However, for the case of pH 8 feed into a pH 6 ion exchanger, there is no difference in the limiting Y_H/Y_{HO} value for 0.15 M and 0.20 M. It is not a problem of the capacity of ion exchanger but rather a case of having a negatively charged hemoglobin feed passing through an eventually negatively charged ion exchanger environment. The equilibrium can be reached very rapidly. So for both 0.15 M and 0.20 M cases, the limiting Y_H/Y_{HO} ratio is approximately one. This signifies passing the negatively charged hemoglobin feed without major retardation through the ion exchanger.

b. Effect of pH. As the capacity of the ion exchanger is also dependent on the number of charged groups it may, therefore, vary with pH. Each protein has its isoelectric point (i.e., a certain pH). When the protein is in a medium of pH below its isoelectric point, the protein will bear a positive charge. If the pH of the medium is above the isoelectric point of the protein, then it will bear a negative charge. The isoelectric point for hemoglobin is 6.7, so that the higher the pH of the buffer is above pH 6.7, the more the hemoglobin molecules will bear negative charges and vice versa for the case below pH 6.7.

(a) pH 8 Feed Into pH 6 Ion Exchanger. A pH 8 hemoglobin feed will carry a net negative charge. At time zero, the pH in column should be in the vicinity of pH 6 (it deviates from pH 6 because of the mixing with 16 cc of pH 8 feed), so that at the beginning, the negatively charged hemoglobin feed will experience a rather low pH ion exchanger therefore tends to be adsorbed to the ion exchanger. Later as more pH 8 feed passes through the ion exchanger, the pH of the ion exchanger will gradually raise to above pH 6.7. This will enable the hemoglobin feed to pass through the ion exchanger freely, or in other words, the Y_H/Y_{HO} will be approximately equal to one, eventually.

Results from Runs 1 and 4 in Figures 4 and 5 do confirm the preceding argument. The abrupt change (overshoot) of Y_H/Y_{HO} ratio in Figure 4 from Run 1 (a 0.15 M system) is due to the fact that a 0.15 M system has a fairly large capacity for the ion exchanger as compared to that for a 0.20 M system, so that at earlier stages the 0.15 M system will adsorb a somewhat larger amount of hemoglobin (during this period, the ion exchanger is still in a medium with pH lower than 6.7). This reasonably accounts for the abrupt overshoot. However, the overshoot is not observed for the 0.20 M system with relatively lower capacity for the ion exchanger.

(b) pH 6 Feed Into pH 6 Ion Exchanger. In this case, there is no difference between the pH of the feed and that of the ion exchanger initially. The effect of pH on the performance of the ion exchanger can be isolated and therefore enable us to focus on the effect of concentration alone on the capacity of the ion exchnager. This has been discussed in previous section. Figure 3 demonstrates the situation.

(c) pH 6 Feed Into pH 8 Ion Exchanger. The pH 6 feed will carry a net positive charge because it is below pH 6.7. Also the initial pH in the ion exchanger will be determined by the 16 cc of pH 8 feed (originally mixed with the sephadex ion exchanger), the condition ensures a negative charge

environment for the ion exchanger. So it is reasonable to expect that the incoming hemoglobin feed to be adsorbed to the ion exchanger before it can reach equilibrium or saturation. Results from Runs 3 and 6 demonstrate the case. Also, Figures 4 and 5 indicate the situation of a pH 6 feed into pH 8 ion exchanger system is somewhat similar to that of Ph 6 feed into pH 6 ion exchanger except that the former experiences a pH change in the ion exchanger at earlier stages.

(d) Comparison Between pH 8 Feed Into pH 6 Column and pH 6 Feed Into pH 8 Column. These were demonstrated in Figures 4 and 5 for 0.15 M and 0.20 M systems respectively.

It can be concluded that for hemoglobin, the Y_H/Y_{HO} profile is S-type, this signifies that at the beginning, hemoglobin was adsorbed to the ion exchanger. As the process goes along, the ion exchanger gradually becomes enriched in hemoglobin until eventually the hemoglobin establishes an equilibrium between the mobile and stationary phases of the sephadex ion exchanger. That is, Y_H/Y_{HO} become a constant.

(2) Study of Equilibrium of Albumin Between the Mobile and Stationary Phase of Sephadex Ion Exchanger

A series of four runs were undertaken to show that the equilibrium of albumin between the mobile and stationary phase of the ion exchanger can be very rapidly established. In Figure 7, it was compared between the equilibrium of hemoglobin and albumin in the sephadex ion exchanger. It clearly pointed out that the Y_A/Y_{AO} profile did not proceed as that of Y_H/Y_{HO}.

For albumin, the Y_A/Y_{AO} profile is random, the observed profile phenomenally implies albumin remain unaffected when it was passed through the sephadex ion exchanger. The equilibrium of albumin between mobile and stationary phase was established very rapidly. From Run 7 through Run 10, the data consistently reflected the situation (Y_A/Y_{AO} reached equilibrium value, i.e., in the vicinity of one so fast (no later than the third sample was collected).

Also, Figure 8 illustrates that separation factors for albumin are approximately one. The separation factors as a function of n (number of cycles) in the semi-continuous pH parametric pumping is defined as the quotient of bottom and top product concentration.

Cycling Zone

This experiment was conducted to further confirm the situation of albumin equilibrium between the mobile and stationary phase of the sephadex ion exchanger.

From the data, it can be concluded that the result does confirm the result in A. Step Testing (2) Equilibrium of Albumin Between the Mobile and Stationary Phase in Sephadex Ion Exchanger. The data in this experiment indicated that again there was no increasing trend for the $^{Y}A/^{Y}AO$ ratio profile. The result convincingly shows that albumin can reach equilibrium very rapidly between the mobile and stationary phase of the ion exchanger. $^{Y}A/^{Y}AO$ is dependent on the sample history for the time period passing through the column. It can be concluded that albumin is not affected by the ion exchanger to any extent of physical significance when it was passed through the ion exchanger.

In conclusion, the $^{Y}H/^{Y}HO$ profile is a function of elution volume (feed volume) before hemoglobin reaches equilibrium. However, this is not the case for albumin. $^{Y}A/^{Y}AO$ values depend on the feed flow history passing through the ion exchanger at that particular instant. The irregularities of the $^{Y}A/^{Y}AO$ profile reflect the situation very clearly. Albumin was never affected by the ion exchanger to any extent with physical significance.

The irregularity of profile for albumin may be due to: (a) noninteracting nature of albumin itself with the ion exchanger, (b) the swollen nature of the ion exchanger causes nonuniform gel structure, (c) stripping channels may have been developed, and (d) backmixing of the incoming feed flow may happen within the ion exchanger.

Experimental parameters for these four runs are tabulated in Table 1. In Runs 7 and 10, the albumin feed experienced no pH change, the data as expected reasonably indicated no retardation of albumin in the ion exchanger since both pH 6 and pH 8 feed are well above the isoelectric point of albumin pH 4.7. In Runs 8 and 9, the albumin feed experienced a pH change at the beginning, later the process was just essentially passing the pH 8 albumin feed through the pH 8 ion exchanger. Runs 8 and 9 differ only in their initial pH in the ion exchanger.

B. Semi-continuous pH Parametric Pumping

(a) Systems with Larger Difference in Concentration.

In Figure 9, It was shown that an increase of concentration from 0.035 M to 0.15 M would certainly up-lift the Y_{HT}/Y_{HO} and Y_{HB}/Y_{HO} ratio profiles. The effect of increasing concentration from 0.035 M to 0.20 M on separation was demonstrated in Figure 10. To be more extensively clarify the phenomena, examining Figures 11 and 12 for 0.20 M and 0.15 M respectively,

it is impressive that the limiting values for Y_{HT}/Y_{HO} are some 0.8 (Figure 11) as exposed to 0.47 for the low concentration of 0.035 M in Figure 13. The higher the Y_{HT}/Y_{HO} implies the more enrichment of hemoglobin in the top product stream and this in turn is due to the fact, a higher concentration system means a relatively lower capacity of the ion exchanger and relatively fewer sites in the ion exchanger are available for hemoglobin. So during the down-flow, hemoglobin in the incoming feed is less adsorbed to the ion exchanger. In other words, hemoglobin is more preferably to stay in the mobile phase and thus cause enrichment of hemoglobin in the top product stream.

(b) Systems with Smaller Difference in Concentration. To be more convincingly illustrate the role of concentration plays on separation , it was aimed at systems with smaller difference in concentration like 0.035 M and 0.10 M. As shown in Figure 14, noticing that the pH of the bottom reservoir of 0.10 M system is pH 6 as compared to pH 8.9 for the 0.035 M system. The introduction of a pH 6 bottom reservoir is certainly a minus factor to the removal of hemoglobin from the ion exchanger. The whole idea behind introducing a minus factor is to dramatize the effect of concentration on separation. In Table 2, it was quite

remarkable the average pH in the bottom products in Experiment 5 (0.10 M system) is relatively lower due to the employment of a pH 6 bottom reservoir. However, the limiting value of Y_{HB}/Y_{HO} is some 1.3 for 0.10 M system as compared to some 0.95 for the 0.035 M system. This concludes that for systems with smaller concentration difference, even though it is smaller, the effect of increasing concentration can still impressively outweigh the lower pH 6 bottom reservoir minus factor. A lower pH bottom reservoir tends to induce a lower pH in the up-flow stream and is supposedly to less uptake the adsorbed hemoglobin from the ion exchanger and thereby decreasing the enrichment of hemoglobin in the bottom product stream, also decreasing the Y_{HB}/Y_{HO}. However, a higher concentration of 0.10 M overwhelmingly and drastically overcome the negative effect resulting from the pH 6 bottom reservoir.

(c) Systems with Smaller Difference in Concentration but Both at Higher Concentration. Now attention will be shifted from systems with lower concentration like 0.035 M and 0.10 M to systems with higher concentration like 0.15 M and 0.20 M. Comparing Figure 11 and 12, it was shown that as far as separation efficiency is concerned, there is no remarkable difference between 0.15 M and 0.20 M. Though 0.20 M system still shows a higher Y_{HB}/Y_{HO} limiting value of some 1.27 as compared to 1.2 for 0.15 M system.

The fact that the effect of concentration on separation is less indicative at higher concentration can be rationalized in this manner; at higher concentrations, the exposure of charged sites in the ion exchanger to hemoglobin does not differ greatly for 0.15 M and 0.20 M systems. So for systems at higher concentration, a smaller difference in concentration will not impressively affect the separation performance of the parametric pump. Controlling factor like pH spread will be emphasized in later discussion.

(2) Effect of pH Spread

(a) Systems with 0.15 M Concentration, Large Vs Small pH Spread. Experiments 9 and 10 were designed to demonstrate that systems with 0.15 M, a larger pH spread of 4.0 (i.e., pH 8.9 and pH 4.9 for bottom and top reservoir respectively) in Experiment 9 shows a better separation than Experiment 10 with a smaller pH spread of 2.9 (i.e., pH 8.9 and pH 6 for bottom and top reservoir respectively), the situation is shown in Figure 15. Also the separation factors curves for hemoglobin are compared in Figure 16 which strongly suggests that a larger pH spread will undoubtedly do a better separation job.

(b) Concentration Vs pH Spread (0.15 M with pH Spread of 4.0 Vs 0.20 M with pH spread of 2.9). In Figure 17, a comparison was made to show that a larger

pH pread of 4.0 will offset a smaller concentration of 0.15 M. The 0.15 M system though, is smaller that 0.20 M in concentration, however, a larger pH spread of 4.0 has tremendously lowered the Y_{HT}/Y_{HO} to some 0.2. The lower the Y_{HT}/Y_{HO} implies that the more the hemoglobin in the feed has been adsorbed to the ion exchanger during the down-flow.

(c) Ideal Separation. The most desirable results in a semi-continuous pH parametric pumping separation of proteins (hemoglobin and albumin mixture) will be the case with Y_{HT}/Y_{HO} limiting value be zero (i.e., all the hemoglobin in the feed will be totally adsorbed to the ion exchanger during the down stream) and during the up-flow cycle, all the hemoglobin adsorbed to the ion exchanger will be totally desorbed as a result of switching to a higher pH 8 which renders the hemoglobin to become negatively charged. With regard to albumin, it is basically not affected by the process because it always bear negative charge. The result from Experiment 9 and separation factors in Figure 16 both indicated the research has been conducted in the right direction.

(d) Discriminating the pH 8 Feed. An attempt was made to discriminate the pH 8 feed system. Figure 18 demonstrates the difference between a pH 6 feed and a pH

8 feed. For a pH 8 feed system in Experiment 7, the limiting value for the Y_{HB}/Y_{HO} is approximately 0.6. These are relatively higher than those for pH 6 feed system in Experiment 8 (i.e., 0.95 for Y_{HB}/Y_{HO} and 0.47 for Y_{HT}/Y_{HO}). A pH 8 feed would imply both hemoglobin and albumin are bearing negative charges. These negatively charged protein molecules should be able to pass through the ion exchanger (with negative charged sites on it) without major retardation. So that during the down-flow, the top product stream will be enriched with hemoglobin. This accounts for the fact of a higher Y_{HT}/Y_{HO} of 0.6 for a pH 8 feed system. In Figure 19, the separation factors for the pH 8 feed system was shown, there were overshoots for both hemoglobin and albumin. Again, this is due to the pH 8 feed. During the first few cycles, hemoglobin and albumin the 16 cc of pH 8 feed originally mixed with sephadex gel would bear negative charges and tended to stay in the mobile phase. As a result of being pushed up and down during the cycle, these protein molecules will move freely along with the product stream thus give a higher Y_{HT}/Y_{HO} and Y_{HB}/Y_{HO}. Naturally a higher Y_{HT}/Y_{HO} is not desirable as has been discussed in the ideal separation. So it can be colcluded that we should discrimainate the pH 8 feed.

As for a pH 6 feed, hemoglobin will carry a net positive charge. It will be adsorbed to the ion exchanger. Also due to the large capacity of a 0.035 M system ion exchanger, the amount of hemoglobin adsorbed to the ion exchanger wcuild be very large. This accounts for the low Y_{HT}/Y_{HO} value during the down-flow. During the up-flow, those previously adsorbed hemoglobin would be eluted less effectively in a pH 6 feed system.

CONCLUSION

The purpose of this research is to study the effect of concentration of the buffer and the pH on the semi-continuous pH parametric pump separation performance.

From the results in the step testing experiments, it can be concluded that the rapidity to establish an equilibrium for hemoglobin between the mobile and stationary phases of the sephadex ion exchanger is dependent on both the buffer concentration and the pH spread between the feed and the initial pH in the ion exchanger.

For albumin, it can pass through the ion exchanger without any major retardation. There is no indication of increasing trend of the product concentration profile. This implies there is no significant adsorption or interaction between the albumin and the ion exchanger to any extent of physical significance.

In the semi-continuous pH parametric pumping experiment, it can be also concluded that an increase in the buffer concentration results in a shifting of position of equilibrium involving the ion exchanger. This in turn results in less uptake of hemoglobin and high concentration in the top product stream. The albumin concentration is practically unaffected by the pumping operation. In both top and bottom product stream, it remains essentially

at the feed concentration.

With regard to the effect of pH spread, due to the fact that the change of pH towards the isoelectric point of the protein render it neutral and thus reduces the interaction with the ion exchanger. The results reflected that less separation was achieved with smaller pH spread. A larger spread in pH will bring up very significant increase in separation.

APPENDIX

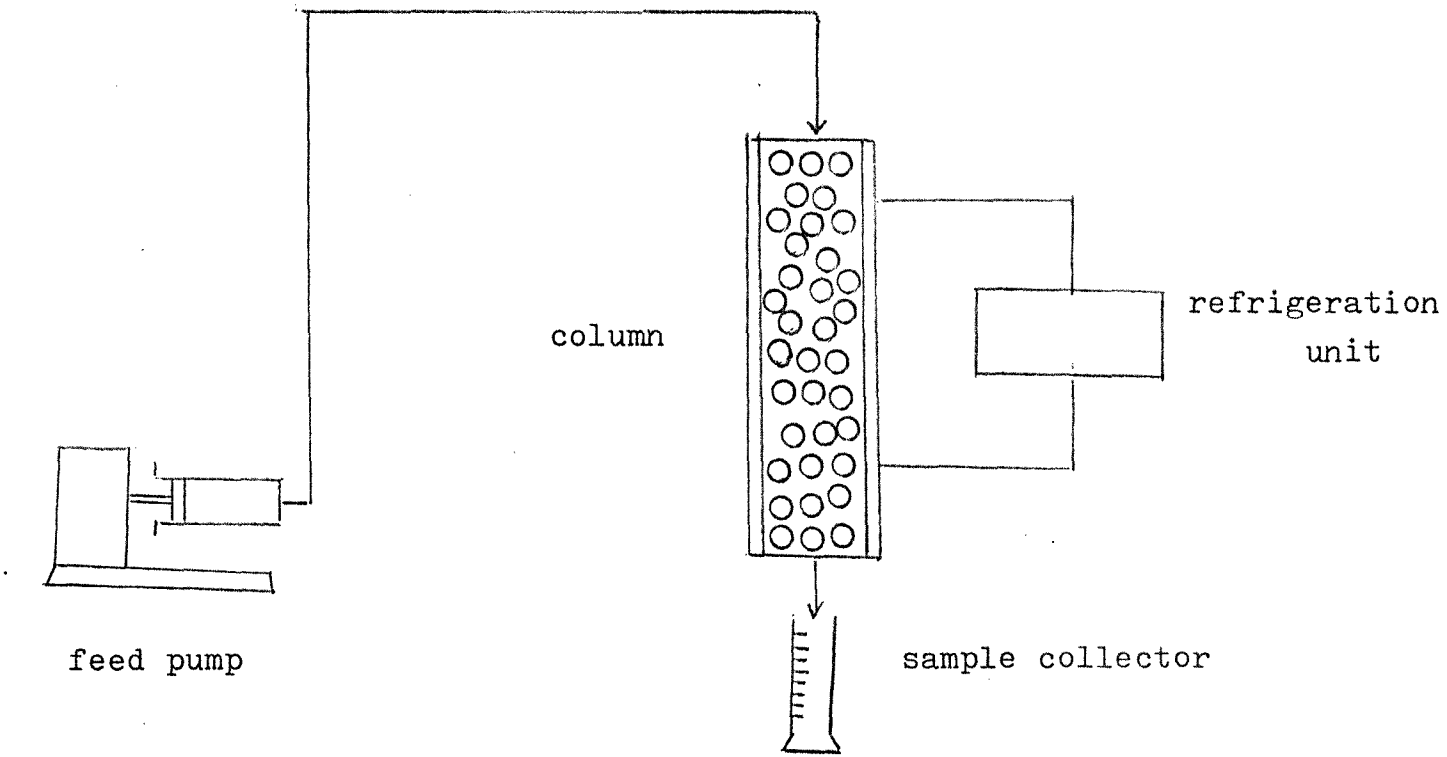
This section consists of two parts:

