

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

The Van Houten library has removed some of the personal information and all signatures from the approval page and biographical sketches of theses and dissertations in order to protect the identity of NJIT graduates and faculty.

INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.
2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.
3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.
4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.
5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

**University
Microfilms
International**

300 N. Zeeb Road
Ann Arbor, MI 48106

8300192

Hollein, Helen Conway

**SEPARATION OF PROTEINS VIA pH PARAMETRIC PUMPING WITH
ELECTRIC FIELD**

New Jersey Institute of Technology

D.ENG.SC.

1982

**University
Microfilms
International** 300 N. Zeeb Road, Ann Arbor, MI 48106

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark .

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages _____
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

University
Microfilms
International

SEPARATION OF PROTEINS VIA
pH PARAMETRIC PUMPING WITH ELECTRIC FIELD

by
Helen Conway Hollein

Dissertation submitted to the Faculty of the Graduate School
at New Jersey Institute of Technology in partial fulfillment
of the requirements for the degree of
Doctor of Engineering Science
1982

ABSTRACT

Title of Dissertation: Separation of Proteins via pH Parametric Pumping with Electric Field

Helen C. Hollein, Doctor of Engineering Science, 1982

Dissertation directed by: Dr. Ching-Rong Huang
Professor and Assistant Chairman
Department of Chemical Engineering

A new semi-continuous parametric pumping process for the separation of protein mixtures has been developed, based on cyclic variation of pH and electric field. The model system used for this process consisted of a mixture of human serum albumin and human hemoglobin in contact with CM Sepharose cation exchanger. Experimental results show that the protein separation via the pH parapump with electric field is two to three times greater than the results which are obtained in batch chromatography or via either single-column or multi-column parapumps with cyclic variation of pH and ionic strength.

The separation in the new process depends on selective adsorption of one protein onto the ion exchanger, and also on a difference in the migration velocities of the two proteins in the presence of an electric field. Protein A (hemoglobin) was stripped from the top stream and enriched in the bottom stream by adsorption at low pH and desorption at high pH. The top stream was thereby enriched in Protein B (albumin) relative to Protein A. Protein B was then stripped from the bottom stream by applying an electric field across the parapump during the desorption stage of each cycle of the process.

Various modes of operation and a number of experimental parameters were considered. A low ionic strength organic buffer (0.05M Tris-Maleate + NaOH) was selected in order to maximize field strength with minimum heat generation, and the optimum fluid displacement for this buffer was determined experimentally. The effect of the electric field on the adsorption and desorption concentration waves and pH profiles was examined for different bulk velocities and alternate field polarities. These results were applied to the final process.

A mathematical model was developed to verify the experimental data, based on finite mass transfer and constant electric mobility. The adsorption isotherm was assumed to be linear in the region of interest. The transport equations were solved by the finite difference method and also by the Stop & Go model. A pH wave lag was necessarily incorporated into the mathematical model. The pH wave velocity was assumed to be constant. The model agrees well with the experimental data. Protein separation was optimized mathematically as a function of top or bottom feed rates and as a function of total production rate.

Various parametric pumping processes have been reported based on temperature, pressure, and pH. The use of electric field polarity as a thermodynamic variable in a parapumping process is a promising new concept in protein separation technology.

APPROVAL SHEET

Title of Dissertation: Separation of Proteins via pH Parametric Pumping with Electric Field

Name of Candidate: Helen C. Hollein
Doctor of Engineering Science, 1982

Dissertation and Abstract Approved:

Dr. Ching Rong Huang	_____
Professor and Assistant Chairman	Date
Department of Chemical Engineering	

Signatures of other
members of the dis-
sertation committee

}	_____	_____
	_____	_____
	_____	_____
	_____	_____

DEDICATION

To my husband, Leo

ACKNOWLEDGEMENTS

The author is eternally grateful to her late advisor, Dr. Hung-Tsung Chen, for his guidance from 1978-81. He had the original concept of using electrophoresis in a parapumping process and was extremely encouraging during the early experimental stages when the concept sometimes seemed to be an unreachable goal. The author is also grateful to Dr.'s Ching-Rong Huang and Frank B. Hill for their guidance during the year since Dr. Chen's tragic death.

The National Science Foundation provided financial support for this research under grant CPE 79-10540. The author thanks Union Carbide Corporation, 1978-81, and Allied Chemical Corporation, 1981-82, for doctoral fellowships. Dr. Emmett Bassett of the College of Medicine and Dentistry of New Jersey kindly loaned the LKB 7900 UNIPHOR apparatus for the experimental work. Portions of this dissertation were presented at the 2nd World Congress of Chemical Engineering, Montreal, 1981, and were published in Industrial and Engineering Chemistry Fundamentals, 1982.

Finally, the author gratefully acknowledges the contributions of her fellow parapumpers and co-workers. Dr. Zikri M. Ahmed and Mr. Robert J. Parisi are responsible for guidance in the state-of-the-art experimental techniques; Mr. Jen-Fu Chao for instructions on polyacrylamide gel electrophoresis; Dr. Charles O. Kerobo for crucial advice on the mechanical modifications of the experimental apparatus and on the mathematical modeling; and Mr. David Ma for help with the data. Special thanks to Mr. James Huang for proofreading this manuscript.

TABLE OF CONTENTS

<u>Chapter</u>	<u>Page</u>
INTRODUCTION.....	1
1. BACKGROUND.....	10
Pressure Swing Adsorption.....	15
Thermal Parametric Pumping.....	17
Cycling Zone Adsorption.....	22
Electrochemical Parametric Pumping and Electropolarization Chromatography.....	30
2. pH PARAMETRIC PUMPING.....	33
Graphical Solution.....	44
Experimental Parameters.....	67
3. ELECTROPOLARIZATION CHROMATOGRAPHY.....	86
4. PARAMETRIC PUMPING WITH pH AND ELECTRIC FIELD....	105
Description of the New Splitting Process.....	111
Experimental Set-Up.....	116
Experimental Results.....	120
5. MATHEMATICAL ANALYSIS OF THE ALBUMIN SEPARATION IN THE NEW PROCESS.....	127
6. MATHEMATICAL ANALYSIS OF THE ELECTRIC FIELD EFFECT ON THE HEMOGLOBIN SEPARATION....	164
The pH Wave Equation.....	177
Calculation of the Concentration Waves.....	185
Hemoglobin Separation in the New Process.....	197
7. PRELIMINARY STUDIES: THE ENRICHMENT PROCESS WITH ELECTRIC FIELD.....	215
8. CONCLUSIONS	222
APPENDIX A. EXPERIMENTAL CALCULATIONS & METHODS.....	224

APPENDIX B. EXPERIMENTAL DATA.....	230
APPENDIX C. COMPUTER CALCULATIONS.....	371
Program #1 - Hemoglobin Concentration Waves.....	372
Program #2 - Parametric Pumping.....	375
Program #3 - Albumin Separation.....	384
Program #4 - Albumin Optimization.....	387
Computer Notation.....	389
Calculated Data.....	394
REFERENCES.....	473
NOMENCLATURE.....	482

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Methods for Adsorption-Desorption.....	3
2. Properties of Hemoglobin and Albumin.....	8
3. Calculation of pH Wave Velocity (Run 9).....	28
4. pH Cyclic Separation Processes.....	34
5. Protein Recovery in Pulse Experiments.....	73
6. Displacement in Parapumping Experiments.....	80
7. Migration Velocities.....	103
8. Operating Conditions for the New Process.....	121
9. Dimensionless pH Wave Velocities.....	179
10. Experimental Parameters for the New Process.....	191
11. Effect of λ on the Overall Separation.....	205
12. Protein Recovery in the New Process.....	212
13. Phosphate Buffer.....	225
14. Tris-maleate / NaOH Buffer.....	226

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Protein Charge as a Function of pH.....	7
2. Recuperative versus Direct Mode Operation.....	12
3. Process Diagram for Pressure Swing Adsorption....	16
4. Thermal Parametric Pumping: Process Diagrams for Recuperative and Direct Modes.....	19
5. Cycling Zone Adsorption with Electric Field (Runs 9-12).....	23
6. Experimental Results for Cycling Zone Adsorption (Run 9).....	24
7. The Two-Stage Semicontinuous pH Parapump.....	39
8. The Four-Stage pH Parapump.....	41
9. The Eight-Stage pH Parapump.....	48
10. The Batch pH Parapump.....	52
11. Graphical Solution for the Batch Process.....	53
12. Graphical Solution for the Semicontinuous Process without Electric Field.....	58
13. Graphical Solution for the Semicontinuous Process with Electric Field.....	61
14. Experimental Results for the Four-Stage pH Parapump (Run 44).....	68
15. Field Strength as a Function of Buffer Concentration and Power Generation.....	74
16. Effect of Buffer Concentration on Hemoglobin Separation (Experimental).....	75
17. Separation of Mixture versus Single Component (Experimental Results).....	76
18. Optimum Displacement from Pulse Experiments.....	81
19. Comparison with Previous Results at Low Displacement Volumes.....	82

<u>Figure</u>	<u>Page</u>
20. Comparison of Separations at Optimum and High Displacement Volumes (Experimental).....	83
21. Experimental Results for the Six-Stage pH Parapump (Run 45).....	84
22. Effect of Circulation on Hemoglobin Separation (Experimental).....	85
23. Effect of Electric Field in Cycling Zone Adsorption.....	87
24. Effect of Bulk Flow Rate on the Concentration Wave in Electropolarization Chromatography....	89
25. Separation of Hemoglobin and Albumin via Polyacrylamide Gel Electrophoresis.....	92
26. Effect of Electric Field in Stage VI.....	99
27. Effect of Electric Field in Stage III.....	100
28. Effect of Electric Field in Stage I.....	101
29. Effect of Electric Field in Stage IV.....	102
30. Batch Separation via Electropolarization Chromatography (Run 6).....	104
31. Process Diagram for the pH Parapump with Electric Field (Mode 7).....	106
32. Optimum Displacements for Stages I & II (Run 13).	108
33. Maximum Upflow for Stage IV of the New Process (Run 8).....	109
34. Schematic Description of the Separation Principle in the New Process (Modes 5-8).....	114
35. Experimental Apparatus (Mode 7).....	117
36. Process Diagram for the pH Parapump with Electric Field (Mode 6).....	122
37. Experimental Results for the New Process.....	123
38. Experimental Separation Factors for the New Process.....	124
39. Regions of Operation based on Feed Volumes.....	129