A. Figures: Figure 1 to Figure 19

B. Tables: Table 8 to Table 32 and cycling zone (sample calculation)

Figure 1

Apparatus for Step Testung



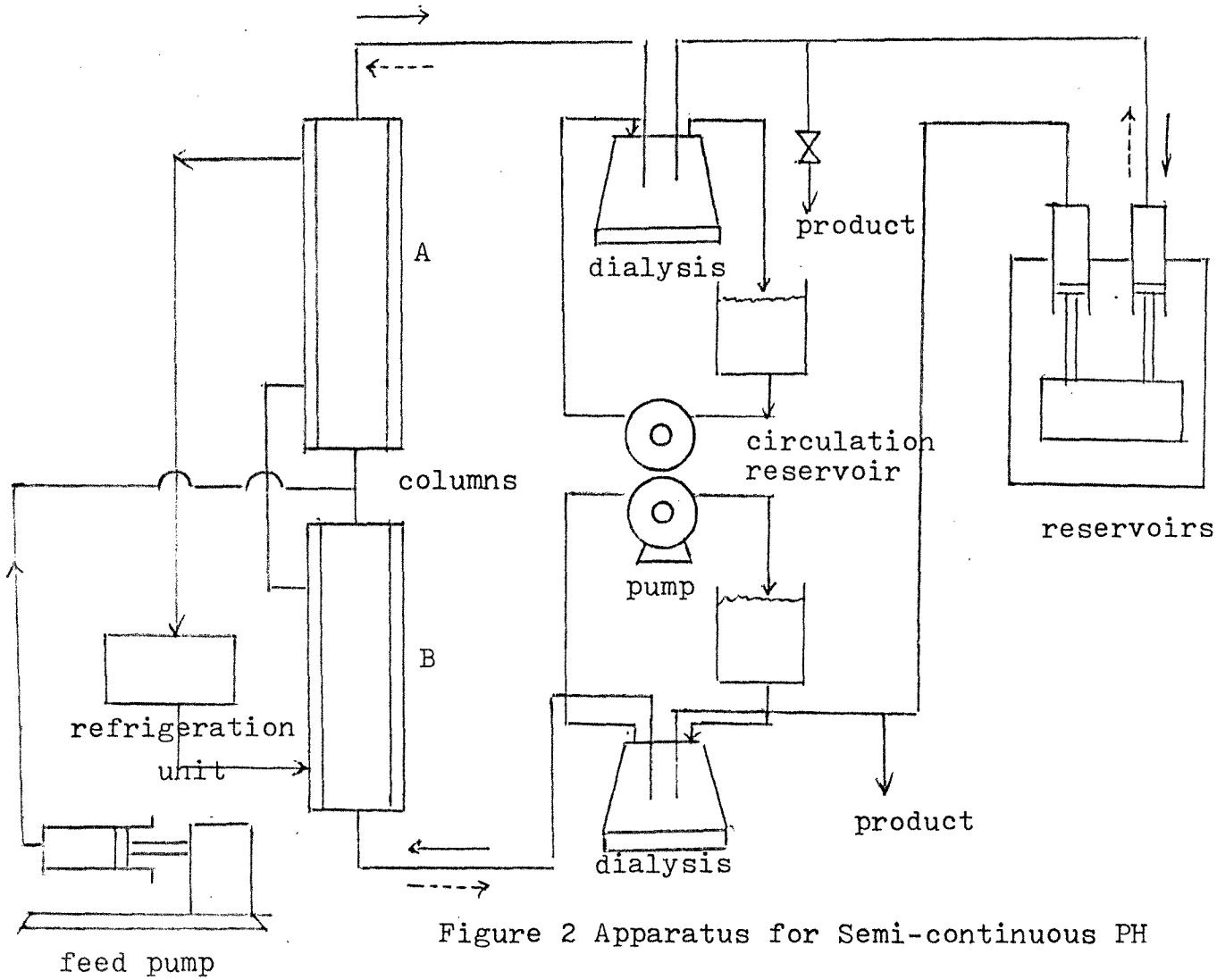


Figure 2 Apparatus for Semi-continuous PH Parametric Pumping

Figure 3

Equilibrium of Hemoglobin 0.15 M vs 0.20 M

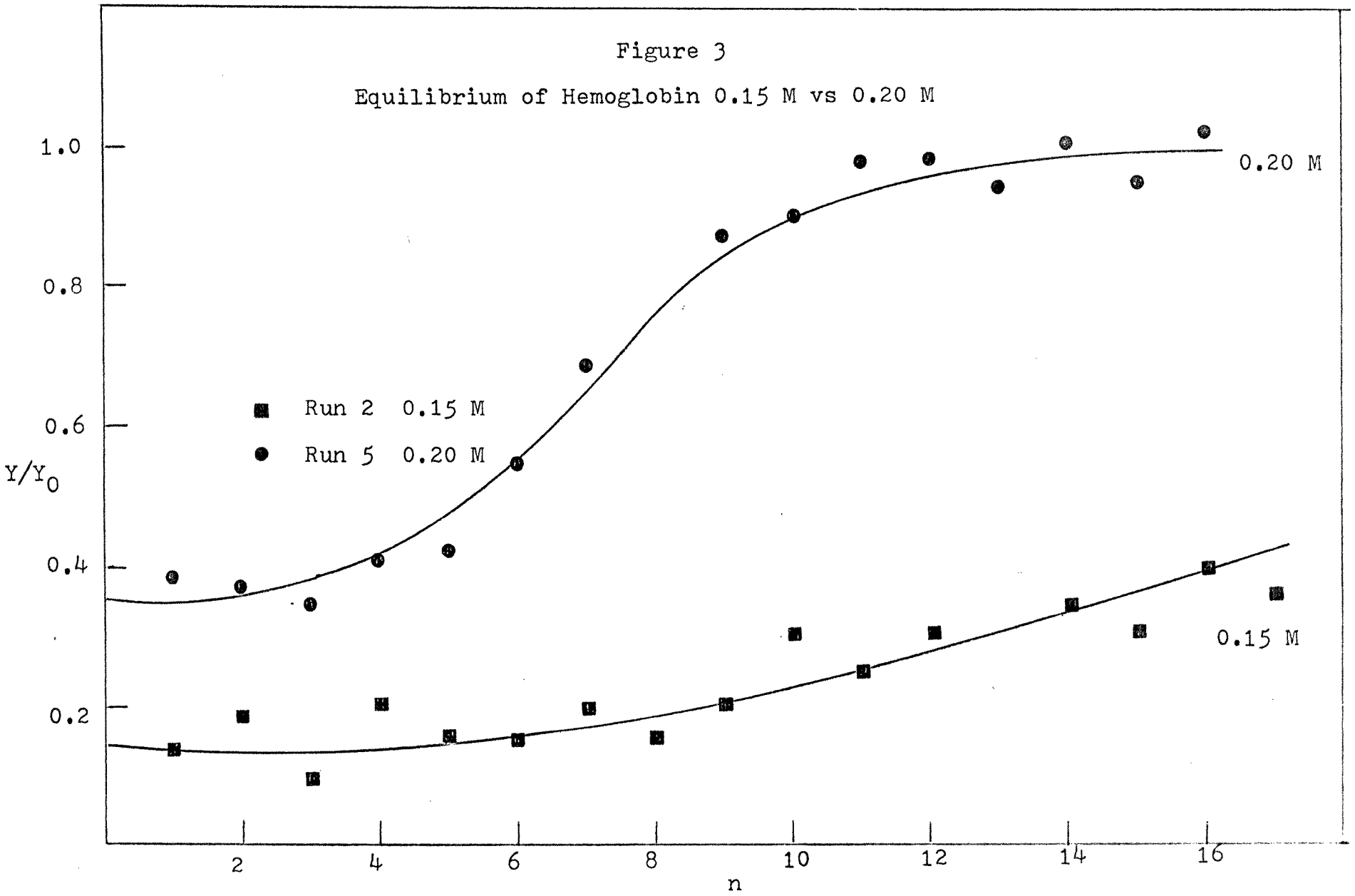


Figure 4

Effect of PH Difference on Equilibrium for 0.15 M Hemoglobin System

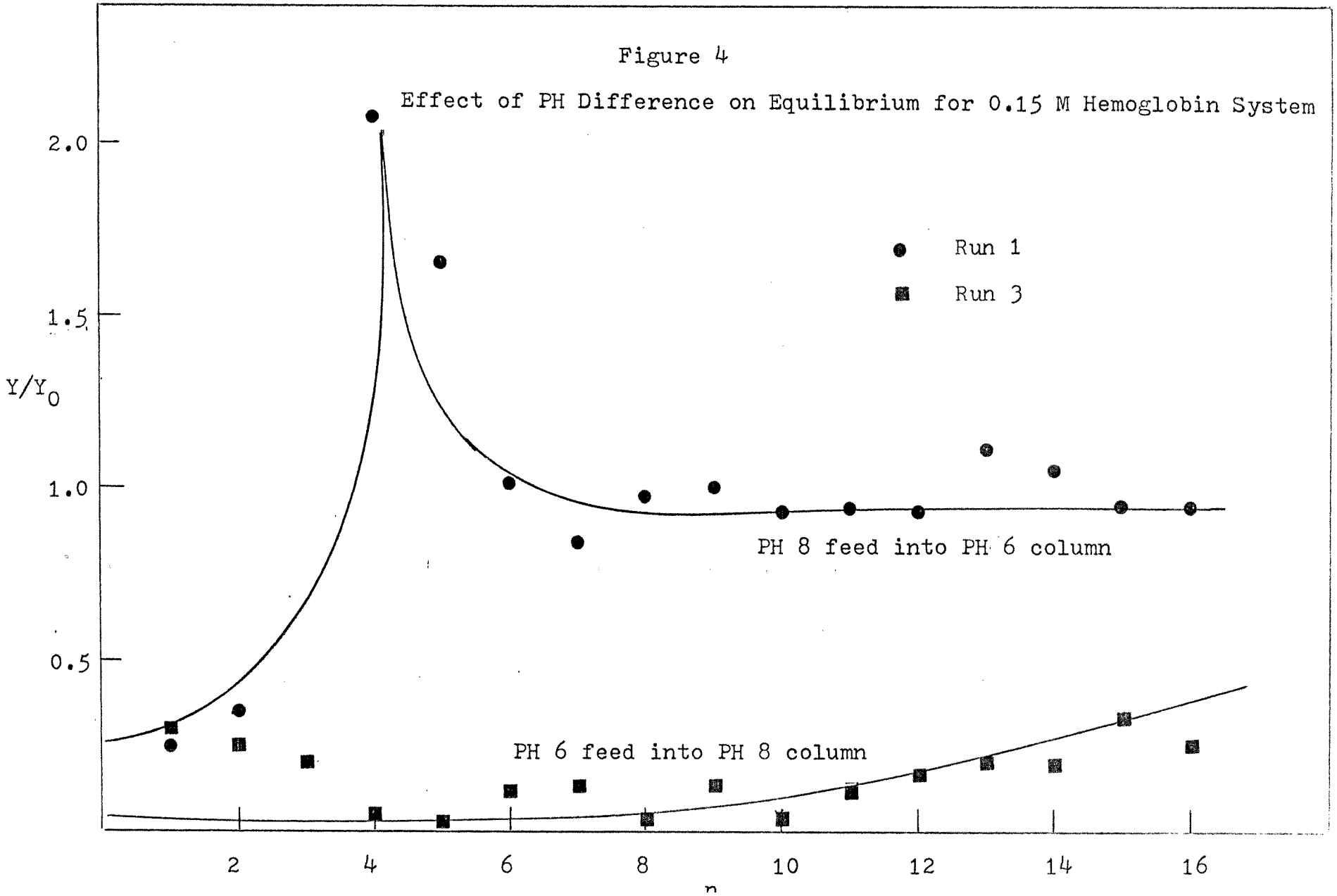


Figure 5

Effect of PH Difference on Equilibrium for 0.20 M Hemoglobin System

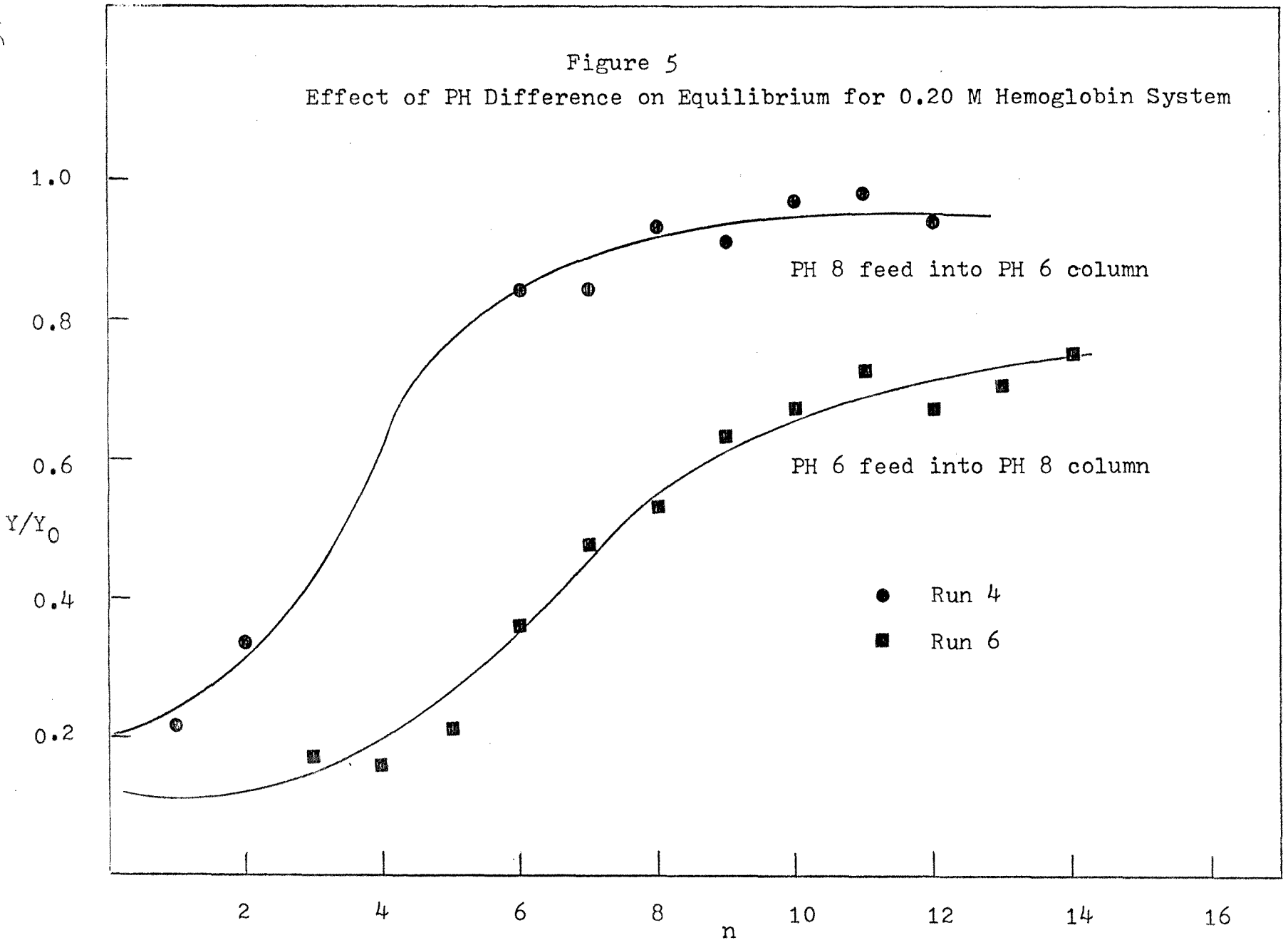


Figure 6

PH 6 Feed into PH 8 Column 0.15 M vs 0.20 M Hemoglobin System

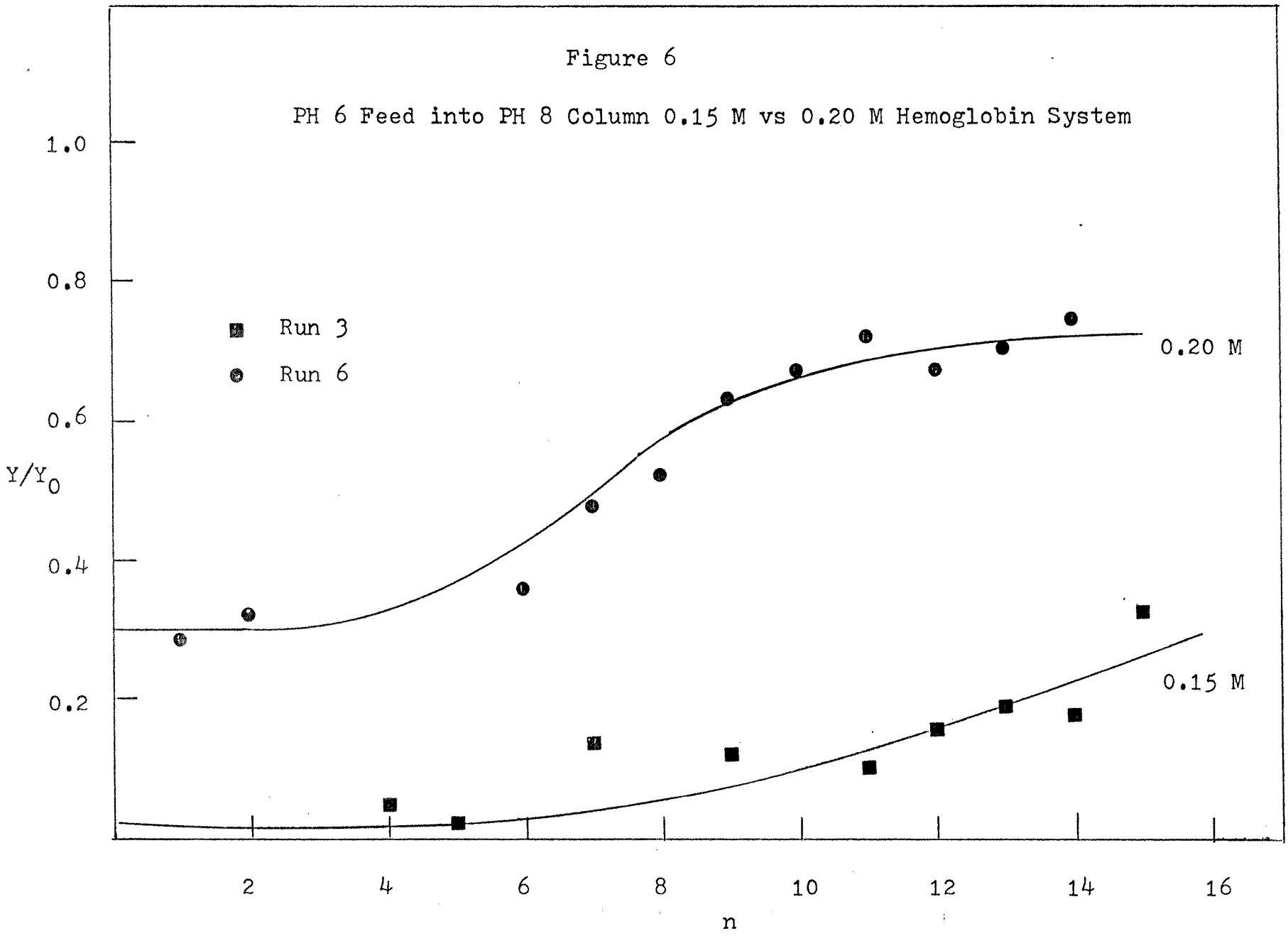


Figure 7

Adsorption of Hemoglobin vs Negligible Adsorption of Albumin

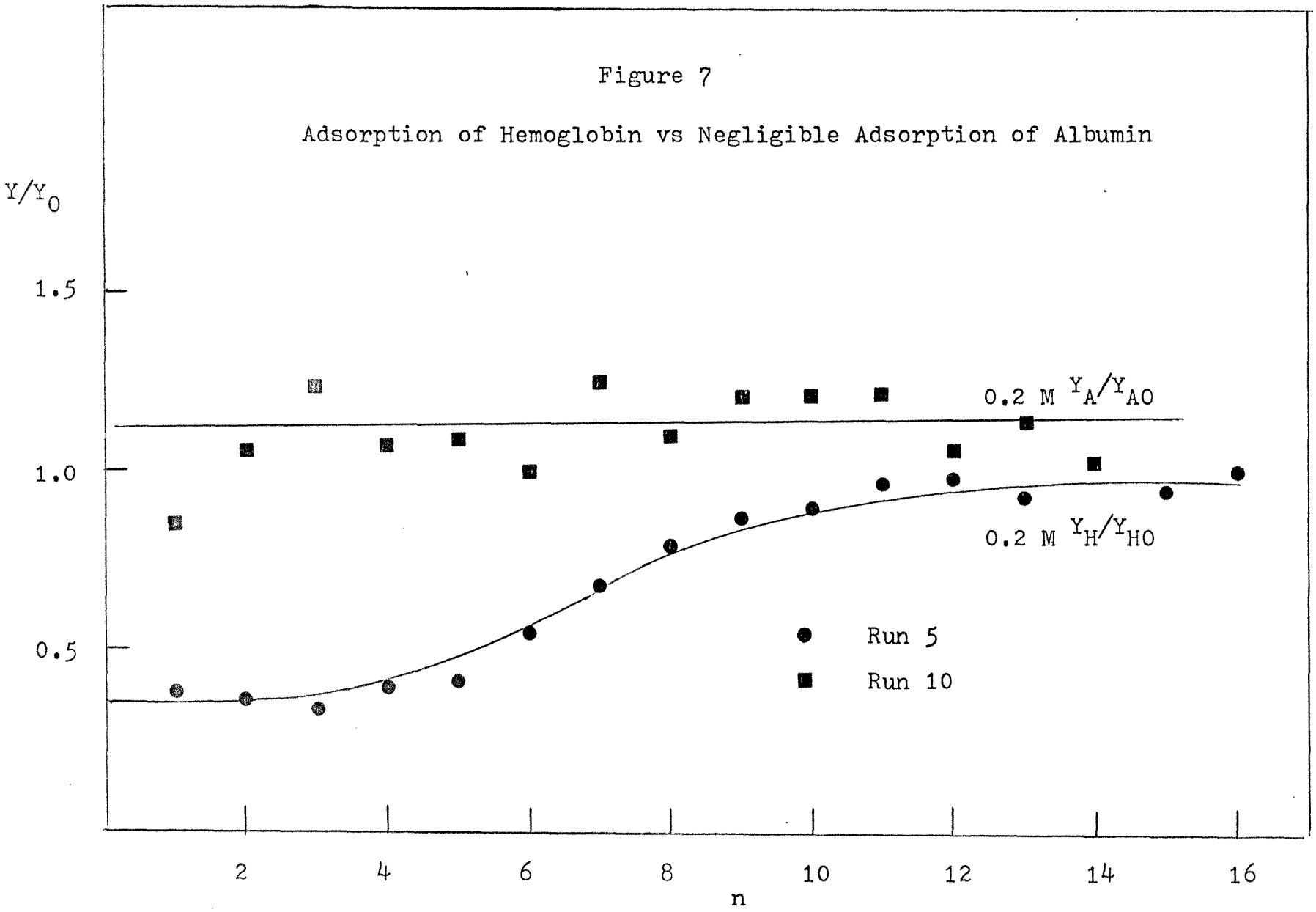


Figure 8

Separation Factors for Experiment 9

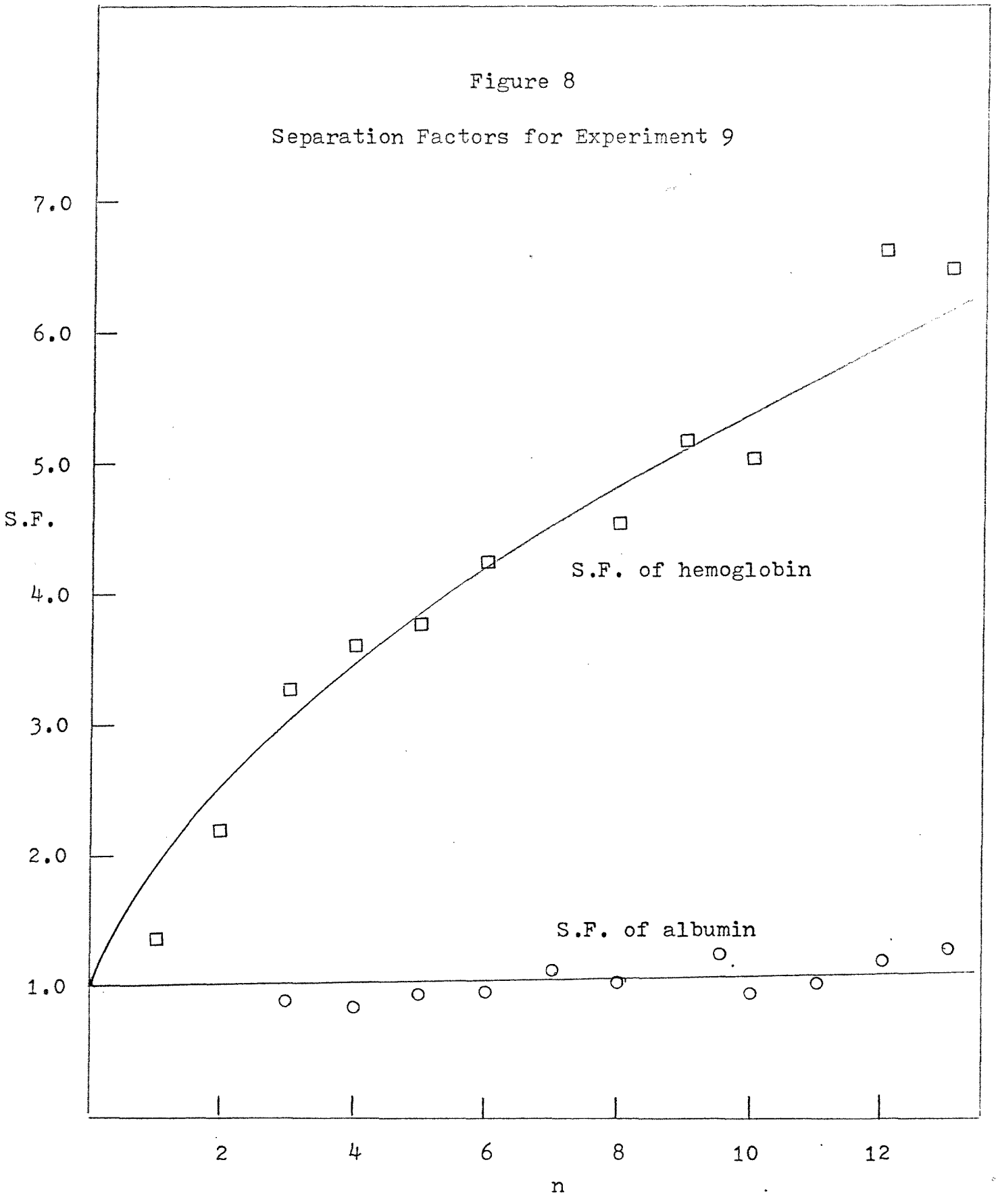


Figure 9

Effect of Concentration on Separation 0.035 M VS 0.15 M

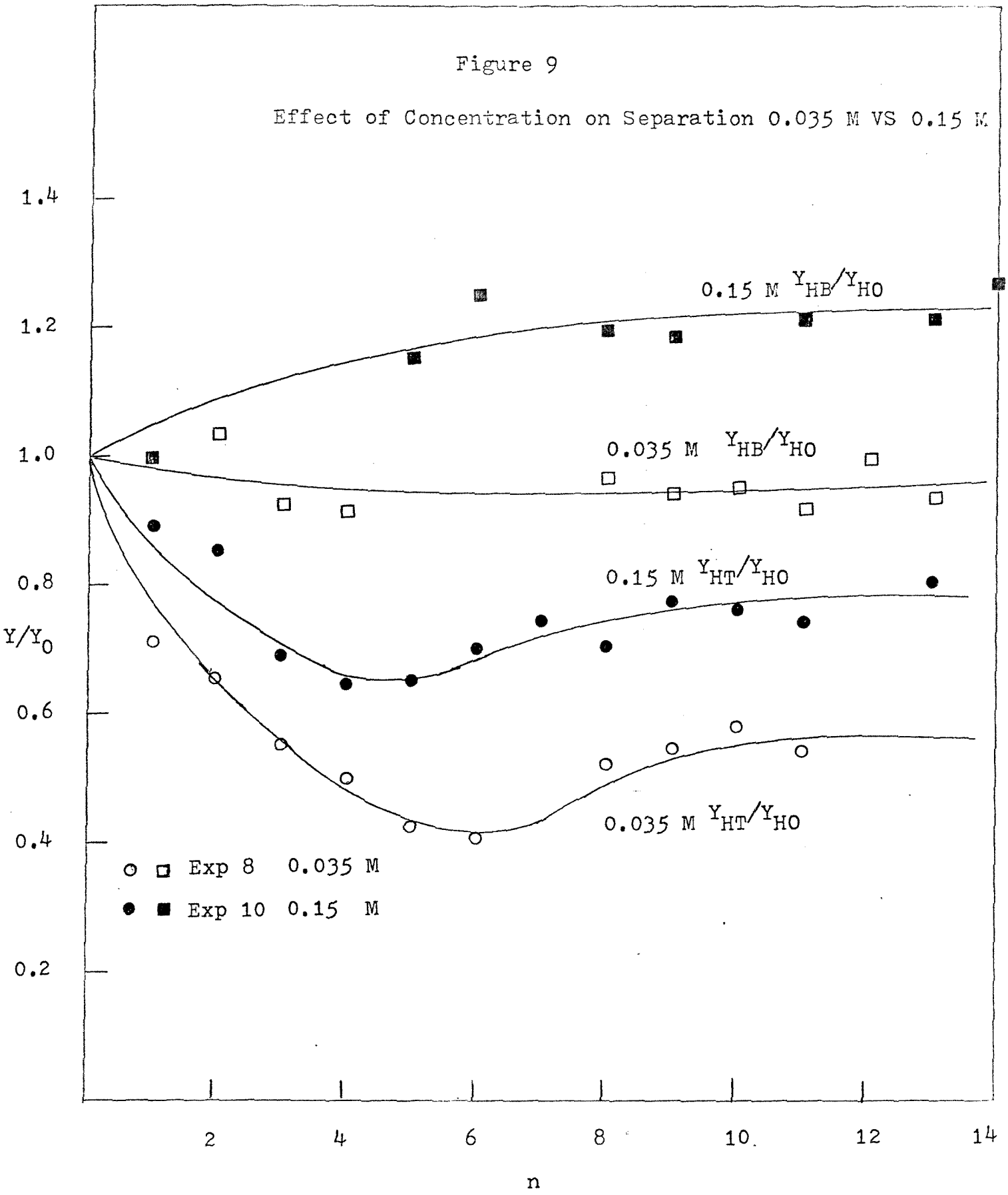


Figure 10

Effect of Concentration on Separation 0.035 M VS 0.20 M

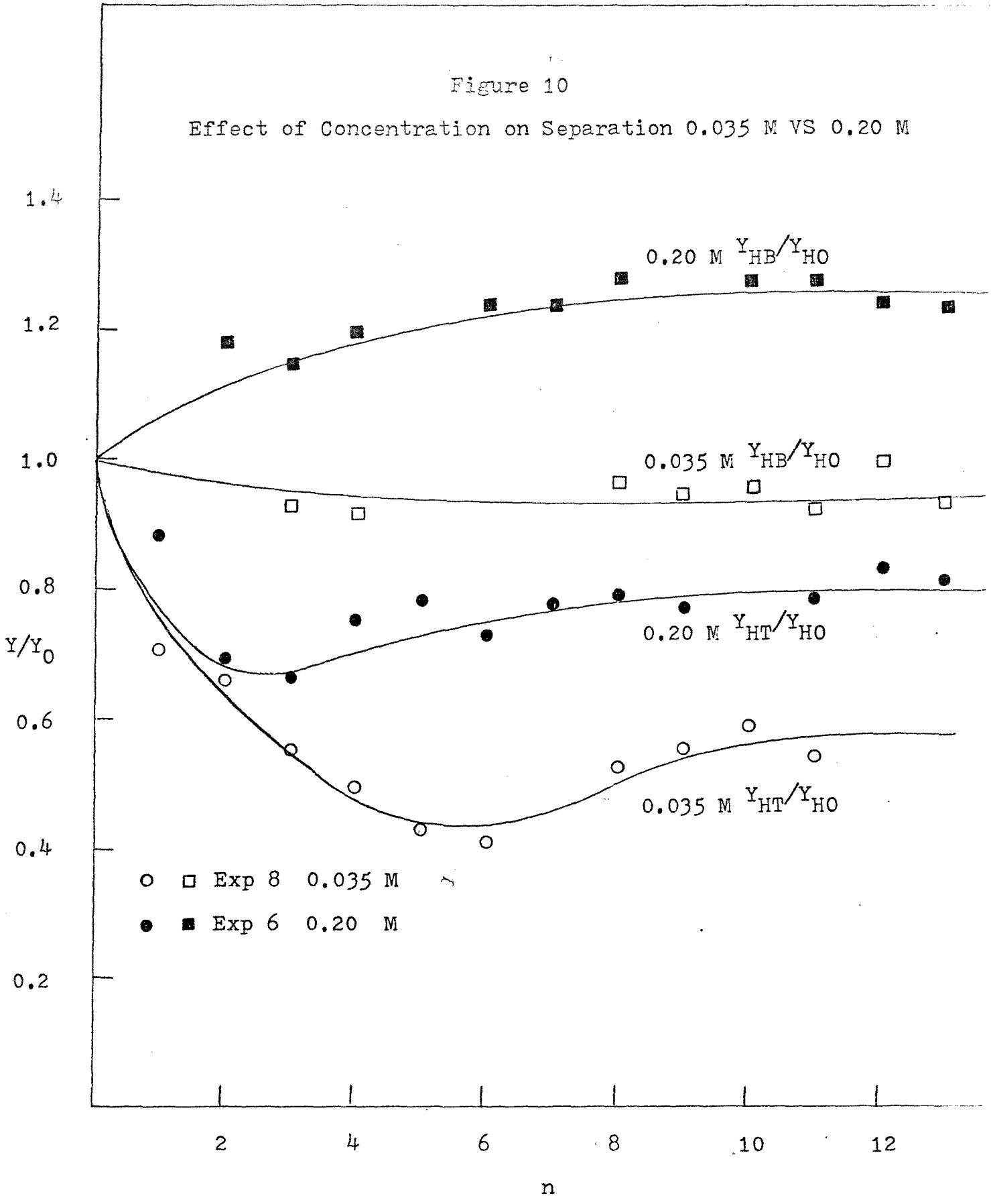


Figure 11

Separation of Hemoglobin in Experiment 6

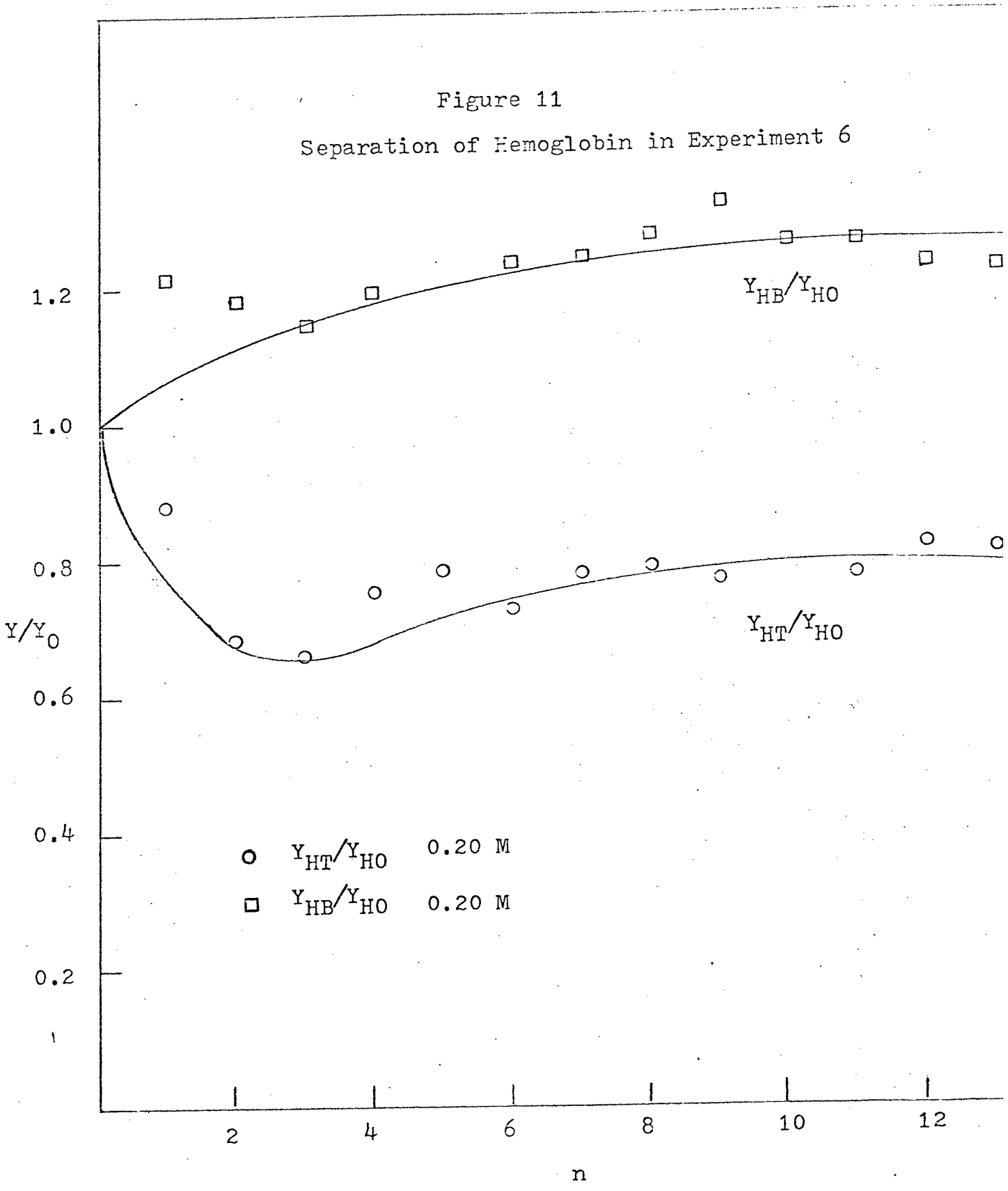


Figure 12

Separation of Hemoglobin in Experiment 10

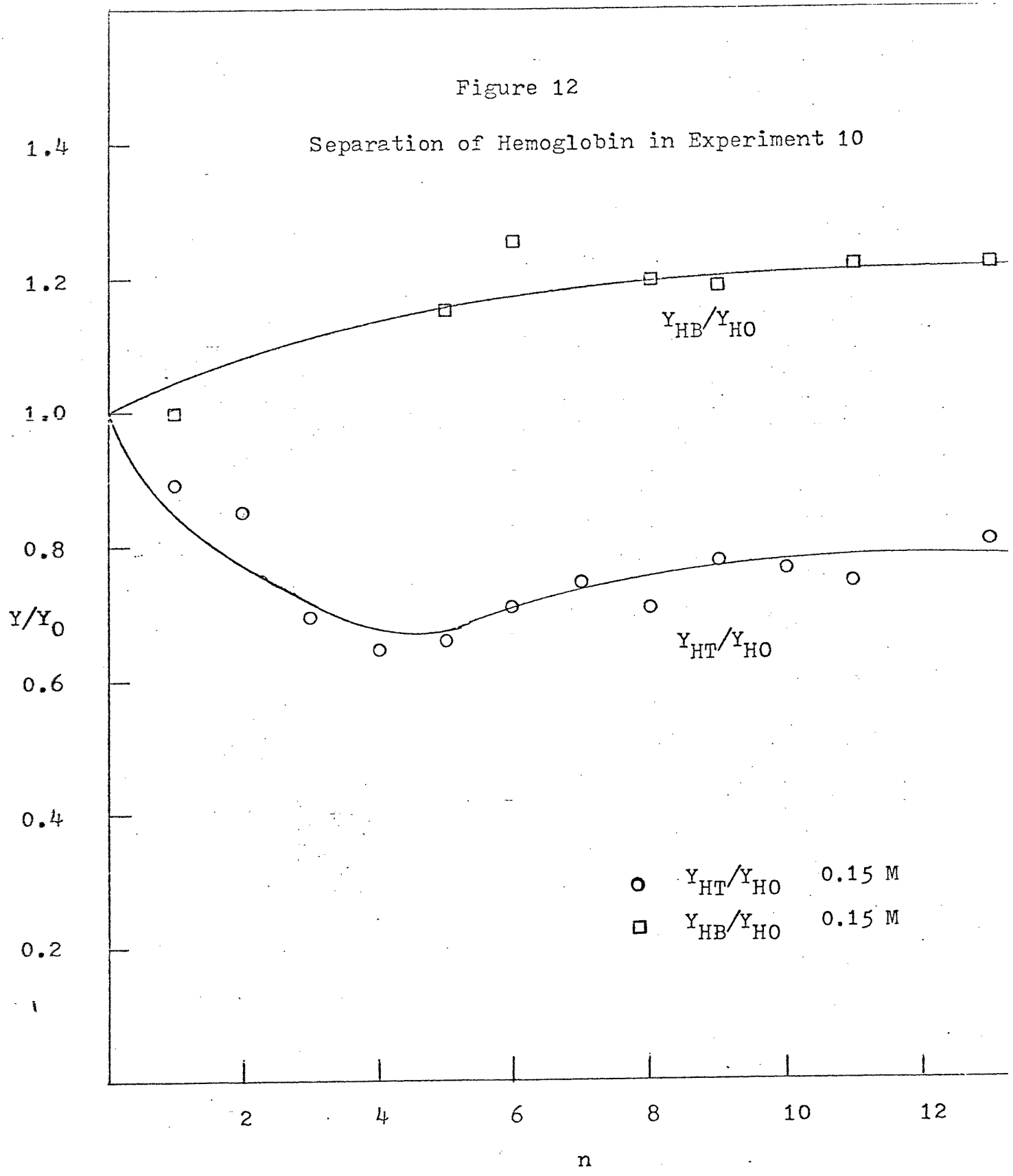


Figure 13

Separation of Hemoglobin in Experiment 8

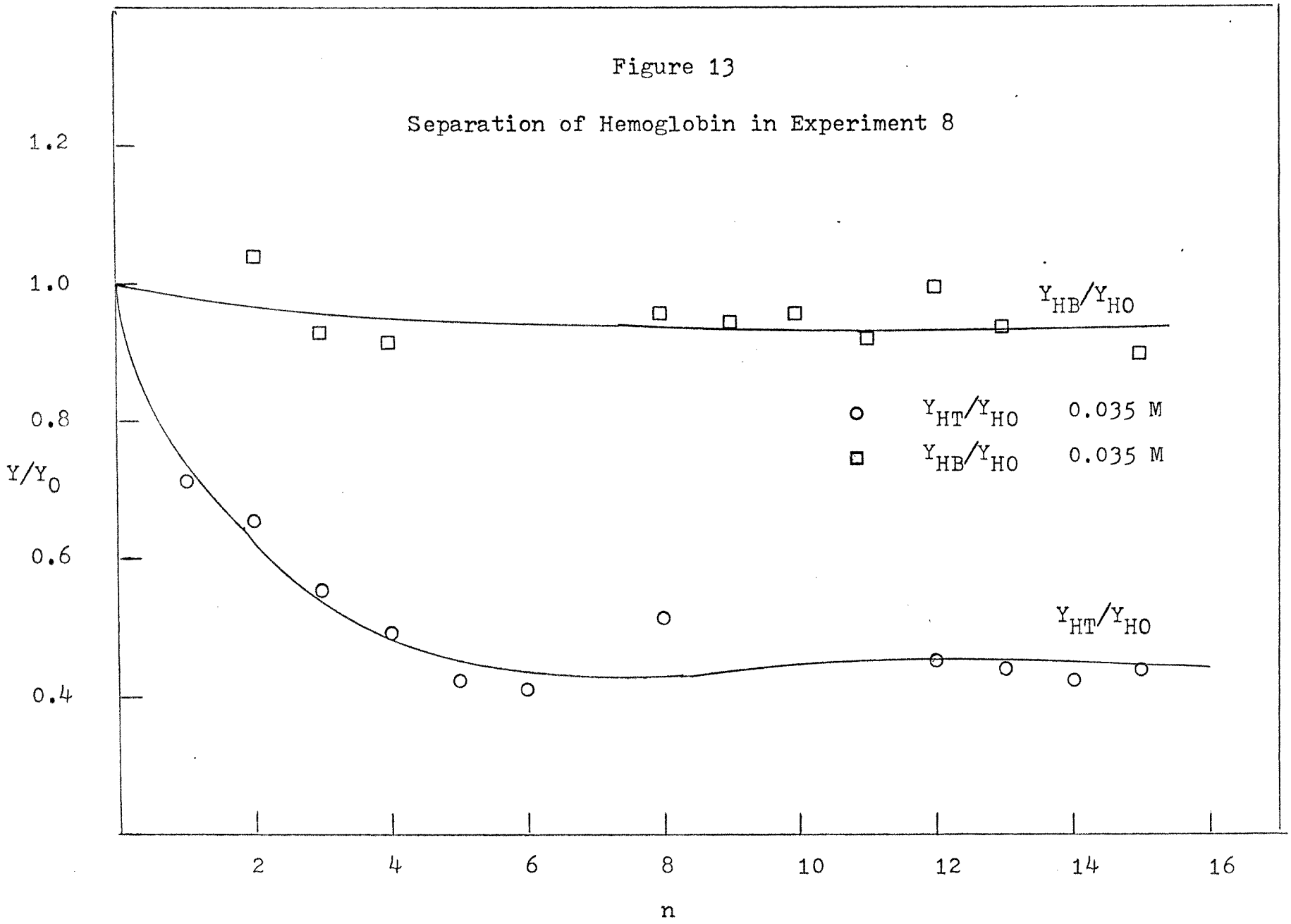


Figure 14

Effect of Concentration on Separation 0.035 M VS 0.100 M

(PH 6 Bottom Reservoir)