<u>Figure</u>	<u>Page</u>
40. Process Diagram for the pH Parapump with Electric Field (Mode 8).....	138
41. Process Diagram for the pH Parapump with Electric Field (Mode 9).....	140
42. Process Diagram for the pH Parapump with Electric Field (Mode 10).....	141
43. Calculated vs. Experimental Albumin Separation for the New Process.....	144
44. Effect of Albumin Migration Velocity on Calculated Results.....	145
45. Calculated Effect of the Reservoir Dead Volume on Albumin Separation (Mode 6).....	146
46. Calculated Effect of Feed Volumes on Albumin Separation in the New Process (Mode 7).....	149
47. Albumin Separation as a Function of Top and Bottom Feed Rates (Runs 66B-69B).....	155
48. Hemoglobin Separation as a Function of Top and Bottom Feed Rates (Runs 66A-69A).....	156
49. Overall Separation as a Function of Top and Bottom Feed Rates (Runs 66A-69A).....	157
50. Optimization of Total Feed Rate Based on Albumin Recovery (top) and Albumin Separation Factor (bottom).....	158
51. Optimization of Total Feed Rate Based on Hemoglobin Recovery (top) and Hemoglobin Separation Factor (bottom).....	159
52. Optimization of Total Feed Rate Based on Protein Recovery (top) and Overall Separation Factor (bottom).....	160
53. Optimization of Stage IV Operation.....	161
54. Optimization of Stage V Operation.....	162
55. Optimum Displacements for Stages I, IV, and V...	163
56. Multi-Cells-in-Series Model with STOP & GO Algorithm.....	170

<u>Figure</u>	<u>Page</u>
57. Determination of Equilibrium Constant at High pH.....	176
58. Titration Curve for Tris-maleate Buffer.....	182
59. pH Waves without Electric Field.....	183
60. pH Waves with Electric Field.....	184
61. Relationship Between pH and Concentration Waves for the Adsorbed Protein.....	190
62. Determination of Equilibrium Constant at Low pH.....	192
63. Shape of Concentration Wave as a Function of Number of Cells used for the Multi-Cells- in-Series Model.....	193
64. Effect of Mass Transfer Coefficient on Shape of Concentration Wave.....	194
65. Calculated Desorption with Electric Field.....	195
66. Calculated Adsorption with Electric Field.....	196
67. Calculated vs. Experimental Concentrations (Mode 7 - Run 55A: $F_B = 52.5$ cc & $F_T = 17.5$ cc)	201
68. Calculated vs. Experimental Results (Mode 7 - Run 55A: $F_B = 52.5$ cc & $F_T = 17.5$ cc).....	202
69. Decrease in Top Product Hemoglobin Concentration with Repeating Cycles.....	203
70. Increase in Bottom Product Hemoglobin Concentration with Repeating Cycles.....	204
71. Calculated vs. Experimental Results (Mode 7 - Run 56A: $F_B = 35.0$ cc & $F_T = 35.0$ cc).....	206
72. Calculated vs. Experimental Results (Mode 7 - Run 57B: $F_B = 17.5$ cc & $F_T = 17.5$ cc).....	207
73. Calculated vs. Experimental Results (Mode 7 - Run 58B: $F_B = 17.5$ cc & $F_T = 52.5$ cc).....	208
74. Calculated vs. Experimental Results (Mode 7 - Run 59B: $F_B = 52.5$ cc & $F_T = 52.5$ cc).....	209
75. Calculated vs. Experimental Results (Mode 8 - Run 60A: $F_B = 35.0$ cc & $F_T = 35.0$ cc).....	210

<u>Figure</u>	<u>Page</u>
76. Calculated vs. Experimental Results (Mode 6 - Run 54B: $F_B = 35.0$ cc & $F_T = 35.0$ cc).....	211
77. Effect of Low Feed Rate on Hemoglobin Recovery and Separation (Experimental).....	213
78. Effect of Buffer Cooling on Hemoglobin Recovery and Separation (Experimental).....	214
79. pH Parametric Pumping with Electric Field: The Enrichment Process.....	217
80. Experimental Results for the Enrichment Process with Electric Field (Mode 3).....	218
81. Experimental Results for the Mixture via Mode 3 Operation.....	219
82. Experimental Enrichment of Hemoglobin via Mode 1 Operation.....	220
83. Experimental Enrichment of Hemoglobin via Mode 2 Operation.....	221
84. pH Correction Curve for Hemoglobin Concentration.....	228
85. Process Diagram for the pH Parapump with Electric Field (Mode 4).....	341
86. Process Diagram for the pH Parapump with Electric Field (Mode 5).....	354

INTRODUCTION

PARAMETRIC PUMPING was introduced by the late Richard H. Wilhelm and co-workers at Princeton University in 1966. It is a member of a group of cyclic separation processes which depend on a change in some thermodynamic variable, such as temperature or pH, for adsorption or desorption. PRESSURE SWING ADSORPTION, a pre-existing industrial process, operates on the same fundamental principle as parametric pumping with pressure as the alternating intensive variable (Shendalman and Mitchell, 1972; and Skarstrom, 1959).

Sabadell and Sweed (1972) formulated the basic requirements for parametric pumping processes to be four-fold.

- "1. The existence of a two-phase system."
- "2. An equilibrium distribution of the component being separated between the phases."
- "3. An alternating relative velocity between the phases."
- "4. An alternating interphase mass flux obtained by periodically changing one or more of the intensive thermodynamic variables that affect equilibrium."

The early parapumping work by Wilhelm and co-workers, 1968, showed enthusiastically high separation factors ranging from 10^3 to 10^5 , where the SEPARATION FACTOR (SF) is defined as follows:

$$[\text{SF}]_i = \frac{y_i \text{ (product enriched in component } i\text{)}}{y_i \text{ (product depleted in component } i\text{)}} \quad (\text{I-1})$$

By coupling the cyclic adsorption/desorption phenomena with changes in flow direction, a build-up of the concentration wave from cycle-to-cycle was observed. Note that the two-phase system incorporates one mobile and one immobile phase, usually a gas or liquid mobile phase and a solid immobile phase.