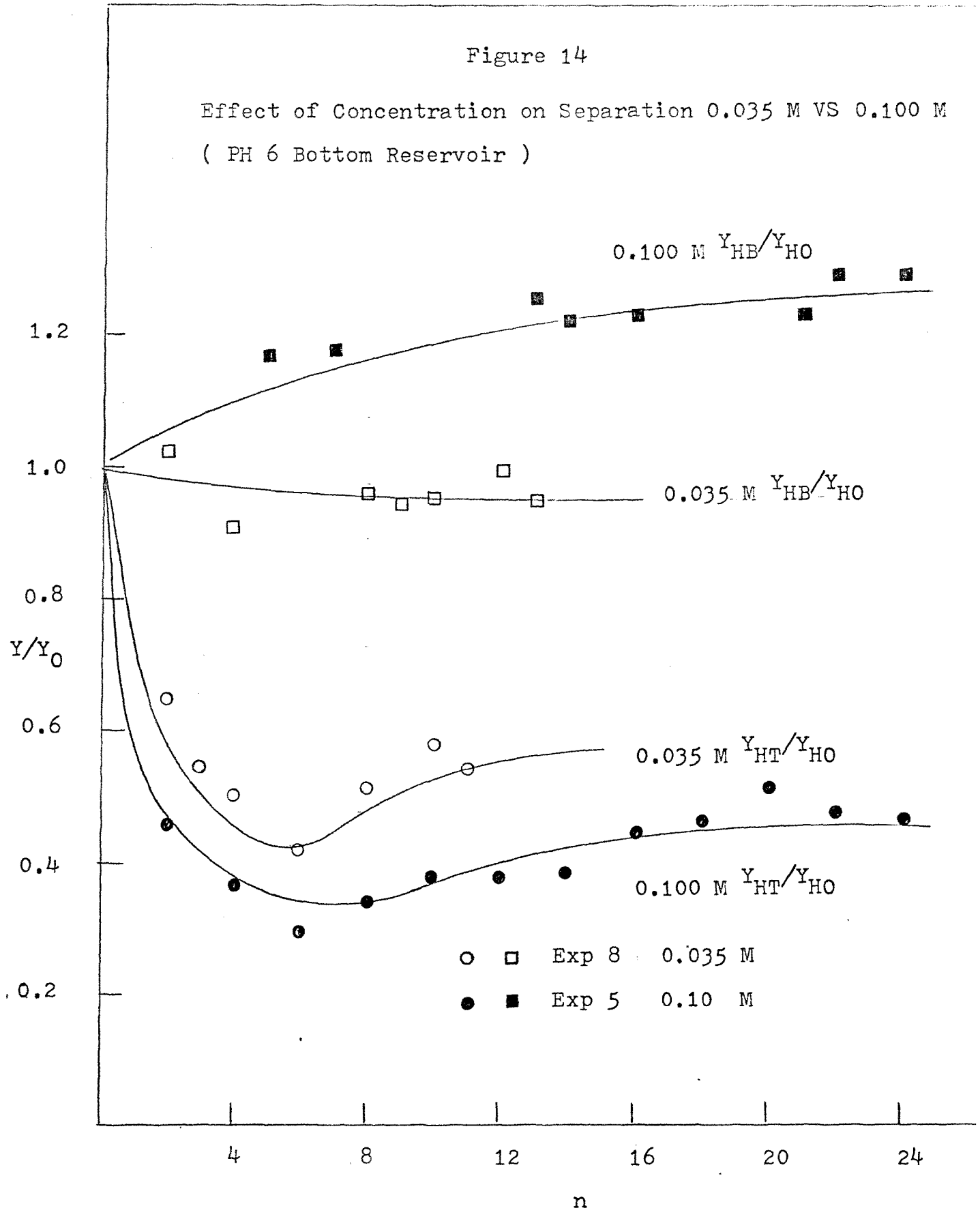


Figure 15

Effect of PH on Separation PH 8.9-4.9 VS PH 8.9-6 for 0.15 M Systems

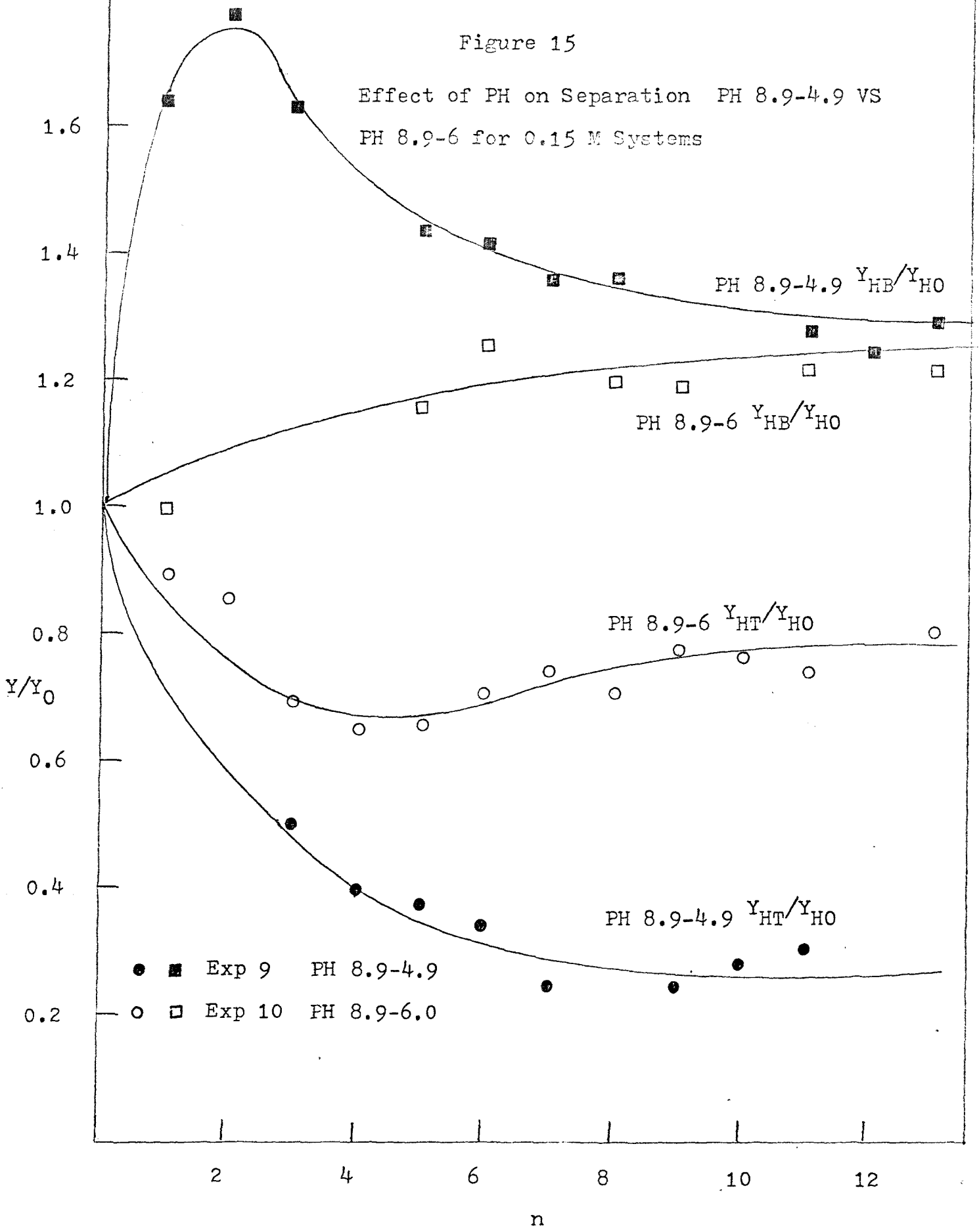


Figure 16

Comparison of S.F. between Exp. 9 and Exp. 10 for Hemoglobin

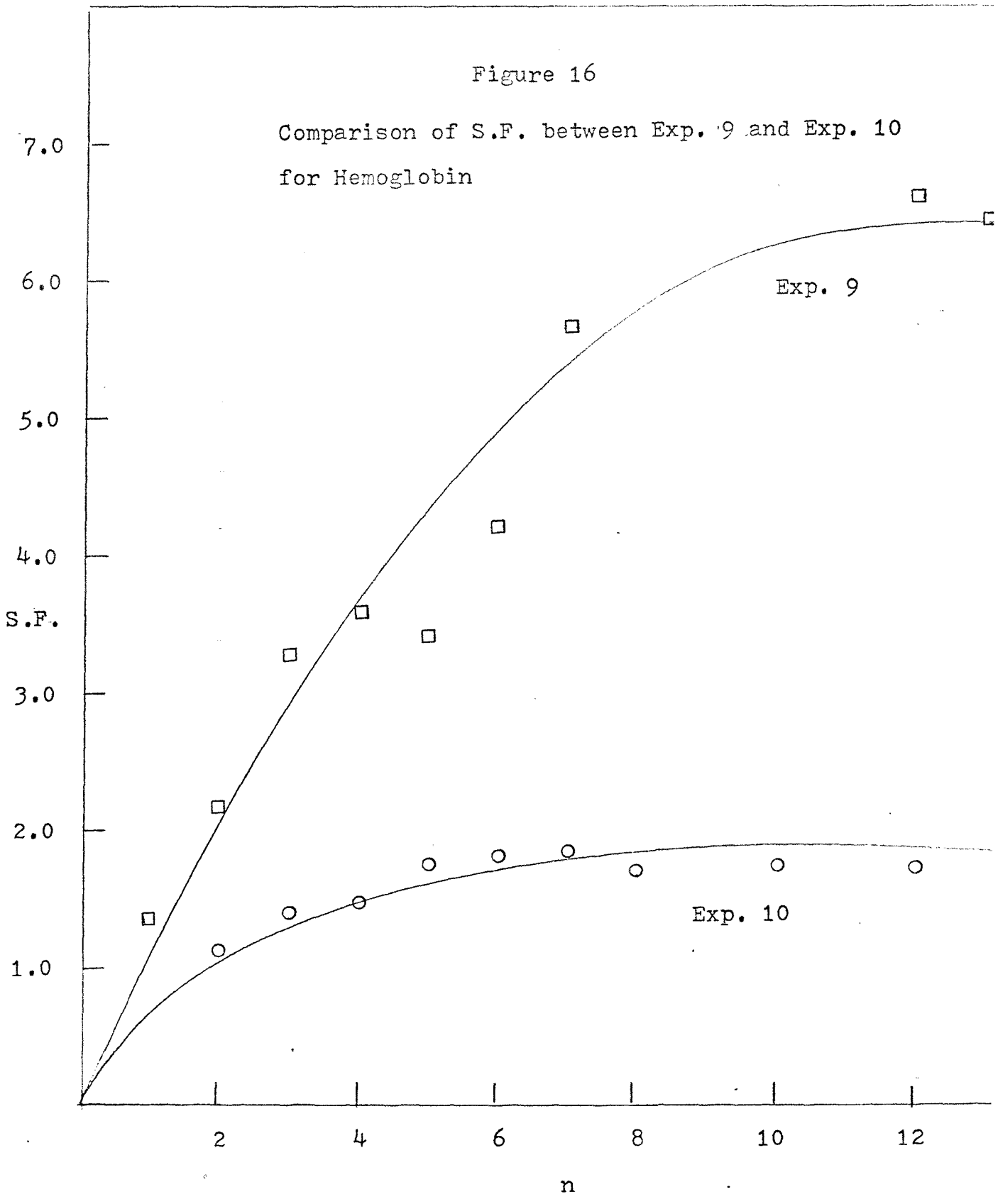


Figure 17

Effect of Concentration VS PH Spread on Separation (0.2 M VS 0.15 M with larger PH spread)

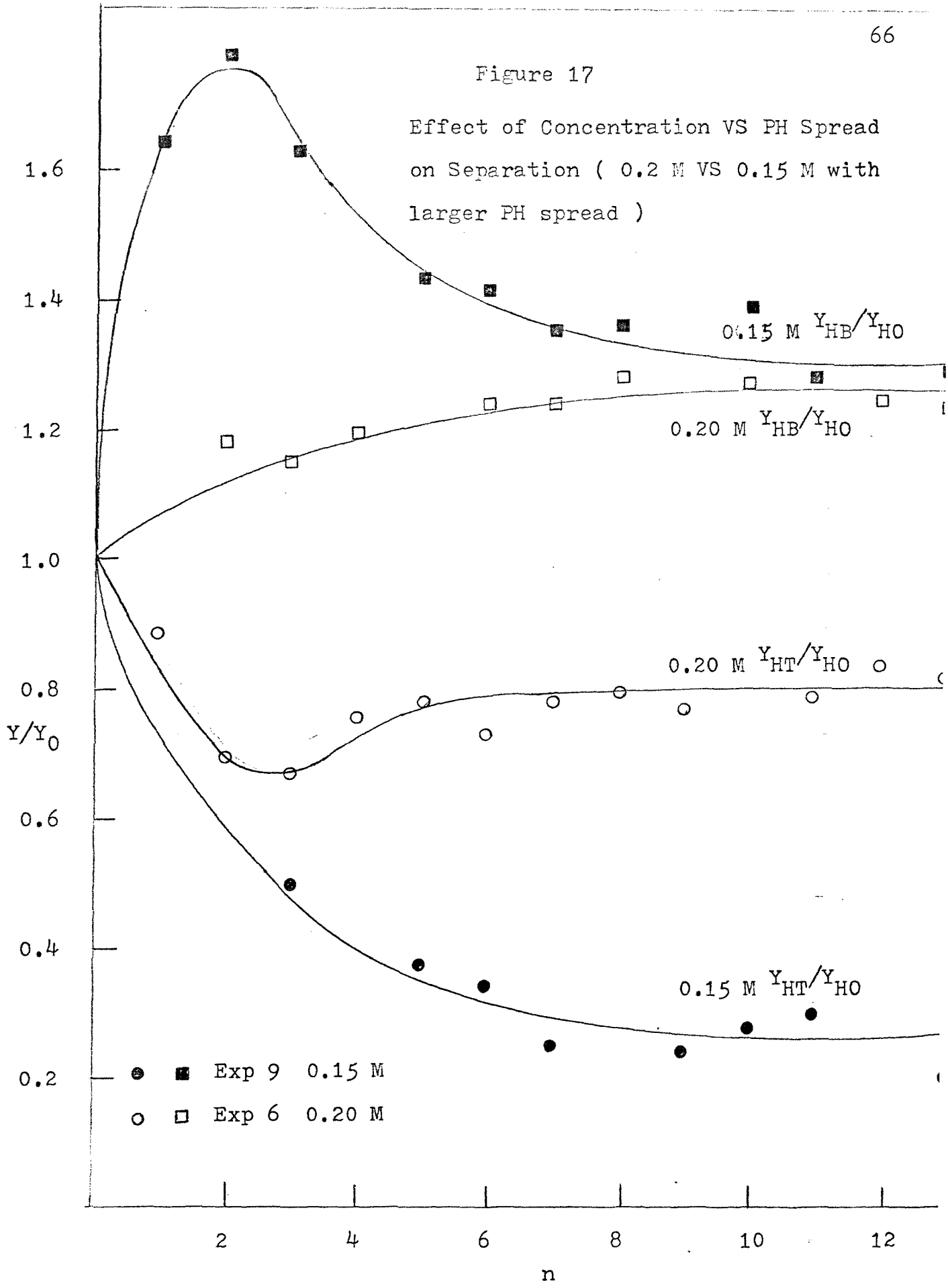


Figure 18

PH 8 Feed VS PH 6 Feed for 0.035 M Systems

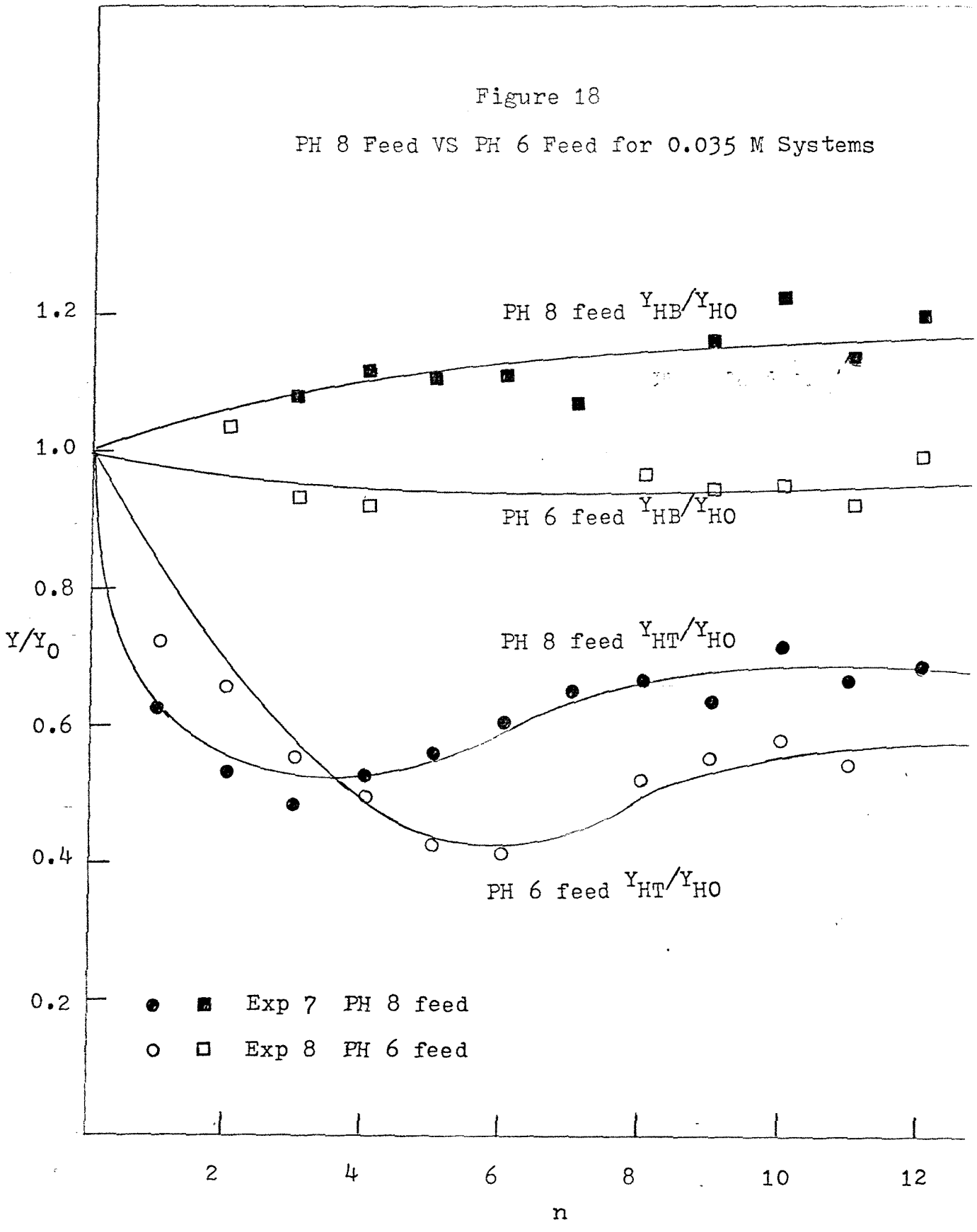


Figure 19

Separation Factors for PH 8 Feed System for Exp 7

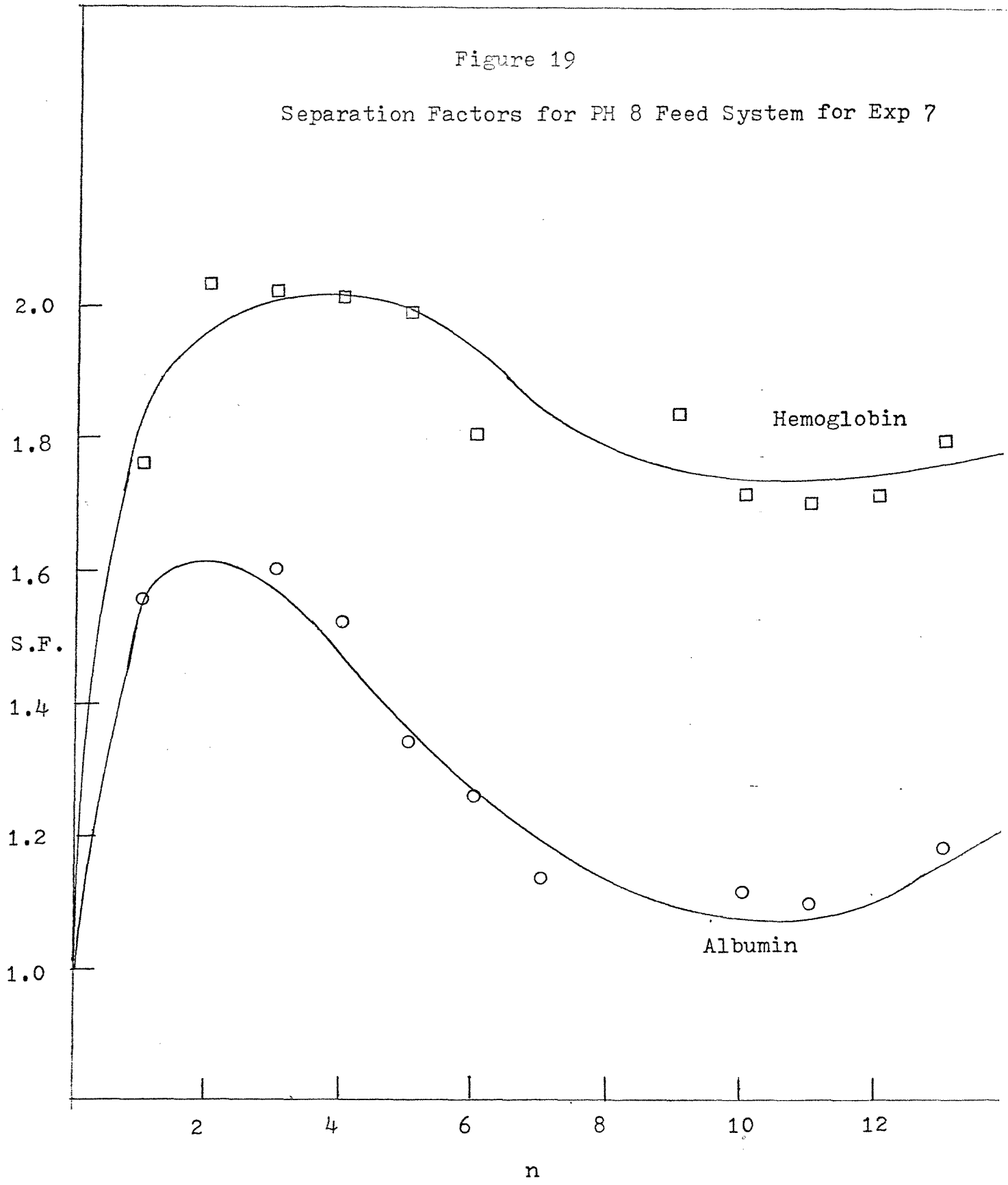


TABLE 8

STEP TESTING EXPERIMENTS RUN 1 403 MU

N	PH	CC	Trans.	Log Trans.	Y_H,%	Y_H/Y_{HO}
1	5.8	3.0	77.8	0.113	0.00250	0.257
2	5.8	3.0	74.8	0.155	0.00343	0.353
3	5.9	3.0	79.8	0.102	0.00226	0.232
4	6.1	3.0	13.0	0.915	0.02260	2.080
5	7.0	3.0	19.2	0.721	0.01600	1.648
6	7.7	3.0	36.0	0.474	0.01050	1.081
7	7.7	3.0	43.2	0.369	0.00816	0.840
8	7.7	3.0	40.2	0.425	0.00941	0.969
9	7.7	3.0	36.8	0.438	0.00970	0.999
10	7.7	3.0	41.2	0.408	0.00903	0.930
11	7.7	3.0	39.0	0.413	0.00914	0.941
12	7.7	3.0	42.0	0.406	0.00899	0.926
13	7.7	3.0	35.0	0.460	0.01080	1.112
14	7.7	3.0	37.0	0.461	0.01020	1.050
15	7.7	3.0	38.8	0.415	0.00919	0.946
16	7.7	3.0	41.0	0.416	0.00922	0.950

Conditions: Buffer Concentration: 0.15 M

PH of Feed: 8

Feed Concentration: Y_{HO}: 0.00971%

PH of Ion Exchanger (initially): 6

TABLE 9

STEP TESTING EXPERIMENTS RUN 2 403 MU

N	PH	CC	Trans.	Log Trans.	Y_H,%	Y_H/Y_{HO}
1	6.0	3.0	81.0	0.0496	0.00083	0.135
2	6.0	3.0	79.2	0.0673	0.00112	0.182
3	6.0	3.0	83.8	0.0348	0.00058	0.094
4	6.0	3.0	78.6	0.0749	0.00125	0.203
5	6.0	3.0	79.0	0.0605	0.00101	0.164
6	6.0	3.0	79.2	0.0555	0.00092	0.149
7	6.0	3.0	80.8	0.0716	0.00119	0.193
8	6.0	3.0	82.0	0.0554	0.00092	0.149
9	6.0	3.0	77.0	0.0716	0.00119	0.193
10	6.0	3.0	72.0	0.1130	0.00188	0.305
11	6.0	3.0	74.0	0.0899	0.00148	0.240
12	6.0	3.0	72.2	0.1120	0.00186	0.302
13	6.0	3.0	58.5	0.1910	0.00318	0.516
14	6.0	3.0	70.2	0.1240	0.00207	0.336
15	6.0	3.0	70.2	0.1120	0.00186	0.302
16	6.0	3.0	67.0	0.1440	0.00240	0.390

Conditions: Buffer Concentration: 0.15 M

PH of Feed: 6

Feed Concentration: Y_{HO}: 0.00616%

PH of Ion Exchanger (initially): 6

Table 9 Continued

N	PH	CC	Trans.	Log Trans.	Y_H,%	Y_H/Y_{HO}
17	6.0	3.0	67.0	0.1320	0.00220	0.357
18	6.0	3.0	64.2	0.1630	0.00271	0.440
19	6.0	3.0	64.5	0.1490	0.00248	0.403
20	6.0	3.0	59.5	0.1960	0.00326	0.529
21	6.0	3.0	63.8	0.1530	0.00255	0.414
22	6.0	3.0	61.2	0.1830	0.00306	0.497
23	6.0	3.0	66.4	0.1360	0.00227	0.369
24	6.0	3.0	65.2	0.1560	0.00260	0.422
25	6.0	3.0	66.0	0.1390	0.00231	0.375
26	6.0	3.0	66.8	0.1460	0.00243	0.394
27	6.0	3.0	67.8	0.1270	0.00211	0.343
28	6.0	3.0	66.8	0.1480	0.00246	0.399
29	6.0	3.0	63.2	0.1570	0.00262	0.425
30	6.0	3.0	61.2	0.1850	0.00308	0.508
31	6.0	3.0	57.8	0.1960	0.00327	0.531
32	6.0	3.0	57.0	0.2140	0.00357	0.580

TABLE 10

STEP TESTING EXPERIMENTS RUN 3 403 MU

N	PH	CC	Trans.	Log Trans.	Y_H,%	Y_H/Y_{HO}
1	7.5	3.0	68.0	0.160	0.00266	0.296
2	7.0	3.0	70.5	0.134	0.00223	0.249
3	6.3	3.0	76.5	0.108	0.00181	0.201
4	6.1	3.0	90.0	0.028	0.00047	0.052
5	6.1	3.0	95.8	0.011	0.00018	0.020
6	6.1	3.0	83.2	0.062	0.00103	0.115
7	6.0	3.0	81.8	0.079	0.00132	0.147
8	6.0	3.0	92.5	0.016	0.00027	0.030
9	6.0	3.0	84.0	0.068	0.00173	0.126
10	6.0	3.0	91.2	0.022	0.00037	0.041
11	6.0	3.0	85.8	0.059	0.00098	0.109
12	6.0	3.0	79.0	0.085	0.00141	0.157
13	6.0	3.0	77.0	0.106	0.00176	0.196
14	6.0	3.0	76.0	0.101	0.00169	0.188
15	6.0	3.0	65.0	0.179	0.00299	0.332
16	6.0	3.0	70.0	0.137	0.00229	0.254
17	6.0	3.0	65.8	0.174	0.00290	0.322
18	6.0	3.0	57.8	0.220	0.00367	0.408
19	6.0	3.0	53.8	0.261	0.00435	0.484
20	6.0	3.0	52.8	0.260	0.00449	0.500

Conditions: Buffer Concentration: 0.15 M

PH of Feed: 6

Feed Concentration: Y_{HO}: 0.00899%

PH of Ion Exchanger (initially): 8

TABLE 11

STEP TESTING EXPERIMENTS RUN 4 403 MU

N	PH	CC	Trans.	Log Trans.	Y_H,%	Y_H/Y_{HO}
1	6.0	3.0	82.0	0.112	0.00249	0.216
2	6.1	3.0	72.0	0.174	0.00384	0.335
3	6.7	3.0	36.5	0.463	0.01030	0.894
4	7.3	3.0	13.5	0.901	0.01990	1.736
5	7.8	3.0	34.0	0.495	0.01090	0.953
6	7.8	3.0	39.5	0.434	0.00961	0.837
7	7.8	3.0	39.2	0.432	0.00958	0.834
8	7.8	3.0	35.2	0.484	0.01070	0.932
9	7.8	3.0	35.8	0.472	0.01050	0.910
10	7.8	3.0	33.8	0.502	0.01110	0.968
11	7.8	3.0	32.8	0.510	0.01130	0.984
12	7.8	3.0	34.8	0.489	0.01083	0.943

Conditions: Buffer Concentrations: 0.2 M

PH of Feed: 8

Feed Concentration: Y_{HO}: 0.01148%

PH of Ion Exchanger (initially): 6

TABLE 12

STEP TESTING EXPERIMENTS RUN 5 403 MU

N	PH	CC	Trans.	Log Trans.	Y_H,%	Y_H/Y_{HO}
1	6.0	3.0	68.0	0.165	0.00275	0.385
2	6.0	3.0	69.0	0.159	0.00265	0.371
3	6.0	3.0	71.0	0.147	0.00245	0.343
4	6.0	3.0	66.5	0.175	0.00292	0.408
5	6.0	3.0	66.0	0.178	0.00297	0.416
6	6.0	3.0	58.0	0.234	0.00391	0.547
7	6.0	3.0	50.8	0.292	0.00487	0.682
8	6.0	3.0	45.5	0.340	0.00566	0.793
9	6.0	3.0	42.2	0.373	0.00620	0.870
10	6.0	3.0	41.0	0.385	0.00642	0.899
11	6.0	3.0	38.0	0.418	0.00697	0.976
12	6.0	3.0	37.8	0.420	0.00701	0.981
13	6.0	3.0	39.5	0.401	0.00669	0.937
14	6.0	3.0	37.0	0.430	0.00716	1.002
15	6.0	3.0	39.0	0.407	0.00678	0.949
16	6.0	3.0	36.5	0.435	0.00726	1.016
17	6.0	3.0	37.8	0.420	0.00701	0.981
18	6.0	3.0	36.0	0.441	0.00736	1.031
19	6.0	3.0	38.0	0.418	0.00697	0.976
20	6.0	3.0	37.2	0.427	0.00712	0.997

Conditions: Buffer Concentration: 0.2 M

PH of Feed: 6

Feed Concentration: Y_{HO}: 0.00714%

PH of Ion Exchanger (initially): 6

TABLE 13

STEP TESTING EXPERIMENTS RUN 6 403 MU

N	PH	CC	Trans.	Log Trans.	Y_H,%	Y_H/Y_{HO}
1	7.6	3.0	75.0	0.123	0.00205	0.287
2	7.3	3.0	72.0	0.140	0.00234	0.328
3	6.5	3.0	84.0	0.074	0.00123	0.172
4	6.0	3.0	85.0	0.068	0.00114	0.160
5	6.1	3.0	80.8	0.090	0.00151	0.211
6	6.0	3.0	70.0	0.153	0.00255	0.357
7	6.1	3.0	62.0	0.205	0.00342	0.480
8	6.0	3.0	59.0	0.227	0.00378	0.530
9	6.0	3.0	53.2	0.272	0.00453	0.635
10	6.0	3.0	51.0	0.290	0.00484	0.678
11	6.0	3.0	48.3	0.314	0.00523	0.733
12	6.0	3.0	51.0	0.290	0.00484	0.678
13	6.0	3.0	49.6	0.302	0.00504	0.706
14	6.0	3.0	47.4	0.322	0.00537	0.752
15	6.0	3.0	44.0	0.354	0.00591	0.827
16	6.0	3.0	47.8	0.318	0.00531	0.743
17	6.0	3.0	45.8	0.337	0.00562	0.787
18	6.0	3.0	45.5	0.340	0.00566	0.793
19	6.0	3.0	47.0	0.326	0.00543	0.760
20	6.0	3.0	44.8	0.347	0.00578	0.809

Conditions: Buffer Concentration: 0.2 M

PH of Feed: 6

Feed Concentration: Y_{HO}: 0.00714%

PH of Ion Exchanger (initially): 8

TABLE 14

STEP TESTING EXPERIMENTS RUN 7 280 MU

N	PH	CC	Trans.	Log Trans.	Y_A,%	Y_A/Y_{AO}
1	8.0	3.0	49.0	0.278	0.0526	1.008
2	8.0	3.0	43.6	0.338	0.0639	1.225
3	8.0	3.0	38.0	0.389	0.0735	1.408
4	8.0	3.0	39.0	0.338	0.0639	1.225
5	8.0	3.0	35.0	0.424	0.0802	1.537
6	8.0	3.0	47.5	0.300	0.0567	1.087
7	8.0	3.0	41.0	0.356	0.0672	1.288
8	8.0	3.0	49.0	0.287	0.0544	1.041
9	8.0	3.0	43.2	0.333	0.0629	1.206
10	8.0	3.0	48.0	0.296	0.0560	1.074
11	8.0	3.0	44.0	0.325	0.0614	1.177
12	8.0	3.0	45.0	0.324	0.0613	1.175

Conditions: Buffer Concentration: 0.2 M

PH of Feed: 8

Feed Concentration: Y_{AO}: 0.0522%

PH of Ion Exchanger (initially): 8

TABLE 15

STEP TESTING EXPERIMENTS RUN 8 280 MU

N	PH	CC	Trans.	Log Trans.	Y_A,%	Y_A/Y_{AO}
1	7.6	3.0	52.5	0.224	0.0424	0.885
2	7.6	3.0	52.0	0.238	0.0450	0.940
3	7.8	3.0	41.0	0.332	0.0627	1.309
4	7.8	3.0	44.5	0.306	0.0578	1.207
5	7.8	3.0	45.0	0.291	0.0551	1.149
6	7.8	3.0	47.0	0.282	0.0533	1.113
7	7.8	3.0	46.0	0.281	0.0533	1.113
8	7.8	3.0	42.5	0.326	0.0616	1.286
9	7.8	3.0	40.5	0.337	0.0637	1.330
10	7.8	3.0	47.0	0.282	0.0533	1.113
11	7.8	3.0	41.0	0.332	0.0627	1.309
12	7.8	3.0	48.0	0.273	0.0516	1.077
13	7.8	3.0	40.5	0.337	0.0637	1.330

Conditions: Buffer Concentration: 0.2 M

PH of Feed: 8

Feed Concentration: Y_{AO}: 0.0479%

PH of Ion Exchanger (initially): 6

TABLE 16

STEP TESTING EXPERIMENTS RUN 9 280 MU

N	PH	CC	Trans.	Log Trans.	Y_A,%	Y_A/Y_{AO}
1	5.5	3.0	62.0	0.152	0.0288	0.600
2	6.7	3.0	38.0	0.374	0.0708	1.478
3	7.5	3.0	39.0	0.353	0.0668	1.395
4	7.6	3.0	45.0	0.301	0.0569	1.188
5	7.6	3.0	44.0	0.301	0.0569	1.188
6	7.7	3.0	51.0	0.247	0.0466	0.973
7	7.7	3.0	45.0	0.291	0.0551	1.149
8	7.7	3.0	47.0	0.282	0.0533	1.113
9	7.7	3.0	47.0	0.272	0.0514	1.075
10	7.7	3.0	46.0	0.291	0.0551	1.150
11	7.7	3.0	41.0	0.332	0.0627	1.309
12	7.7	3.0	42.0	0.341	0.0645	1.348
13	7.7	3.0	45.0	0.291	0.0551	1.149
14	7.7	3.0	50.0	0.255	0.0483	1.007

Conditions: Buffer Concentration: 0.2 M

PH of Feed: 8

Feed Concentration: Y_{AO}: 0.0479%

PH of Ion Exchanger (initially): 4.9

TABLE 17

STEP TESTING EXPERIMENTS RUN 10 280 MU

N	PH	CC	Trans.	Log Trans.	Y_A,%	Y_A/Y_{AO}
1	6.0	3.0	58.2	0.239	0.0453	0.858
2	6.0	3.0	48.0	0.299	0.0566	1.071
3	6.0	3.0	45.5	0.346	0.0655	1.240
4	6.0	3.0	47.8	0.301	0.0569	1.078
5	6.0	3.0	50.0	0.305	0.0577	1.093
6	6.0	3.0	50.0	0.381	0.0532	1.008
7	6.0	3.0	45.0	0.351	0.0664	1.257
8	6.0	3.0	47.0	0.308	0.0583	1.104
9	6.0	3.0	46.2	0.339	0.0642	1.216
10	6.0	3.0	44.0	0.337	0.0637	1.207
11	6.0	3.0	46.0	0.341	0.0646	1.223
12	6.0	3.0	48.0	0.299	0.0566	1.071
13	6.0	3.0	48.5	0.319	0.0602	1.141
14	6.0	3.0	49.5	0.288	0.0545	1.033

Conditions: Buffer Concentration: 0.2 M

PH of Feed: 6

Feed Concentration: Y_{AO}: 0.0528%

PH of Ion Exchanger (initially): 6

CYCLING ZONE EXPERIMENT 280 MU

N	PH	CC	Log Trans.	Y_A,%	Y_A/Y_{AO}
1	6.0	2.0	- - - -	- - - -	- - - -
2	6.0	2.0	0.113	0.0214	1.363
3	6.0	2.0	0.038	0.0072	0.459
4	6.0	2.0	0.093	0.0176	1.121
5	6.0	2.0	0.105	0.0198	1.261
6	6.0	2.0	0.093	0.0176	1.121
7	6.0	2.0	0.072	0.0136	0.866
8	6.0	2.0	0.080	0.0151	0.962
9	5.9	2.0	0.076	0.0144	0.917
10	5.9	2.0	0.071	0.0132	0.764
11	5.9	2.0	0.092	0.0174	1.108
12	5.9	2.0	0.081	0.0153	0.974
13	5.9	2.0	0.082	0.0155	0.987
14	5.9	2.0	0.086	0.0163	1.038
15	5.9	2.0	0.106	0.0200	1.274
16	6.0	2.0	0.093	0.0176	1.121
17	6.0	2.0	0.106	0.0200	1.274
18	6.0	2.0	0.086	0.0163	1.038
19	6.0	2.0	0.113	0.0214	1.363
20	6.0	2.0	0.111	0.0210	1.338
21	6.2	2.0	0.087	0.0164	1.197
22	6.6	2.0	0.083	0.0137	1.000