Wankat, 1973, has shown that the parapumping principle may also be extended to liquid-liquid extraction systems.

A similar separation process which depends on cyclic variation of an intensive variable but no change in flow direction is known as CYCLING ZONE ADSORPTION. This process was introduced by Robert L. Pigford and co-workers, 1969a. It was reported to have the capability of cyclic separation with higher production rates than the oscillating flow processes. These processes were compared experimentally for the purification of the enzyme alkaline phosphatase by Chen et al., 1981b, and Ahmed, 1981. Under identical operating conditions, parametric pumping gave higher purification factors and larger percent enzyme activity recovered, while cycling zone adsorption had a higher production rate. The enzyme purification in both processes was two to three times the commercially available purity.

The general methods for conventional and cyclic adsorptive separations are outlined in Table 1. The choice of adsorbent depends on the physical properties of the components to be separated. Common substances such as silica gel, activated carbon, natural and activated clays, and polymeric resins are all physical adsorbents. Physical adsorption results from intermolecular attraction between the molecules of the fluid and solid due to van der Waal's forces. Constitutents of the fluid phase may be separated if their polarities are slightly different. Chemisorption involves chemical bonding of the molecules from the solute to the adsorbent, and is generally used for catalysis rather than separation.

TABLE 1Methods for Adsorption-Desorption**ADSORPTION**

- I. Physical Adsorbents
- II. Chemical Adsorbents
- III. Molecular Sieves
- IV. Ion Exchange Resins

DESORPTION**Conventional Processes**

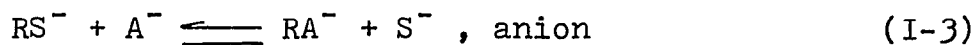
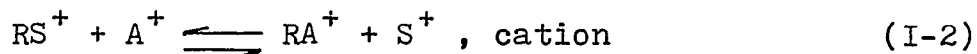
- I. Regenerate with -- INERT FLUIDS
- II. " " STRONGLY ADSORBED CHEMICALS
- III. " " WEAKLY ADSORBED CHEMICALS

Cyclic Processes

- I. Regenerate by Changing -- PRESSURE
- II. " " " TEMPERATURE
- III. " " " pH
- IV. " " " MOLECULAR AFFINITY
- V. " " " IONIC STRENGTH
- VI. " " " ELECTRIC FIELD

Molecular sieves or zeolite resins are adsorbents with a controlled pore size in the range of 3 to 10 Å, and are used to separate substances with different molecular diameters. The Linde Division of Union Carbide Corporation is a major sieve manufacturer (Collins, 1968). The ISOSIV process by Union Carbide (Guccione, 1965; Griesmer et al., 1965; and Hydrocarbon Processing, 1976) uses 5 Å molecular sieves in a pressure swing adsorption process to separate normal and branched hydrocarbons. Zeolite resins are also used in the LINDOX pressure swing system (Breck, 1974) for the separation of oxygen and nitrogen from air feed streams, and in U.O.P.'s SORBEX or PAREX process (Broughton et al., 1975) for separation of p-xylene from a mixture of C₈-aromatics.

Ion exchange resins can be used to separate substances such as proteins which carry an ionic charge. These resins are available in cationic, anionic, or mixed ionic forms. A charged solute particle A⁺ or A⁻ replaces the counterion S⁺ or S⁻. The adsorption is reversible.



At low fluid concentration of the counter ion S⁺, adsorption is favored.



At high fluid concentration of S⁺, desorption is favored.



Thus one method of controlling the adsorption/desorption of charged particles A⁺ or A⁻ in a cyclic adsorption process is

alternately changing the ionic strength of the fluid phase, where the fluid phase includes a buffer solution of counterion S^+ or S^- . Chen et al., 1981, and Ahmed, 1981 have developed a parametric pumping process for the purification of alkaline phosphatase which is based on a combination of pH and ionic strength.

Conventional separation processes depend on the use of extraneous chemicals for desorption (Table 1). For example, phenolic wastewater streams are cleaned by adsorption of phenol onto a polymeric resin and desorption of phenol with methanol or acetone, followed by a water wash to remove the desorbent from the resin (Fox, 1979). This creates a new separation problem in that distillation must be used to remove the phenol from the methanol or acetone and water. Costa et al., 1982, have suggested a thermal parapumping process for purification of phenolic wastewaters in order to avoid the distillation step and the associated energy costs. It is also desirable to avoid the use of extraneous chemicals in some adsorptive separations in order to avoid final product contamination. This is particularly true with food-grade or analytical-grade chemicals or pharmaceuticals.

Desorption is accomplished in cyclic separation processes by changing temperature, pressure, pH, ionic strength or some other thermodynamic variable as indicated in Table 1. This alters the equilibrium distribution of a given component between the solid and fluid phases. The choice of variable depends on the properties of the mixture under consideration.

Either temperature or pressure swing systems are feasible for separating mixtures of gases or low molecular weight hydrocarbons. Pressure is the variable used in industrial processes such as ISOSIV and LINDOX because of mechanical and economic superiority as compared with temperature variation in industrial-size packed beds. A number of liquid-solid processes with temperature changes, i.e., thermal parametric pumping, have been proposed in the literature for the separation of liquid organic mixtures. Both the gas-solid and the liquid-solid thermal systems will be discussed in more detail in Chapter 1.

A pure end product is essential in protein separations. Protein separation and purification generally entails a series of steps including extraction, ion exchange chromatography, cytoprecipitation, gel filtration, ultrafiltration, and crystallization. For examples, see the purification of d-serine dehydratase from *E. coli* (Shafer, 1981) and the fractionation of albumin from blood plasma (Pharmacia). Parametric pumping has the potential of replacing a number of intermediate steps in these lengthy separation schemes. Proteins are biological polymers and cannot be vaporized without decomposition. Heating denatures proteins -- so temperature and pressure are not feasible thermodynamic variables for protein parapumping separations. Appropriate variables for cyclic separation of protein mixtures include pH, molecular affinity, ionic strength, or electric field (Table 1).

Proteins and enzymes carry acid (COOH) and amine (NH₂)

functional groups. In solution, these groups interact with hydrogen ions to give charged protein ions as shown in Figure 1.

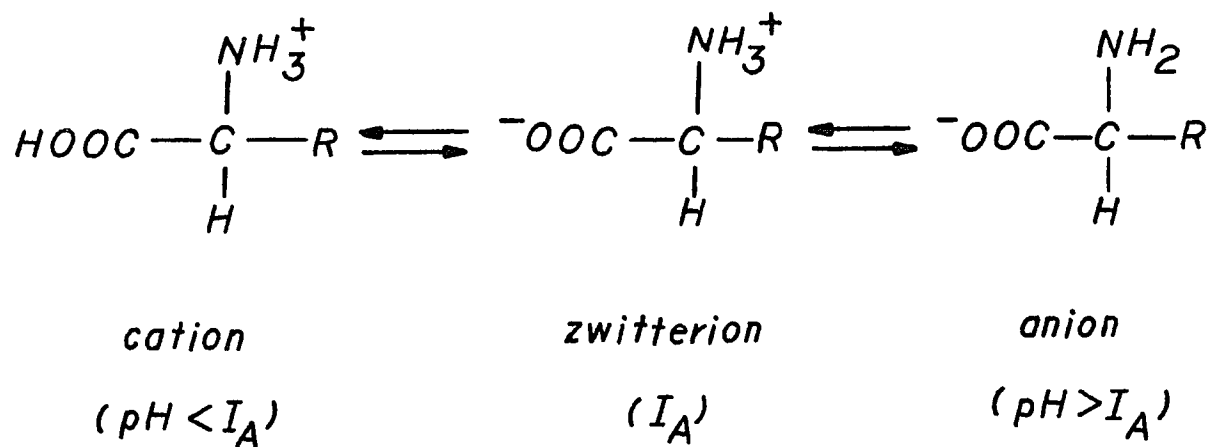


FIGURE 1: Protein Charge as a Function of pH

At low pH, the molecule carries a net positive charge and may be adsorbed on a cation exchanger or attracted towards the negative electrode in an electric field. At high pH, the protein molecule carries a net negative charge and may be adsorbed on an anion exchanger or attracted towards the cathode in an electric field. At some intermediate pH known as the ISOELECTRIC POINT (I_A), the protein exists as a zwitterion or double ion with a net charge of zero. Each protein species carries a number of these charged groups in its molecular structure, the summation of which gives it a unique value for its isoelectric point. The isoelectric points and molecular weights of a large number of proteins have been tabulated by Righetti and Caravaggio, 1976.

In 1975, Shaffer and Hamrin demonstrated that pH changes could be used in conjunction with an affinity resin as a basis

for the separation or purification of enzyme mixtures via parametric pumping. The late Hung T. Chen and co-workers (1977; 1979a; 1980a,b,c; 1981a,b,c); Chao et al., 1981; and Hollein et al., 1982, have done extensive work on protein separations via parametric pumping with pH and other thermodynamic variables. Four Doctor of Engineering Science dissertations (Wu, 1981; Yang, 1981; Ahmed, 1981; and Pancharoen, 1982) and numerous Master of Science theses have been completed on various aspects of this field of research at New Jersey Institute of Technology. This work was reviewed by Chen et al., 1981d, and will be explained in Chapter 2.

The present investigation utilizes both electric field polarity and pH changes for protein separation. A mixture of human hemoglobin and human serum albumin on CM Sepharose cation exchanger was chosen as the model system for the experimental work. These two proteins have similar properties except for their isoelectric points, as shown in Table 2.

TABLE 2

<u>Properties of Hemoglobin and Albumin</u>			
<u>Protein</u>	<u>Molecular Weight</u> ^a	<u>I_A</u> ^a	<u>D_{AB} (cm²/sec)</u> ^b
Hemoglobin	64500	6.95	7.6 x 10 ⁻⁷
Albumin	69000	5.85	7.0 x 10 ⁻⁷
References:	(a) Righetti and Carvaggio, 1976		
	(b) Keller et al., 1971 - @ 25 °C & 1.0 weight %		

In previous work, Chen and co-workers have reported the isoelectric point of albumin as 4.6-4.7, based on the value

given by Worthington, 1977. This lower value is closer to the value reported for bovine albumin by Righetti and Carvaggio, 1976. Irregardless of the exact value of the isoelectric point of albumin, I_B is less than I_A .