280 MU

N	PH	CC	Log Trans.	Y_A,%	Y_A/Y_{AO}
23	7.4	2.0	0.100	0.0165	1.205
24	7.8	2.0	0.094	0.0155	1.131
25	7.8	2.0	0.104	0.0172	1.255
26	7.8	2.0	0.087	0.0144	1.051
27	7.8	2.0	0.103	0.0170	1.241
28	7.8	2.0	0.092	0.0152	1.109
29	7.8	2.0	0.101	0.0167	1.219
30	7.7	2.0	0.105	0.0173	1.263
31	7.5	2.0	0.107	0.0177	1.292
32	6.9	2.0	0.102	0.0168	1.226
33	6.0	2.0	0.103	0.0195	1.242
34	6.0	2.0	0.110	0.0208	1.325
35	6.0	2.0	0.091	0.0172	1.096
36	6.0	2.0	0.086	0.0163	1.038
37	6.0	2.0	0.103	0.0195	1.242
38	6.0	2.0	0.081	0.0153	0.975
39	6.0	2.0	0.077	0.0146	0.930
40	6.0	2.0	0.089	0.0168	1.070
41	6.1	2.0	0.081	0.0153	1.117
42	6.1	2.0	0.083	0.0157	1.146
43	6.4	2.0	0.107	0.0202	1.474
44	7.0	2.0	0.094	0.0155	1.131

280 MU

N	PH	CC	Log Trans.	Y_A,%	Y_A/Y_{AO}
45	7.5	2.0	0.084	0.0139	1.015
46	7.6	2.0	0.082	0.0135	0.985
47	7.7	2.0	0.077	0.0127	0.927
48	7.7	2.0	0.080	0.0132	0.964
49	7.8	2.0	0.092	0.0152	1.109
50	7.8	2.0	0.120	0.0198	1.445
51	7.7	2.0	0.083	0.0137	1.000
52	7.6	2.0	0.128	0.0211	1.540
53	7.4	2.0	0.098	0.0162	1.182
54	6.8	2.0	0.101	0.0167	1.219
55	6.2	2.0	0.123	0.0203	1.482
56	5.9	2.0	0.083	0.0157	1.000
57	5.9	2.0	0.082	0.0155	0.987
58	5.9	2.0	0.107	0.0202	1.287
59	5.9	2.0	0.089	0.0168	1.070
60	5.9	2.0	0.067	0.0127	0.809
61	6.0	2.0	0.116	0.0219	1.395
62	5.9	2.0	0.122	0.0231	1.471
63	5.9	2.0	0.095	0.0180	1.146
64	5.9	2.0	0.128	0.0242	1.541
65	6.1	2.0	0.081	0.0134	0.978
66	6.8	2.0	0.120	0.0198	1.445

TABLE 18

PARAMETRIC PUMPING EXPERIMENT 1

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.2	2.9	32.0	0.478	0.00797	0.876
2	6.1	3.0	35.8	0.422	0.00703	0.733
3	6.0	3.0	42.0	0.360	0.00600	0.660
4	6.0	3.0	48.0	0.294	0.00490	0.538
5	6.0	3.0	49.8	0.285	0.00477	0.524
6	6.2	3.0	42.0	0.352	0.00587	0.645
7	6.0	3.0	38.8	0.394	0.00657	0.722
8	6.2	3.0	38.8	0.394	0.00657	0.722
9	6.2	2.8	43.2	0.340	0.00567	0.623
10	6.2	2.8	43.2	0.340	0.00567	0.623

Experimental Conditions:

Buffer Concentration: 0.035 M, Cycle Time: 72 min.

Displacement: 18 CC, Feed PH: 6

Reservoir PH: Top: 6 Bottom: 8

Feed Conc.: Y_{HO}: 0.00910%, Y_{AO}: 0.0660%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	7.1	3.0	39.2	0.398	0.00881	0.968
2	7.0	3.0	47.4	0.312	0.00690	0.758
3	7.0	3.0	45.0	0.338	0.00748	0.822
4	7.2	3.0	47.0	0.316	0.00698	0.767
5	7.3	3.0	48.7	0.303	0.00672	0.738
6	7.4	3.0	44.5	0.339	0.00750	0.824
7	7.6	3.0	45.0	0.338	0.00747	0.820
8	7.6	3.0	45.2	0.333	0.00736	0.809
9	7.6	3.0	45.7	0.331	0.00733	0.805
10	7.6	3.0	45.2	0.333	0.00736	0.809

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.2	2.9	27.0	0.578	0.0765	1.159
2	6.1	3.0	27.0	0.578	0.0765	1.159
3	6.0	3.0	32.0	0.479	0.0659	0.998
4	6.0	3.0	32.0	0.479	0.0659	0.998
5	6.0	3.0	41.4	0.392	0.0710	1.076
6	6.2	3.0	41.4	0.392	0.0710	1.076
7	6.0	3.0	38.8	0.398	0.0483	0.732
8	6.2	3.0	38.8	0.398	0.0483	0.732
9	6.2	3.0	43.0	0.376	0.0478	0.724
10	6.2	3.0	43.0	0.376	0.0478	0.724

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	7.1	3.0	22.0	0.667	0.0867	1.314
2	7.0	3.0	34.0	0.449	0.0558	0.845
3	7.0	3.0	38.2	0.428	0.0508	0.770
4	7.2	3.0	41.8	0.359	0.0408	0.618
5	7.3	3.0	36.0	0.454	0.0571	0.865
6	7.4	3.0	28.2	0.530	0.0676	1.024
7	7.6	3.0	27.8	0.566	0.0736	1.115
8	7.6	3.0	29.8	0.506	0.0640	0.970
9	7.6	3.0	31.8	0.478	0.0594	0.900
10	7.6	3.0	32.3	0.501	0.0633	0.959

TABLE 19

PARAMETRIC PUMPING EXPERIMENT 2

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.2	4.0	35.3	0.439	0.00732	0.808
2	6.3	4.0	33.2	0.472	0.00787	0.869
3	6.2	4.0	43.5	0.349	0.00582	0.642
4	6.2	4.0	41.6	0.373	0.00622	0.687
5	6.2	4.0	39.0	0.396	0.00660	0.728
6	6.2	4.0	43.5	0.354	0.00590	0.651
7	6.3	4.0	42.0	0.365	0.00608	0.671
8	6.2	4.0	43.9	0.351	0.00585	0.646
9	6.2	4.1	47.4	0.312	0.00520	0.574
10	6.2	4.0	46.0	0.330	0.00550	0.607
11	6.2	4.0	51.3	0.278	0.00463	0.511
12	6.2	4.0	51.5	0.281	0.00468	0.517

Table 19 Continued

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
13	6.2	4.0	54.0	0.255	0.00425	0.469
14	6.2	4.0	51.2	0.282	0.00473	0.522
15	6.2	4.0	53.4	0.260	0.00433	0.478
16	6.2	4.0	51.3	0.282	0.00470	0.519
17	6.2	4.0	53.8	0.257	0.00428	0.472
18	6.2	3.5	53.8	0.262	0.00436	0.481
19	6.2	3.7	56.2	0.238	0.00397	0.438
20	6.2	3.8	57.0	0.237	0.00395	0.436

Experimental Conditions:

Buffer Conc. : 0.035 M, Cycle Time: 72 min.

Displacement: 18 CC, Feed PH: 6

Reservoir PH: Top: 6, Bottom: 8

Feed Conc. : Y_{HO}: 0.00906%, Y_{AO}: 0.0522%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	7.4	3.5	43.9	0.347	0.00768	0.848
2	7.3	4.0	44.7	0.346	0.00766	0.845
3	7.4	4.0	40.9	0.378	0.00836	0.923
4	7.3	4.0	43.7	0.356	0.00788	0.870
5	7.4	4.0	41.7	0.370	0.00819	0.904
6	7.6	4.0	41.7	0.376	0.00832	0.918
7	7.6	4.0	42.1	0.366	0.00810	0.894
8	7.6	3.9	45.4	0.339	0.00750	0.828
9	7.6	3.7	38.7	0.402	0.00890	0.982
10	7.6	4.0	42.0	0.373	0.00825	0.911
11	7.6	4.1	40.7	0.382	0.00845	0.933
12	7.6	4.1	45.3	0.340	0.00752	0.830
13	7.6	4.0	36.0	0.474	0.00960	1.060
14	7.6	4.1	34.9	0.453	0.01000	1.103
15	7.7	4.0	39.9	0.389	0.00861	0.950
16	7.6	4.0	40.5	0.389	0.00861	0.950
17	7.6	4.0	42.0	0.367	0.00812	0.896
18	7.6	4.0	40.9	0.384	0.00850	0.938
19	7.6	4.0	46.4	0.323	0.00715	0.789
20	7.6	4.0	45.8	0.335	0.00741	0.818

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.2	4.0	22.3	0.652	0.0933	1.787
2	6.3	4.0	20.1	0.704	0.0880	0.686
3	6.2	4.0	38.5	0.415	0.0477	0.914
4	6.2	4.0	26.3	0.588	0.0748	1.433
5	6.2	4.0	24.5	0.610	0.0883	1.692
6	6.2	4.0	28.8	0.548	0.0795	1.523
7	6.3	4.0	33.0	0.481	0.0661	1.266
8	6.2	4.0	33.3	0.460	0.0630	1.207
9	6.2	4.1	35.1	0.455	0.0647	1.172
10	6.2	4.0	29.1	0.543	0.0802	1.536
11	6.2	4.0	42.8	0.369	0.0508	0.973
12	6.2	4.0	39.0	0.416	0.0595	1.140
13	6.2	4.0	44.7	0.350	0.0488	0.935
14	6.2	4.0	41.5	0.389	0.0542	1.038
15	6.2	4.0	39.5	0.403	0.0585	1.121
16	6.2	4.0	41.3	0.391	0.0547	1.048
17	6.2	4.0	46.1	0.336	0.0460	0.881
18	6.2	3.5	46.6	0.338	0.0461	0.883
19	6.2	3.7	49.5	0.305	0.0414	0.793
20	6.2	3.8	48.5	0.314	0.0432	0.828

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	7.4	3.5	31.8	0.496	0.0615	1.178
2	7.3	4.0	27.5	0.547	0.0699	1.339
3	7.4	4.0	28.7	0.541	0.0671	1.285
4	7.3	4.0	29.4	0.518	0.0573	1.098
5	7.4	4.0	28.8	0.540	0.0674	1.291
6	7.6	4.0	28.9	0.525	0.0646	1.238
7	7.6	4.0	32.0	0.493	0.0599	1.148
8	7.6	3.9	31.2	0.492	0.0613	1.174
9	7.6	3.7	23.8	0.622	0.0791	1.515
10	7.6	4.0	28.8	0.528	0.0653	1.251
11	7.6	4.1	26.8	0.569	0.0715	1.370
12	7.6	4.1	26.0	0.571	0.0743	1.423
13	7.6	4.0	21.3	0.670	0.0851	1.630
14	7.6	4.0	23.6	0.613	0.0747	1.431
15	7.7	4.0	26.2	0.580	0.0729	1.397
16	7.6	4.0	26.3	0.566	0.0706	1.352
17	7.6	4.0	31.0	0.507	0.0622	1.192
18	7.6	4.0	32.1	0.480	0.0567	1.086
19	7.6	4.0	36.9	0.431	0.0522	1.000
20	7.6	4.0	36.8	0.420	0.0497	0.952

TABLE 20

PARAMETRIC PUMPING EXPERIMENT 3

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.0	4.0	27.1	0.554	0.00923	0.932
2	6.0	4.2	31.9	0.482	0.00803	0.812
3	6.0	4.0	42.0	0.364	0.00606	0.612
4	6.1	4.0	54.8	0.247	0.00412	0.416
5	6.0	4.2	69.4	0.145	0.00242	0.245
6	6.0	4.0	75.2	0.110	0.00183	0.185
7	6.0	4.0	75.2	0.111	0.00184	0.186
8	6.0	4.0	78.0	0.094	0.00156	0.158
9	6.0	4.1	78.0	0.095	0.00158	0.159
10	6.0	4.0	79.0	0.088	0.00147	0.149
11	6.0	4.0	81.0	0.078	0.00130	0.132
12	6.0	4.0	82.4	0.070	0.00166	0.118
13	6.0	4.0	81.8	0.074	0.00123	0.125
14	6.0	4.0	81.0	0.077	0.00129	0.130
15	6.0	4.0	78.8	0.090	0.00150	0.152

Experimental Conditions:

Buffer Conc. : 0.10 M, Cycle Time: 104 min.

Displacement: 26 CC, Feed PH: 6

Reservoir PH: Top: 6, Bottom: 8.5

Feed Conc. : Y_{HO}: 0.00990%, Y_{AO}: 0.0532%

Hemoglobin-Top 403 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
16	6.0	4.0	72.0	0.129	0.00214	0.216
17	6.0	4.1	90.5	0.030	0.00050	0.051
18	6.0	4.0	90.0	0.032	0.00053	0.053
19	6.0	3.2	91.0	0.028	0.00046	0.047
20	6.0	4.0	88.8	0.037	0.00062	0.063
21	6.0	4.0	92.6	0.020	0.00034	0.034
22	6.0	4.0	94.6	0.010	0.00017	0.017
23	6.0	4.0	88.4	0.040	0.00067	0.068
24	6.0	3.9	95.0	0.008	0.00014	0.014
25	6.0	4.0	92.0	0.023	0.00038	0.039
26	6.0	4.0	91.5	0.024	0.00041	0.041
27	6.0	4.0	94.5	0.009	0.00015	0.015
28	6.0	4.0	95.0	0.008	0.00014	0.014
29	6.0	4.0	84.0	0.062	0.00104	0.105
30	6.0	4.0	89.0	0.036	0.00061	0.061

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	7.6	4.0	33.2	0.540	0.01190	1.202
2	7.3	4.1	62.0	0.249	0.00551	0.557
3	7.6	4.1	64.5	0.251	0.00556	0.561
4	7.8	4.2	63.6	0.238	0.00527	0.532
5	8.0	4.1	64.2	0.253	0.00560	0.565
6	8.2	4.0	61.2	0.255	0.00563	0.569
7	8.2	4.0	72.8	0.199	0.00439	0.444
8	8.2	4.0	70.0	0.196	0.00434	0.439
9	8.2	4.2	62.0	0.268	0.00594	0.600
10	8.2	4.0	62.0	0.249	0.00551	0.557
11	8.2	4.2	60.5	0.279	0.00617	0.623
12	8.0	4.0	43.5	0.403	0.00892	0.901
13	8.1	4.0	62.6	0.264	0.00584	0.590
14	8.0	4.0	58.2	0.276	0.00612	0.618
15	8.0	4.2	58.0	0.297	0.00658	0.664

Hemoglobin-Bottom 403 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
16	8.0	4.1	55.4	0.298	0.00659	0.666
17	8.0	4.0	54.6	0.324	0.00716	0.723
18	8.0	4.2	54.0	0.309	0.00684	0.691
19	8.0	3.0	58.0	0.297	0.00658	0.664
20	8.0	4.0	57.4	0.282	0.00625	0.631
21	8.0	4.0	57.2	0.303	0.00671	0.678
22	8.0	4.0	58.2	0.276	0.00612	0.618
23	8.0	4.0	52.2	0.343	0.00759	0.767
24	8.0	4.0	55.4	0.298	0.00659	0.666
25	8.0	4.0	55.0	0.320	0.00709	0.716
26	8.0	4.0	58.0	0.278	0.00615	0.621
27	8.0	4.0	59.0	0.290	0.00641	0.648
28	8.0	4.0	60.5	0.260	0.00575	0.580
29	8.0	4.0	55.0	0.320	0.00709	0.716
30	8.0	4.0	58.8	0.272	0.00602	0.608

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.0	4.0	25.0	0.596	0.0748	1.406
2	6.0	4.2	22.5	0.643	0.0886	1.665
3	6.0	4.0	30.5	0.507	0.0710	1.334
4	6.1	4.0	44.2	0.349	0.0491	0.923
5	6.0	4.0	57.2	0.234	0.0343	0.645
6	6.0	4.0	63.5	0.192	0.0288	0.541
7	6.0	4.0	65.6	0.174	0.0253	0.476
8	6.0	4.0	72.8	0.133	0.0187	0.352
9	6.0	4.1	65.2	0.177	0.0270	0.507
10	6.0	4.0	67.5	0.165	0.0252	0.473
11	6.0	4.0	67.3	0.164	0.0257	0.483
12	6.0	4.0	68.8	0.157	0.0229	0.430
13	6.0	4.0	70.0	0.146	0.0226	0.424
14	6.0	4.0	68.2	0.161	0.0251	0.473
15	6.0	4.0	64.0	0.185	0.0288	0.542

Albumin-Top 280 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
16	6.0	4.0	48.4	0.310	0.0498	0.937
17	6.0	4.1	78.5	0.096	0.0161	0.302
18	6.0	4.0	78.0	0.103	0.0173	0.325
19	6.0	3.2	81.2	0.082	0.0132	0.256
20	6.0	4.0	77.6	0.105	0.0197	0.325
21	6.0	4.0	82.0	0.077	0.0203	0.247
22	6.0	4.0	77.0	0.108	0.0136	0.371
23	6.0	4.0	74.0	0.122	0.0203	0.382
24	6.0	3.9	83.2	0.075	0.0136	0.256
25	6.0	4.0	78.0	0.099	0.0172	0.322
26	6.0	4.0	77.5	0.105	0.0182	0.341
27	6.0	4.0	86.2	0.056	0.0100	0.187
28	6.0	4.0	83.8	0.072	0.0130	0.245
29	6.0	4.0	68.0	0.159	0.0258	0.485
30	6.0	4.0	76.0	0.114	0.0190	0.357

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	7.6	4.0	16.0	0.805	0.1013	1.905
2	7.3	4.1	35.6	0.436	0.0573	1.078
3	7.6	4.1	45.4	0.352	0.0434	0.815
4	7.8	4.2	47.6	0.310	0.0372	0.699
5	8.0	4.1	49.4	0.316	0.0373	0.701
6	8.2	4.0	48.4	0.302	0.0349	0.656
7	8.2	4.0	57.2	0.252	0.0300	0.563
8	8.2	4.0	56.6	0.234	0.0271	0.510
9	8.2	4.2	44.2	0.364	0.0443	0.833
10	8.2	4.0	46.8	0.317	0.0377	0.709
11	8.2	4.2	43.5	0.371	0.0449	0.844
12	8.0	4.0	20.5	0.675	0.0878	1.650
13	8.1	4.0	47.5	0.333	0.0395	0.742
14	8.0	4.0	40.8	0.377	0.0460	0.865
15	8.0	4.2	41.8	0.388	0.0466	0.876

Albumin-Bottom 280 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
16	8.0	4.1	32.0	0.482	0.0621	1.167
17	8.0	4.0	36.8	0.444	0.0543	1.021
18	8.0	4.2	36.8	0.421	0.0514	0.965
19	8.0	3.0	42.6	0.380	0.0453	0.851
20	8.0	4.0	43.2	0.352	0.0415	0.780
21	8.0	4.0	42.0	0.386	0.0459	0.863
22	8.0	4.0	42.0	0.364	0.0438	0.824
23	8.0	4.0	37.6	0.434	0.0515	0.968
24	8.0	4.0	40.4	0.381	0.0454	0.854
25	8.0	4.0	43.0	0.376	0.0433	0.813
26	8.0	4.0	44.0	0.344	0.0405	0.761
27	8.0	4.0	43.2	0.374	0.0447	0.841
28	8.0	4.0	48.2	0.304	0.0349	0.657
29	8.0	4.0	42.0	0.386	0.0449	0.844
30	8.0	4.0	47.0	0.315	0.0360	0.677

TABLE 21

PARAMETRIC PUMPING EXPERIMENT 4

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.2	3.0	32.4	0.483	0.00804	0.924
2	6.1	3.0	35.8	0.441	0.00735	0.845
3	6.1	3.0	48.0	0.312	0.00520	0.598
4	6.1	3.1	45.2	0.340	0.00567	0.652
5	6.0	3.0	57.0	0.238	0.00397	0.456
6	6.0	3.0	60.8	0.211	0.00351	0.403
7	6.1	3.0	60.5	0.212	0.00353	0.406
8	6.0	3.0	60.8	0.210	0.00351	0.403
9	6.0	3.0	61.2	0.207	0.00344	0.395
10	6.0	3.0	62.0	0.202	0.00337	0.387

Experimental Conditions:

Buffer Conc. : 0.035 M, Cycle Time: 72 min.