It must be emphasized that the goal in this investigation is not to separate the specific proteins, hemoglobin and albumin. It is rather to study the fundamental principles underlying pH parametric pumping as a protein separation technique, and to develop new separation processes which can be applied to any mixture of proteins with different isoelectric points I_A and I_B . Engineering proof of a basic understanding of the fundamentals lies in mathematical justification of the experimental data.

Chapter 1

BACKGROUND

A great deal of research has been done on the various cyclic separation techniques since parametric pumping was introduced in 1966. Recent review articles have been written by Richard G. Rice, 1976, Phillip C. Wankat, 1974a & 1978a, and Hung T. Chen, 1979b. Wankat's 1978 paper covers the three areas of interest, i.e., parametric pumping, cycling zone adsorption, and pressure swing adsorption, with 127 references discussed. Wankat has a CHEMI module in press on the same topics, which will presumably update and expand this work. Rice's 1976 paper concentrates on parametric pumping and is especially informative on the mathematical modeling which has been done on these processes. Only selected references are discussed in the present work.

The dichotomy which exists between fundamental research and industrial application is immediately apparent in the field of parametric pumping. Wilhelm's thermal parapumping process has generated a continuous flurry of publications since its debut in 1966, including recent papers by Rice and Foo, 1981, and Costa et al., 1982. No known industrial applications have resulted from this work, however. On the other hand, pressure swing adsorption has been practiced industrially for more than twenty years (Skarstrom, 1959), but little fundamental research has appeared in the open literature (Chan et al., 1981). The existing industrial processes specifically apply to mixtures which are difficult and/or expensive to separate by the more

common methods such as filtration, distillation, crystallization, adsorption, etc.

Thermal parametric pumping may be operated by either the DIRECT MODE or the RECUPERATIVE MODE as shown in Figure 2. pH Parametric pumping is operated in the recuperative mode. The temperature in the direct mode of operation is changed across the entire packed bed at the same time by changing the temperature in the heating or cooling jacket. Most of the bench-scale processes have been operated in the so-called direct mode. If these processes were to be scaled-up to industrial-sized packed beds, large segments of down-time would be required in order to change the temperatures for the heating and cooling half-cycles (Stokes, 1976). Stokes and Chen, 1979, have proposed a commercial parapump with multiple smaller tubes in a heat exchanger shell in order to facilitate direct thermal operation.

In the recuperative mode of operation (Figure 2), the appropriate thermodynamic variable is set at the desired level in the entering streams. For thermal operation, the inlet streams are heated or cooled and the temperature change moves across the packed bed as the entering stream penetrates the column. The recuperative thermal mode has been studied by Wilhelm et al., 1966 & 1968; Rolke and Wilhelm, 1969; Gregory, 1974; Sweed and Rigaudeau, 1975; and Wankat, 1978b. The early experimental work by Wilhelm and co-workers indicated that the separations via the direct mode were far superior to those which could be obtained by the recuperative mode of operation.

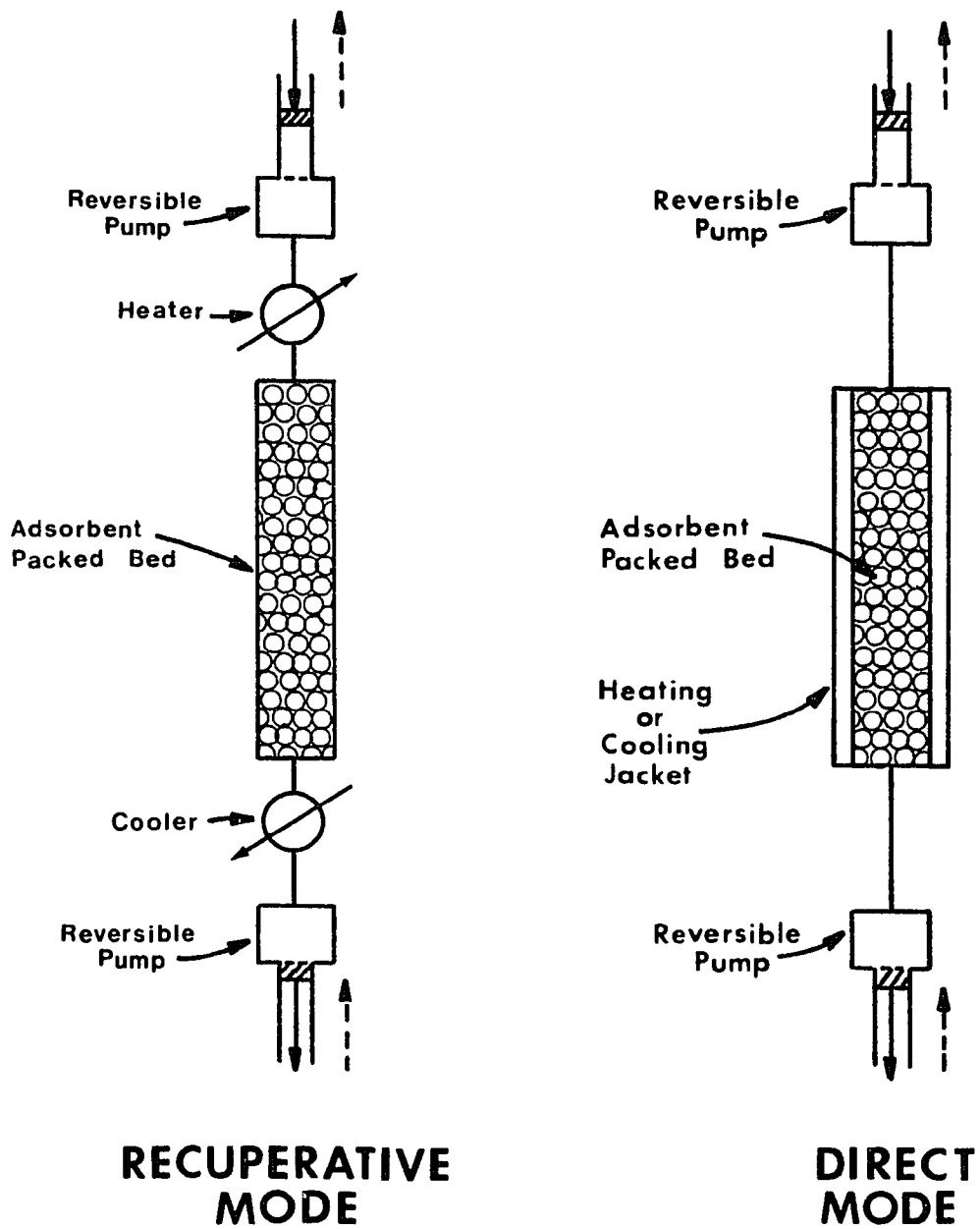


FIGURE 2. Recuperative versus Direct Mode Operation

Wankat, 1978b, has shown mathematically that under certain experimental conditions, the direct and recuperative modes will give identical separations. This study is based on Wankat's experience with the analogous traveling wave mode of cycling zone adsorption. High separation factors have also been predicted for recuperative thermal parapumping by Sweed and Rigaudeau, 1975, but experimental verification is lacking. The difficulty in realizing good separability for the recuperative mode seems to be due to heat losses, i.e., the desired operating temperature is never reached (Kerobo, unpublished work). Sweed and Rigaudeau circumvent this problem by adding supplementary heat exchangers along the column. They calculate that the recuperative mode separation will approach the direct mode separation when the added heat exchangers are used.

pH Parametric pumping has the mechanically attractive feature of recuperative mode operation, and pH losses are not a problem. Experimental separations in the region of 10^2 have been achieved in the pH parapump with electric field (Hollein et al., 1982). The author does not believe, however, that the mechanical advantages of recuperative versus direct operational modes are necessarily a panacea for the question of industrial application. Rather, the feasibility of pH parametric pumping or any other cyclic separation process must hinge on their potential for difficult or tedious separations to be carried-out in a more economic manner.

Stokes and Chen, 1979, compared direct thermal parapumping with distillation and calculated that distillation required

less energy for moderate separations, but that parametric pumping was more energy efficient for good separations. Current industrial applications occur in areas where parametric pumping (or pressure swing adsorption) is indeed more energy efficient. Union Carbide's ISOSIV process, 1976, for the separation of normal and branched paraffins replaces a superfractionator. The pressure swing adsorption process for the removal of methane from hydrogen streams is an alternative to a cryogenic separation process (Alexis, 1967, and Doshi et al., 1971).

Frank B. Hill and co-workers have developed pressure swing and thermal swing processes for the separation of hydrogen isotopes (Wong and Hill, 1979; Wong et al., 1980; Chan et al., 1981; and Hill et al., 1982). Earlier work on the separation of hydrogen isotopes was done by Weaver and Hamrin, 1974. This mixture is difficult to separate under any circumstances, so that any process which promises good separability is attractive. The important advantage of parametric pumping in this case is the phenomena of increasing separation from cycle-to-cycle, with substantial improvement over separability in one-way processes.

Although energy efficiency is an important advantage for parametric pumping processes in general, it is not an important consideration for protein separations. As mentioned previously, protein separations are generally tedious procedures involving many intermediate separation steps. pH Parametric pumping has the potential of improved separation of proteins via a comparatively simple procedure. Furthermore, typical

analytical procedures such as affinity chromatography or ion exchange chromatography may be scaled-up to a preparative scale in the parametric pumping process since the underlying separation principles are identical.

Pressure Swing Adsorption

The parapumping process contains four distinct operations per cycle, which may take place in separate stages or may be combined so that two of the operations occur simultaneously.