Displacement: 18 CC, Feed PH 6

Reservoir PH: Top: 6, Bottom: 8

Feed Conc. : Y_{HO}: 0.00870%, Y_{AO}: 0.0422%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	7.0	3.0	54.4	0.256	0.00566	0.651
2	7.0	2.9	62.3	0.193	0.00427	0.491
3	7.1	3.0	59.5	0.217	0.00480	0.552
4	7.2	3.0	58.0	0.224	0.00496	0.570
5	7.4	3.0	58.2	0.226	0.00500	0.575
6	7.6	3.0	54.2	0.254	0.00562	0.646
7	7.6	3.0	56.5	0.239	0.00529	0.608
8	7.6	3.0	57.0	0.232	0.00513	0.590
9	7.6	3.0	58.2	0.226	0.00500	0.575
10	7.6	3.0	60.0	0.210	0.00464	0.533

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.2	3.0	35.4	0.438	0.0498	1.180
2	6.1	3.0	35.8	0.456	0.0561	1.329
3	6.1	3.0	41.2	0.372	0.0490	1.161
4	6.1	3.1	46.8	0.340	0.0410	0.971
5	6.0	3.0	48.5	0.301	0.0406	0.962
6	6.0	3.0	49.0	0.320	0.0461	1.092
7	6.1	3.0	48.8	0.298	0.0419	0.993
8	6.0	3.0	52.0	0.294	0.0412	0.976
9	6.0	3.0	53.8	0.256	0.0343	0.813
10	6.0	3.0	53.8	0.280	0.0391	0.926

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	7.0	3.0	33.0	0.461	0.0611	1.447
2	7.0	2.9	36.8	0.434	0.0603	1.429
3	7.1	3.0	41.0	0.366	0.0477	1.130
4	7.2	3.0	43.7	0.360	0.0463	1.097
5	7.4	3.0	44.0	0.336	0.0422	1.000
6	7.6	3.0	39.0	0.409	0.0526	1.246
7	7.6	3.0	44.4	0.332	0.0408	0.967
8	7.6	3.0	43.0	0.367	0.0470	1.113
9	7.6	3.0	45.0	0.326	0.0406	0.962
10	7.6	3.0	47.5	0.323	0.0410	0.971

TABLE 22

PARAMETRIC PUMPING EXPERIMENT 5

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.0	4.0	36.5	0.432	0.00720	0.791
2	6.1	4.0	55.0	0.246	0.00410	0.451
3	6.0	4.0	62.0	0.202	0.00337	0.370
4	6.0	4.0	62.3	0.200	0.00334	0.367
5	6.0	4.1	62.0	0.202	0.00337	0.370
6	6.0	4.0	66.6	0.163	0.00272	0.299
7	6.0	3.6	60.0	0.217	0.00362	0.398
8	6.0	4.0	63.0	0.187	0.00312	0.343
9	6.1	4.0	67.0	0.169	0.00282	0.310
10	6.0	4.0	60.0	0.209	0.00348	0.382
11	6.1	4.0	61.5	0.215	0.00358	0.393
12	6.2	3.8	61.6	0.207	0.00345	0.379
13	6.2	3.3	60.2	0.215	0.00358	0.393

Experimental Conditions:

Buffer Conc. : 0.10 M, Cycle Time: 80 min.

Displacement: 20 CC, Feed PH: 6

Reservoir PH: Top: 6, Bottom: 8.9

Feed Conc. : Y_{HO}: 0.00910%, Y_{AO}: 0.0596%

Hemoglobin-Top 403 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
14	6.2	3.6	59.8	0.210	0.00350	0.385
15	6.2	3.8	56.8	0.240	0.00400	0.440
16	6.1	3.5	56.0	0.238	0.00396	0.435
17	6.0	4.0	56.0	0.247	0.00412	0.453
18	6.1	3.6	54.2	0.253	0.00422	0.464
19	6.0	3.6	54.0	0.262	0.00437	0.480
20	6.0	3.4	51.0	0.279	0.00465	0.511
21	6.1	3.5	55.2	0.253	0.00422	0.486
22	6.1	3.6	53.0	0.262	0.00437	0.480
23	6.0	3.8	53.6	0.265	0.00442	0.486
24	6.0	4.0	54.0	0.254	0.00423	0.465
25	6.0	4.0	55.0	0.254	0.00423	0.465

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	6.6	4.0	36.8	0.436	0.00965	1.060
2	6.9	3.4	40.8	0.401	0.00887	0.975
3	6.8	3.0	48.0	0.328	0.00726	0.798
4	6.8	3.0	48.2	0.329	0.00728	0.800
5	7.1	3.6	33.6	0.483	0.01070	1.175
6	7.1	3.4	33.2	0.491	0.01090	1.199
7	7.3	3.0	33.3	0.487	0.01080	1.187
8	7.3	3.0	40.8	0.401	0.00881	0.968
9	7.3	3.2	43.0	0.376	0.00832	0.914
10	7.5	3.4	44.0	0.369	0.00817	0.898
11	7.4	3.2	38.5	0.424	0.00938	1.031
12	7.5	3.0	38.0	0.432	0.00956	1.051
13	7.8	3.1	32.0	0.504	0.01150	1.264

Hemoglobin-Bottom 403 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
14	7.7	3.0	32.2	0.501	0.01110	1.220
15	7.6	3.0	31.0	0.518	0.01150	1.264
16	7.7	2.9	32.0	0.507	0.01120	1.231
17	7.7	3.0	28.0	0.556	0.01230	1.352
18	7.7	3.0	34.0	0.480	0.01060	1.165
19	7.8	3.2	28.2	0.559	0.01240	1.363
20	7.8	2.0	33.5	0.487	0.01080	1.187
21	7.8	3.0	31.8	0.507	0.01120	1.231
22	7.8	3.0	30.0	0.535	0.01180	1.297
23	8.0	4.0	29.8	0.533	0.01180	1.297
24	8.2	3.2	30.0	0.535	0.01180	1.297
25	7.9	3.3	29.2	0.544	0.01200	1.319

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.0	4.0	35.8	0.436	0.0530	0.889
2	6.1	4.0	46.5	0.314	0.0466	0.782
3	6.0	4.0	50.8	0.284	0.0399	0.669
4	6.0	4.0	54.0	0.251	0.0338	0.567
5	6.0	4.1	56.0	0.242	0.0320	0.537
6	6.0	4.0	56.0	0.233	0.0329	0.552
7	6.0	3.6	51.0	0.283	0.0387	0.649
8	6.0	4.0	42.0	0.358	0.0549	0.921
9	6.1	4.0	54.0	0.258	0.0372	0.624
10	6.0	4.0	47.5	0.305	0.0434	0.728
11	6.1	4.0	49.2	0.298	0.0417	0.708
12	6.2	3.8	48.2	0.298	0.0422	0.763
13	6.2	3.3	47.0	0.318	0.0455	0.763

Albumin-Top 280 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
14	6.2	3.6	46.2	0.317	0.0456	0.765
15	6.2	3.8	46.5	0.322	0.0445	0.747
16	6.1	3.5	42.8	0.350	0.0500	0.839
17	6.0	4.0	4.0	43.2	0.0503	0.844
18	6.1	3.6	42.8	0.350	0.0489	0.820
19	6.0	3.6	42.2	0.365	0.0511	0.857
20	6.0	3.4	38.0	0.401	0.0568	0.953
21	6.1	3.5	42.4	0.363	0.0513	0.861
22	6.1	3.6	38.3	0.398	0.0574	0.963
23	6.0	3.8	38.0	0.410	0.0594	0.997
24	6.0	4.0	36.3	0.419	0.0619	1.039
25	6.0	4.0	37.0	0.422	0.0625	1.049

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	6.6	4.0	31.2	0.496	0.0563	0.945
2	6.9	3.4	35.0	0.447	0.0503	0.844
3	6.8	3.0	39.2	0.397	0.0463	0.777
4	6.8	3.0	38.5	0.406	0.0477	0.800
5	7.1	3.6	33.0	0.472	0.0495	0.831
6	7.1	3.4	34.2	0.457	0.0465	0.780
7	7.3	3.0	28.6	0.534	0.0595	0.998
8	7.3	3.0	31.8	0.489	0.0573	0.961
9	7.4	3.2	33.0	0.472	0.0559	0.938
10	7.5	3.4	33.8	0.462	0.0546	0.916
11	7.4	3.2	30.8	0.502	0.0580	0.973
12	7.5	3.0	31.8	0.489	0.0554	0.930
13	7.8	3.1	24.2	0.607	0.0697	1.169

Albumin-Bottom 280 MU Continued

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
14	7.7	3.0	23.9	0.612	0.0716	1.201
15	7.6	3.0	25.0	0.592	0.0672	1.128
16	7.7	2.9	26.0	0.576	0.0654	1.097
17	7.7	3.0	26.5	0.567	0.0610	1.023
18	7.7	3.0	26.5	0.567	0.0655	1.099
19	7.8	3.2	25.6	0.582	0.0632	1.060
20	7.8	2.0	27.0	0.560	0.0638	1.070
21	7.8	3.0	25.0	0.592	0.0680	1.141
22	7.8	3.0	23.0	0.629	0.0725	1.216
23	8.0	4.0	20.6	0.676	0.0803	1.347
24	8.2	3.2	22.5	0.639	0.0742	1.245
25	7.9	3.3	21.0	0.668	0.0784	1.315

TABLE 23

PARAMETRIC PUMPING EXPERIMENT 6

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.0	3.5	37.2	0.421	0.00702	0.878
2	6.0	4.0	46.2	0.328	0.00547	0.684
3	6.0	4.0	47.6	0.316	0.00527	0.659
4	6.0	4.0	43.0	0.360	0.00600	0.751
5	6.0	3.8	41.5	0.376	0.00627	0.784
6	6.0	4.0	44.0	0.350	0.00583	0.729
7	6.0	4.0	41.8	0.373	0.00622	0.778
8	6.0	4.0	40.8	0.382	0.00637	0.797
9	6.0	4.0	42.2	0.369	0.00615	0.770
10	6.0	4.0	37.5	0.419	0.00698	0.874
11	6.0	4.0	41.6	0.375	0.00625	0.782
12	6.0	4.0	39.4	0.397	0.00662	0.829
13	6.0	4.0	40.0	0.392	0.00653	0.817
14	6.0	2.2	43.0	0.360	0.00600	0.751
15	6.0	4.0	44.6	0.344	0.00573	0.717

Experimental Conditions:

Buffer Conc. : 0.20 M, Cycle Time: 80 min.

Displacement: 20 CC, Feed PH: 6

Reservoir PH: Top: 6, Bottom: 8.9

Feed Conc. : Y_{HO}: 0.00800%, Y_{AO}: 0.0552%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	8.0	4.0	36.2	0.436	0.00965	1.208
2	8.0	4.0	36.4	0.427	0.00945	1.183
3	7.8	4.0	37.8	0.415	0.00918	1.149
4	7.8	4.0	36.2	0.430	0.00952	1.191
5	7.8	4.0	33.2	0.471	0.01040	1.301
6	7.8	4.0	34.8	0.447	0.00989	1.238
7	7.9	4.0	35.0	0.448	0.00991	1.240
8	7.8	4.0	33.6	0.462	0.01020	1.277
9	7.8	4.0	32.6	0.479	0.01060	1.327
10	7.9	4.0	33.0	0.470	0.01020	1.277
11	7.9	4.0	34.0	0.461	0.01020	1.277
12	7.9	4.0	34.6	0.449	0.00994	1.244
13	7.9	4.0	35.2	0.446	0.00987	1.235
14	7.8	4.0	34.0	0.457	0.01011	1.265
15	7.9	4.0	36.0	0.435	0.00963	1.205

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.0	3.5	29.6	0.517	0.0690	1.250
2	6.0	4.0	35.0	0.441	0.0610	1.105
3	6.0	4.0	38.0	0.409	0.0558	1.011
4	6.0	4.0	32.0	0.480	0.0662	1.199
5	6.0	3.8	33.6	0.462	0.0617	1.118
6	6.0	4.0	32.2	0.429	0.0572	1.036
7	6.0	4.0	32.8	0.473	0.0640	1.159
8	6.0	4.0	32.2	0.477	0.0641	1.101
9	6.0	4.0	32.0	0.483	0.0662	1.199
10	6.0	4.0	29.0	0.522	0.0701	1.270
11	6.0	4.0	32.8	0.472	0.0631	1.143
12	6.0	4.0	31.8	0.482	0.0640	1.159
13	6.0	4.0	31.2	0.494	0.0667	1.208
14	6.0	2.2	35.0	0.441	0.0588	1.065
15	6.0	4.0	38.0	0.409	0.0539	0.976

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	8.0	4.0	28.6	0.538	0.0632	1.145
2	8.0	4.0	29.0	0.499	0.0573	1.038
3	7.8	4.0	29.8	0.520	0.0615	1.114
4	7.8	4.0	28.8	0.502	0.0576	1.043
5	7.8	4.0	26.8	0.561	0.0660	1.196
6	7.8	4.0	28.3	0.510	0.0580	1.051
7	7.9	4.0	26.2	0.576	0.0688	1.246
8	7.8	4.0	26.2	0.544	0.0628	1.138
9	7.8	4.0	26.8	0.566	0.0633	1.147
10	7.9	4.0	25.0	0.563	0.0659	1.194
11	7.9	4.0	25.0	0.596	0.0713	1.292
12	7.9	4.0	25.4	0.557	0.0656	1.188
13	7.9	4.0	26.0	0.580	0.0696	1.261
14	7.8	4.0	26.8	0.534	0.0613	1.111
15	7.9	4.0	28.0	0.548	0.0649	1.175

TABLE 24

PARAMETRIC PUMPING EXPERIMENT 7

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.2	4.0	39.5	0.386	0.00643	0.624
2	6.2	3.5	48.6	0.326	0.00543	0.527
3	6.2	4.0	48.2	0.299	0.00498	0.483
4	6.3	4.0	47.7	0.324	0.00540	0.524
5	6.3	4.0	43.5	0.344	0.00573	0.556
6	6.3	4.0	42.0	0.379	0.00632	0.614
7	6.3	4.0	37.8	0.405	0.00675	0.655
8	6.3	3.0	39.0	0.412	0.00687	0.667
9	6.3	3.8	39.0	0.391	0.00652	0.633
10	6.3	4.0	36.0	0.446	0.00743	0.721
11	6.3	4.0	37.0	0.414	0.00690	0.670
12	6.2	3.8	38.0	0.422	0.00703	0.683
13	6.2	4.0	38.0	0.389	0.00671	0.651
14	6.1	4.0	41.0	0.390	0.00650	0.631
15	6.1	4.0	41.4	0.365	0.00608	0.590

Experimental Conditions:

Buffer Conc. : 0.035 M, Cycle Time: 80 min.

Displacement: 20 CC, Feed PH: 8

Reservoir PH: Top: 6, Bottom: 8.9

Feed Conc. : Y_{HO}: 0.01030%, Y_{AO}: 0.0833%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	7.9	4.0	30.6	0.509	0.0113	1.097
2	8.0	4.0	27.8	0.568	0.0126	1.223
3	8.2	4.0	31.0	0.503	0.0111	1.078
4	8.3	4.0	31.2	0.518	0.0115	1.117
5	8.2	4.0	30.0	0.517	0.0114	1.107
6	8.2	4.0	31.5	0.514	0.0114	1.107
7	8.2	4.0	31.4	0.497	0.0110	1.068
8	8.2	4.0	33.0	0.493	0.0109	1.048
9	8.1	4.0	28.5	0.539	0.0119	1.167
10	8.0	4.0	27.7	0.570	0.0126	1.235
11	8.0	4.0	29.2	0.529	0.0117	1.136
12	8.0	4.0	28.2	0.561	0.0124	1.204
13	8.0	4.0	28.2	0.544	0.0120	1.165
14	8.0	4.0	29.4	0.544	0.0120	1.165
15	8.0	4.0	26.4	0.572	0.0126	1.223

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB} %	Y_{AB}/Y_{AO}
1	7.9	4.0	15.5	0.825	0.1062	1.275
2	8.0	4.0	17.2	0.785	0.0962	1.155
3	8.2	4.0	17.2	0.781	0.0995	1.194
4	8.3	4.0	18.0	0.765	0.0958	1.150
5	8.2	4.0	18.0	0.761	0.0954	1.145
6	8.2	4.0	19.0	0.742	0.0922	1.107
7	8.2	4.0	20.0	0.715	0.0889	1.067
8	8.2	4.0	22.8	0.662	0.0803	0.964
9	8.1	4.0	14.8	0.845	0.1079	1.295
10	8.0	4.0	14.4	0.862	0.1089	1.307
11	8.0	4.0	15.0	0.840	0.1076	1.292
12	8.0	4.0	14.8	0.850	0.1074	1.289
13	8.0	4.0	15.0	0.840	0.1068	1.282
14	8.0	4.0	15.2	0.838	0.1065	1.279
15	8.0	4.0	14.0	0.870	0.1100	1.321

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	6.2	4.0	31.0	0.499	0.0680	0.816
2	6.2	3.5	36.3	0.443	0.0615	0.738
3	6.2	4.0	35.8	0.436	0.0621	0.745
4	6.3	4.0	35.8	0.449	0.0628	0.754
5	6.3	4.0	31.0	0.499	0.0709	0.851
6	6.3	4.0	30.0	0.525	0.0734	0.881
7	6.3	4.0	26.8	0.562	0.0786	0.944
8	6.3	3.0	26.5	0.579	0.0814	0.977
9	6.3	3.8	25.8	0.578	0.0826	0.992
10	6.3	4.0	21.0	0.680	0.0982	1.179
11	6.3	4.0	21.0	0.668	0.0980	1.176
12	6.2	3.8	20.8	0.684	0.1005	1.206
13	6.2	4.0	23.4	0.621	0.0900	1.080
14	6.1	4.0	24.6	0.612	0.0891	1.070
15	6.1	4.0	25.4	0.585	0.0857	1.029

TABLE 25

PARAMETRIC PUMPING EXPERIMENT 8

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.1	4.0	47.6	0.324	0.00540	0.707
2	6.2	3.9	49.5	0.301	0.00502	0.657
3	6.2	3.8	56.2	0.252	0.00420	0.550
4	6.0	4.0	58.6	0.228	0.00380	0.497
5	6.0	3.8	64.2	0.194	0.00323	0.423
6	6.0	3.9	64.2	0.188	0.00313	0.410
7	6.0	3.8	53.0	0.277	0.00462	0.605
8	6.0	4.0	57.0	0.240	0.00400	0.524
9	6.0	4.0	56.2	0.252	0.00420	0.550
10	6.0	3.2	53.2	0.270	0.00450	0.589
11	6.0	3.6	56.4	0.250	0.00417	0.546
12	6.0	3.8	61.2	0.209	0.00348	0.455
13	6.0	3.6	63.0	0.202	0.00337	0.441
14	6.0	3.7	63.2	0.195	0.00325	0.425
15	6.0	3.8	62.6	0.205	0.00342	0.448

Experimental Conditions:

Buffer Conc. : 0.035 M, Cycle Time: 80 min.

Displacement: 20 CC, Feed PH: 6

Reservoir PH: Top: 6, Bottom: 8.9

Feed Conc. : Y_{HO}: 0.00764%, Y_{AO}: 0.0497%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	7.8	4.0	39.0	0.408	0.00903	1.182
2	7.8	4.0	45.2	0.358	0.00792	1.037
3	7.9	4.0	47.8	0.320	0.00708	0.927
4	7.9	4.0	49.8	0.316	0.00699	0.915
5	7.9	4.0	52.0	0.283	0.00626	0.819
6	7.8	4.0	53.6	0.284	0.00628	0.822
7	7.8	3.8	53.2	0.273	0.00604	0.791
8	7.8	3.9	48.0	0.332	0.00735	0.962
9	7.8	4.0	47.0	0.327	0.00724	0.948
10	7.8	4.0	48.2	0.330	0.00730	0.955
11	7.8	3.4	48.0	0.318	0.00704	0.921
12	7.8	3.4	46.6	0.344	0.00761	0.996
13	7.8	3.8	47.4	0.323	0.00715	0.936
14	7.8	3.6	45.0	0.360	0.00797	1.043
15	7.8	3.6	49.0	0.309	0.00684	0.895

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.1	4.0	32.0	0.505	0.0734	1.477
2	6.2	3.9	35.2	0.483	0.0708	1.425
3	6.2	3.8	36.2	0.452	0.0683	1.374
4	6.0	4.0	29.0	0.567	0.0917	1.845
5	6.0	3.8	49.0	0.320	0.0473	0.952
6	6.0	3.9	49.0	0.339	0.0513	1.032
7	6.0	3.8	34.0	0.479	0.0716	1.441
8	6.0	4.0	36.0	0.473	0.0731	1.471
9	6.0	4.0	39.0	0.419	0.0620	1.247
10	6.0	3.2	36.0	0.473	0.0710	1.429
11	6.0	3.6	38.6	0.424	0.0631	1.270
12	6.0	3.8	48.0	0.348	0.0516	1.038
13	6.0	3.6	47.8	0.331	0.0488	0.982
14	6.0	3.7	42.0	0.406	0.0635	1.287
15	6.0	3.8	45.0	0.357	0.0535	1.076

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	7.8	4.0	27.8	0.555	0.0677	1.362
2	7.8	4.0	35.0	0.469	0.0564	1.135
3	7.9	4.0	36.7	0.434	0.0529	1.064
4	7.9	4.0	38.0	0.433	0.0529	1.064
5	7.8	4.0	41.0	0.386	0.0471	0.948
6	7.8	4.0	43.2	0.377	0.0456	0.918
7	7.8	3.8	43.0	0.366	0.0444	0.893
8	7.8	3.9	28.0	0.566	0.0739	1.487
9	7.8	4.0	26.8	0.571	0.0750	1.509
10	7.8	4.0	33.2	0.492	0.0619	1.245
11	7.8	3.4	36.6	0.436	0.0533	1.072
12	7.8	3.4	36.5	0.451	0.0543	1.093
13	7.8	3.8	29.0	0.537	0.0697	1.402
14	7.8	3.6	28.8	0.553	0.0701	1.410
15	7.8	3.6	41.0	0.386	0.0456	0.918

TABLE 26

PARAMETRIC PUMPING EXPERIMENT 9

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	5.2	4.0	31.0	0.505	0.00842	1.194
2	5.2	3.9	45.0	0.344	0.00574	0.814
3	4.9	4.0	61.0	0.211	0.00352	0.499
4	4.9	4.0	68.0	0.165	0.00275	0.390
5	5.5	4.0	69.0	0.158	0.00263	0.373
6	5.6	3.8	71.5	0.143	0.00238	0.338
7	5.6	3.8	78.2	0.103	0.00172	0.244
8	5.6	4.0	74.2	0.126	0.00212	0.301
9	5.6	4.0	78.6	0.101	0.00168	0.238
10	5.6	3.8	76.0	0.117	0.00195	0.277
11	5.4	3.7	73.8	0.128	0.00213	0.302
12	5.3	4.0	82.8	0.079	0.00132	0.187
13	5.4	4.0	81.6	0.085	0.00141	0.200
14	5.8	4.0	79.2	0.099	0.00165	0.234
15	5.8	4.0	81.0	0.088	0.00147	0.209
16	5.6	3.8	78.0	0.105	0.00175	0.248
17	5.6	3.6	75.0	0.121	0.00202	0.287
18	5.6	3.7	77.0	0.111	0.00185	0.262

Experimental Conditions:

Buffer Conc. : 0.15 M, Cycle Time: 80 min.

Displacement: 20 CC, Feed PH: 6

Reservoir PH: Top: 4.9, Bottom: 8.9

Feed Conc. : Y_{HO}: 0.00705%, Y_{AO}: 0.0436%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	8.0	4.0	30.4	0.522	0.01160	1.645
2	8.2	4.0	26.8	0.572	0.01260	1.787
3	8.2	4.0	30.5	0.521	0.01150	1.631
4	8.0	3.9	36.0	0.444	0.00983	1.394
5	8.0	3.8	35.2	0.458	0.01010	1.433
6	8.0	4.0	35.2	0.453	0.01000	1.418
7	7.9	4.0	37.8	0.431	0.00953	1.352
8	7.9	3.7	37.0	0.432	0.00956	1.356
9	7.8	3.2	41.4	0.384	0.00859	1.218
10	7.5	3.8	36.0	0.444	0.00983	1.394
11	7.5	3.9	39.8	0.405	0.00896	1.271
12	7.5	3.4	40.6	0.395	0.00874	1.240
13	7.5	4.0	39.4	0.410	0.00907	1.287
14	7.6	4.0	40.7	0.390	0.00863	1.224
15	7.8	3.9	40.0	0.403	0.00892	1.265
16	7.8	3.9	41.0	0.397	0.00879	1.247
17	7.8	3.9	41.2	0.390	0.00863	1.224
18	7.8	3.9	41.4	0.383	0.00848	1.203

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	5.2	4.0	10.2	0.987	0.1521	3.497
2	5.2	3.9	26.7	0.572	0.0847	1.943
3	4.9	4.0	41.2	0.381	0.0576	1.321
4	4.9	4.0	48.0	0.318	0.0489	1.137
5	5.5	4.0	48.8	0.307	0.0473	1.116
6	5.6	3.8	49.8	0.302	0.0474	1.102
7	5.6	3.8	57.0	0.240	0.0383	0.903
8	5.6	4.0	50.2	0.298	0.0476	1.107
9	5.6	4.0	56.0	0.247	0.0398	0.913
10	5.6	3.8	49.0	0.308	0.0503	1.154
11	5.4	3.7	49.0	0.305	0.0490	1.124
12	5.3	4.0	56.8	0.244	0.0407	0.933
13	5.4	4.0	58.4	0.229	0.0375	0.860
14	5.8	4.0	57.6	0.238	0.0383	0.878
15	5.8	4.0	58.0	0.232	0.0379	0.869
16	5.6	3.8	52.8	0.276	0.0450	1.032
17	5.6	3.6	50.4	0.293	0.0471	1.080
18	5.6	3.7	53.0	0.274	0.0442	1.015

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	8.0	4.0	35.4	0.459	0.0450	1.032
2	8.2	4.0	32.1	0.468	0.0468	1.073
3	8.2	4.0	32.0	0.503	0.0525	1.204
4	8.0	3.9	38.0	0.412	0.0420	0.963
5	8.0	3.8	35.8	0.454	0.0482	1.106
6	8.0	4.0	34.8	0.451	0.0479	1.099
7	7.9	4.0	37.8	0.430	0.0457	1.048
8	7.9	3.7	34.2	0.458	0.0503	1.154
9	7.8	3.2	36.0	0.451	0.0517	1.186
10	7.5	3.8	35.8	0.438	0.0462	1.060
11	7.5	3.9	36.0	0.451	0.0507	1.162
12	7.5	3.4	35.4	0.443	0.0500	1.147
13	7.5	4.0	36.8	0.442	0.0489	1.122
14	7.6	4.0	38.0	0.412	0.0451	1.034
15	7.8	3.9	38.0	0.427	0.0468	1.078
16	7.8	3.9	39.0	0.401	0.0429	0.984
17	7.8	3.9	41.0	0.394	0.0422	0.968
18	7.8	3.9	41.2	0.375	0.0394	0.904

TABLE 27

PARAMETRIC PUMPING EXPERIMENT 10

HEMOGLOBIN-TOP 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HT},%	Y_{HT}/Y_{HO}
1	6.0	3.0	34.8	0.442	0.00736	0.894
2	6.3	3.0	36.0	0.422	0.00703	0.854
3	6.4	3.0	43.8	0.342	0.00570	0.692
4	6.3	3.0	45.6	0.319	0.00532	0.646
5	6.3	3.0	45.5	0.325	0.00542	0.658
6	6.0	3.0	42.8	0.347	0.00578	0.702
7	6.0	2.9	41.1	0.369	0.00616	0.748
8	6.0	3.0	42.6	0.349	0.00581	0.706
9	6.0	3.0	40.0	0.381	0.00635	0.772
10	6.0	3.0	40.0	0.376	0.00627	0.762
11	6.0	3.0	41.1	0.369	0.00616	0.748
12	6.0	3.0	45.7	0.318	0.00530	0.644
13	6.0	3.0	38.5	0.397	0.00661	0.804
14	6.0	3.0	42.0	0.355	0.00592	0.719
15	6.0	3.0	39.4	0.383	0.00638	0.775

Experimental Conditions:

Buffer Conc. : 0.15 M, Cycle Time: 80 min.

Displacement: 20 CC, Feed PH: 6

Reservoir PH: Top: 6, Bottom: 8.9

Feed Conc. : Y_{HO}: 0.00823%, Y_{AO}: 0.0608%

HEMOGLOBIN-BOTTOM 403 MU

N	PH	CC	Trans.	Log Trans.	Y_{HB},%	Y_{HB}/Y_{HO}
1	7.3	3.0	40.0	0.370	0.00819	0.995
2	7.7	3.0	41.0	0.360	0.00797	0.969
3	8.0	3.0	40.6	0.364	0.00805	0.978
4	8.0	3.0	41.2	0.358	0.00793	0.963
5	8.0	3.0	35.0	0.428	0.00947	1.151
6	7.6	3.0	32.0	0.468	0.01040	1.258
7	7.6	3.0	29.2	0.507	0.01120	1.363
8	7.5	3.0	33.8	0.444	0.00983	1.194
9	7.6	3.0	34.0	0.441	0.00975	1.185
10	7.5	3.0	29.8	0.499	0.01100	1.341
11	7.6	3.0	33.2	0.451	0.00998	1.212
12	7.6	3.0	36.0	0.417	0.00922	1.121
13	7.6	3.0	33.2	0.451	0.00998	1.213
14	7.6	3.0	31.5	0.474	0.01050	1.277
15	7.6	3.0	36.0	0.416	0.00920	1.118

ALBUMIN-TOP 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AT},%	Y_{AT}/Y_{AO}
1	6.0	3.0	28.0	0.543	0.0725	1.193
2	6.3	3.0	27.2	0.552	0.0755	1.242
3	6.4	3.0	30.8	0.502	0.0715	1.176
4	6.3	3.0	34.8	0.445	0.0623	1.025
5	6.3	3.0	37.0	0.422	0.0576	0.947
6	6.0	3.0	30.8	0.498	0.0704	1.158
7	6.0	2.9	30.8	0.502	0.0696	1.145
8	6.0	3.0	31.8	0.484	0.0677	1.113
9	6.0	3.0	33.4	0.467	0.0622	1.023
10	6.0	3.0	33.4	0.463	0.0618	1.017
11	6.0	3.0	34.0	0.459	0.0615	1.012
12	6.0	3.0	38.8	0.398	0.0535	0.880
13	6.0	3.0	33.0	0.472	0.0621	1.022
14	6.0	3.0	36.8	0.421	0.0553	0.910
15	6.0	3.0	31.0	0.499	0.0682	1.122

ALBUMIN-BOTTOM 280 MU

N	PH	CC	Trans.	Log Trans.	Y_{AB},%	Y_{AB}/Y_{AO}
1	7.3	3.0	33.4	0.454	0.0532	0.875
2	7.7	3.0	33.0	0.450	0.0531	0.873
3	8.0	3.0	33.0	0.459	0.0544	0.895
4	8.0	3.0	33.0	0.450	0.0533	0.877
5	8.0	3.0	26.2	0.559	0.0672	1.105
6	7.6	3.0	17.2	0.732	0.0932	1.532
7	7.6	3.0	21.6	0.634	0.0749	1.232
8	7.5	3.0	24.5	0.579	0.0695	1.143
9	7.6	3.0	21.8	0.639	0.0796	1.309
10	7.5	3.0	16.0	0.764	0.0969	1.593
11	7.6	3.0	24.4	0.590	0.0709	1.166
12	7.6	3.0	27.2	0.534	0.0637	1.048
13	7.6	3.0	24.0	0.590	0.0709	1.166
14	7.6	3.0	24.6	0.577	0.0674	1.109
15	7.6	3.0	31.6	0.478	0.0545	0.896

TABLE 28

SEPARATION FACTORS

Exp 1			Exp 2		
N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}	N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}
1	1.105	1.134	1	1.050	0.659
2	0.981	0.729	2	0.972	0.794
3	1.245	0.772	3	1.437	1.406
4	1.426	0.619	4	1.266	0.766
5	1.408	0.804	5	1.242	0.763
6	1.278	0.952	6	1.410	0.813
7	1.136	1.523	7	1.332	0.907
8	1.120	1.325	8	1.282	0.973
9	1.292	1.324	9	1.711	1.293
10	1.294	1.243	10	1.501	0.814
			11	1.826	1.408
			12	1.605	1.248
			13	2.260	1.743
			14	2.113	1.379
			15	1.987	1.246
			16	1.830	1.290
			17	1.898	1.353
			18	1.950	1.230
			19	1.801	1.261
			20	1.876	1.150

TABLE 29

SEPARATION FACTORS

Exp 3			Exp 4		
N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}	N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}
1	1.290	1.355	1	0.705	1.226
2	0.686	0.647	2	0.581	1.075
3	0.917	0.611	3	0.923	0.973
4	1.279	0.757	4	0.874	1.129
5	2.306	1.087	5	1.261	1.040
6	3.076	1.212	6	1.603	1.141
7	2.387	1.183	7	1.498	0.974
8	2.778	1.449	8	1.464	1.140
9	3.774	1.643	9	1.456	1.230
10	3.738	1.499	10	1.378	1.049
11	2.940	1.747	21	19.941	3.494
12	7.636	3.837	22	36.353	2.221
13	4.720	1.750	23	11.279	2.534
14	4.753	1.829	24	47.571	3.336
15	4.368	1.616	25	18.359	2.525
16	3.083	1.245	26	15.146	2.232
17	14.176	3.381	27	43.200	4.497
18	13.038	2.969	28	41.429	2.682
19	14.128	3.324	29	6.819	1.740
20	10.016	2.400	30	9.967	1.896

TABLE 30

SEPARATION FACTORS

Exp 5			Exp 6		
N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}	N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}
1	1.340	1.063	1	1.376	0.916
2	2.162	1.079	2	1.730	0.939
3	2.157	1.161	3	1.744	1.102
4	2.180	1.411	4	1.586	0.870
5	3.176	1.547	5	1.659	1.070
6	4.010	1.413	6	1.698	1.014
7	2.982	1.538	7	1.594	1.075
8	2.822	1.043	8	1.602	0.980
9	2.948	1.503	9	1.723	0.940
10	2.351	1.258	10	1.461	1.130
11	2.623	1.390	11	1.633	0.965
12	2.773	1.313	12	1.501	1.025
13	3.216	1.532	13	1.512	1.044
14	3.169	1.570	14	1.684	1.043
15	2.873	1.510	15	1.681	1.204
16	2.830	1.308	21	2.533	1.325
17	2.985	1.212	22	2.533	1.263
18	2.511	1.340	23	2.669	1.351
19	2.787	1.237	24	2.789	1.194
20	2.323	1.123	25	2.837	1.254

TABLE 31

SEPARATION FACTORS

	Exp 7			Exp 8	
N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}	N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}
1	1.758	1.563	1	1.672	0.912
2	2.321	1.565	2	1.578	0.796
3	2.232	1.603	3	1.685	0.774
4	2.132	1.525	4	1.841	0.577
5	1.991	1.345	5	1.936	0.996
6	1.803	1.257	6	2.004	0.890
7	1.631	1.130	7	1.307	0.620
8	1.571	0.987	8	1.836	1.011
9	1.696	1.099	9	1.724	1.210
10	1.713	1.109	10	1.621	0.871
11	1.696	1.099	11	1.687	0.844
12	1.706	1.069	12	2.189	1.053
13	1.790	1.187	13	2.122	1.428
14	1.846	1.195	14	2.454	1.103
15	2.073	1.284	15	1.998	0.853

TABLE 32

SEPARATION FACTORS

Exp 9			Exp 10		
N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}	N	Y_{HB}/Y_{HT}	Y_{AB}/Y_{AT}
1	1.383	0.293	1	1.113	0.733
2	2.195	0.552	2	1.134	0.703
3	3.280	0.911	3	1.413	0.761
4	3.590	0.847	4	1.491	0.856
5	3.378	0.991	5	1.749	1.167
6	4.206	0.997	6	1.792	1.323
7	5.667	1.161	7	1.822	1.076
8	4.533	1.042	8	1.691	1.027
9	5.118	1.299	9	1.535	1.280
10	5.032	0.919	10	1.760	1.566
11	4.209	1.034	11	1.620	1.152
12	6.631	1.229	12	1.740	1.191
13	6.435	1.305	13	1.509	1.141
14	5.231	1.178	14	1.776	1.218
15	6.053	1.240	15	1.443	0.799
16	5.028	0.953			
17	4.265	0.896			
18	4.592	0.891			

NOMENCLATURE

I_A = Isoelectric Point of Albumin

I_H = Isoelectric Point of Hemoglobin

N = Number of Cycles of Pump

η = Slope of Log Transmittance vs Concentration

β = Weight % of Concentration

L = Log Transmittance

Y_H = Concentration of Hemoglobin

Y_{HO} = Concentration of Hemoglobin Feed

Y_A = Concentration of Albumin

Y_{AO} = Concentration of Albumin Feed

Y = Concentration of Protein either Albumin or Hemoglobin

Y_O = Feed Concentration of Protein either Albumin or Hemoglobin

Y_{HT} = Top Product Concentration of Hemoglobin

Y_{HB} = Bottom Product Concentration of Hemoglobin

Y_{AT} = Top Product Concentration of Albumin

Y_{AB} = Bottom Product Concentration of Albumin

Q = Displacement cc/min.

$\frac{Q}{W}$ = Cycle Time in min.

S.F. = Separation Factor

MU = Millimicron

REFERENCES CITED

1. Aris, R., "Equilibrium Theory of Parametric Pump," Ind. Eng. Chem. Fundam., 8, 603 (1968).
2. Chen, H.T. and F.B. Hill, "Characteristics of Batch, Semi-continuous and Continuous Equilibrium Parametric Pumps," Separation Science, 6, 411 (1971).
3. Chen, H.T., J.L. Rak, J.D. Stokes and F.B. Hill, "Separation Via Continuous Parametric Pumping," AIChE J., 18, 356 (1972).
4. Chen, H.T., E.H. Reiss, J.D. Stokes and F.B. Hill, "Separation Via Semi-continuous Parametric Pumping," AIChE J., 19, 589 (1973).
5. Chen, H.T., J.A. Park and J.L. Rak, "Equilibrium Parametric Pumps," Separation Science, 9, 35 (1974a).
6. Chen, H.T., W.W. Lin, J.D. Stokes and W.R. Fabisiak, "Separation of Multicomponent Mixtures Via Thermal Parametric Pumping," AIChE J., 20, 306 (1974b).
7. Chen, H.T. and J.A. Manganaro, "Optimal Performance of Equilibrium Parametric Pumps," AIChE J., 20, 1020 (1974c).
8. Chen, H.T. and V.J. D'Emidio, "Separation of Isomers Via Thermal Parametric Pumping," AIChE J., 21, 813 (1975).
9. Chen, H.T. "Parametric Pumping" in Handbook of Separation Techniques for Chemical Engineers, McGraw Hill (1976a).

10. Chen, H.T., A. Rastogi, A. Kim and J. L. Rak, "Non-Equilibrium Parametric Pumps," Separation Science, 11, 333 (1976b).

11. Lehninger, A.L. Biochemistry First Edition, Worth Inc.

12. Pigford, R. L., B. Baker and E.D. Blum, "An Equilibrium Theory of the Parametric Pump," Ind. Eng. Chem. Fundam., 8, 144 (1969).

13 Pigford, R. L., B. Baker and E.D. Blum, "Cycling Zone Adsorption, a New Separation Process," Ind. Eng. Chem. Fundam., 8, 848 (1969).

14. Pharmacia Fine Chemicals, Sephadex Ion Exchanger, A Guide to Ion Exchange Chromatography.

15. Sabadell, J.E. and N.H. Sweed, "Parametric Pumping with PH," Separation Science, 5, 171 (1970).

16. Shaffer, A.G. and C.E. Hamrin, "Enzyme Separation by Parametric Pumping," AIChE J., 21, 782 (1975).

17. Sweed, N.H. and R.A. Gregory, "Parametric Pumping: Modeling Direct Thermal Separations of Sodium Chloride-Water in Open and Closed System," AIChE J., 17, 171 (1971).

18. Wankat, P.C. and M.E. Busbice, "A Preparative Technique for Counter-Current Distribution and Chromatography," J. of Chromatography, 114, 369 (1975).

19. Weaver, K. and C.E. Hamrin, "Separation of Hydrogen Isotopes by Heatless Adsorption," Chem. Eng. Sci., 29, 1873 (1974).

20. Wilhelm, R.H., A.W. Rice and A.R. Bendelius, "Parametric Pumping: A Dynamic Principle for Separating Fluid Mixture," Ind. Eng. Chem. Fundam., 5, 141 (1968).

21. Wilhelm, R.H. and N.H. Sweed, "Parametric Pumping: Separation of Mixture of Toluene and n-Heptane," Science, 159, 522 (1968).

22. Wilhelm, R.H., A.W. Rice, D.W. Rolke and N.H. Sweed, "Parametric Pumping," Ind. Eng. Chem. Fundam., 7, 337 (1968).