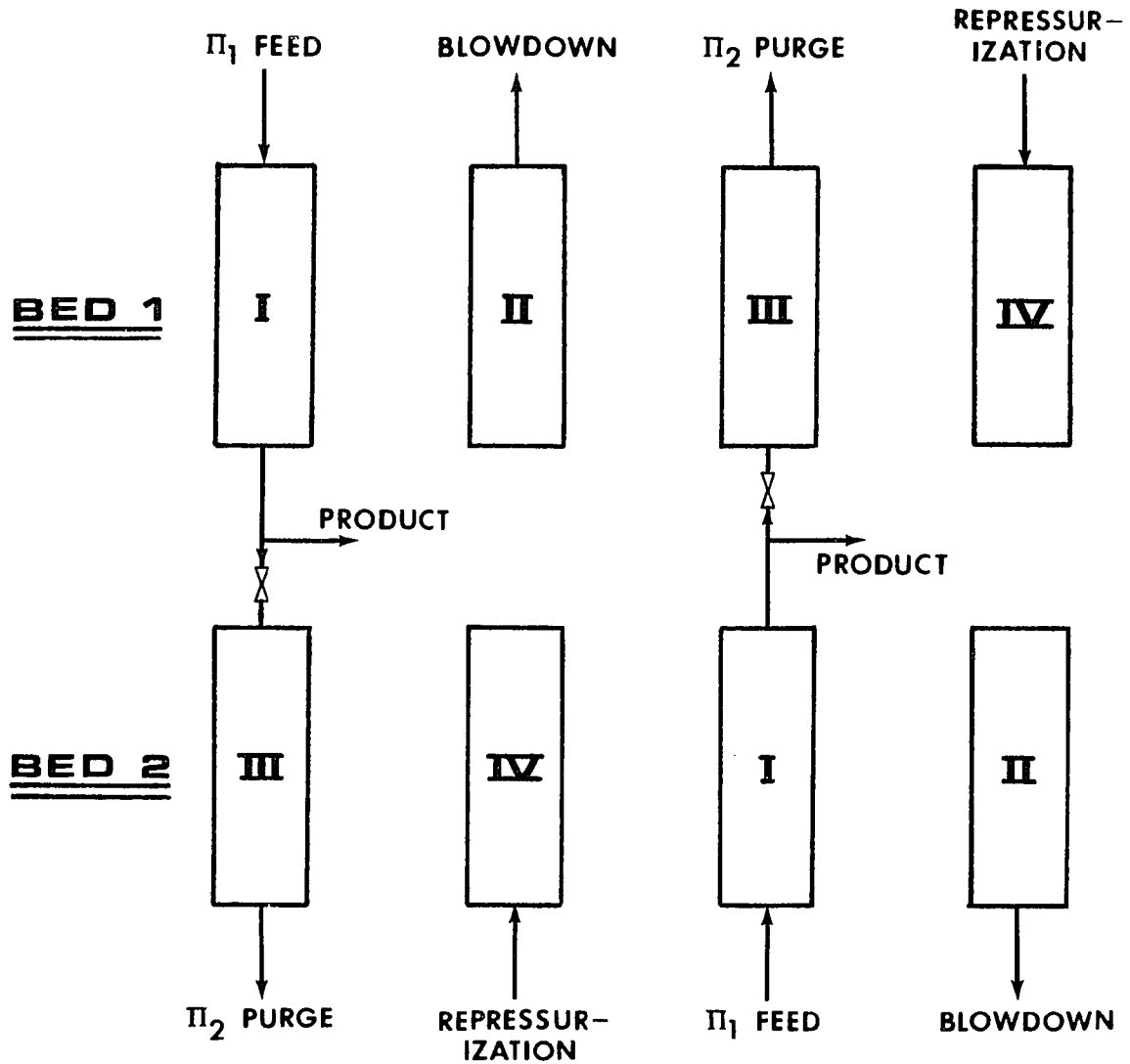
- I. ADSORPTION.
- II. Change of thermodynamic variable to desorptive level.
- III. DESORPTION.
- IV. Change of thermodynamic variable to adsorptive level.

Additional feed stages may be added or the feed may be introduced during the adsorption or desorption stages.

A typical pressure swing adsorption process is shown in Figure 3 (Doshi et al., 1971; Shendalman and Mitchell, 1972; and Wong et al., 1980). Adsorption occurs at high pressure and desorption at low pressure. Four separate stages are used:

- I. ADSORPTION at high feed pressure & DOWNFLOW.
- II. Blowdown to reduce pressure.
- III. DESORPTION at low pressure & UPFLOW.
- IV. Repressurization to feed pressure.

Two or more packed beds are used so that one bed is adsorbing while the other bed is desorbing. Two beds approximate continuous operation since the blowdown and repressurization time periods are minimal compared to adsorption and desorption times.



Π_1 = High Pressure (ADSORB)

Π_2 = Low Pressure (DESORB)

FIGURE 3. Process Diagram for Pressure Swing Adsorption

Thermal Parametric Pumping

Both open- and closed-systems for recuperative mode parapumping were introduced by Wilhelm et al. in 1966. In the CLOSED-SYSTEM, the fluid phase is cycled up-and-down across the solid phase between a top reservoir and a bottom reservoir. This mode of operation is analogous to total reflux in distillation and is commonly referred to as a BATCH parametric pumping process. In the OPEN-SYSTEM, fresh feed is added and product is withdrawn during each cycle. If fresh feed and product withdrawal occur during every stage in the cycle, the process is said to be CONTINUOUS. If feed input and product withdrawal occur only during some portion of each cycle, the process is designated as SEMI-CONTINUOUS. The term SEMI-BATCH is occasionally used instead of semi-continuous.

Wilhelm and Sweed reported separations of $10^{5.1}$ for a mixture of toluene and n-heptane on silica gel in a report in Science magazine in 1968. A direct-mode batch parapump was used. Wilhelm must be credited with the invention of the batch parapump, and the experimentally proven separation capability excited the scientific world. The basic steps for thermal parametric pumping are identical to pressure swing adsorption (Figure 3), where the latter is classified as an open-system. It is easily proved that the ultimate separation is higher for a batch or closed-system than an open-system. This calculation will be presented for the pH-driven parapump in Chapter 2. Both the open and closed thermal systems were examined in detail by Wilhelm et al. in 1968.

The following comments are characteristic of the high praises heaped on Wilhelm's invention,

"It is rare indeed when an entirely novel separation device enters the literature of chemical engineering, but the concept of parametric pumping developed by Wilhelm and co-workers (1966) is just such a device."Rice, 1973.

With "20-20 hindsight", however, one questions the novelty of this process. The similarity between Wilhelm's open-system (recuperative mode) and Skarstrom's pressure swing system are quite apparent. Wilhelm did not seem to be aware of this similarity in the two short reports (Wilhelm et al., 1966, and Wilhelm and Sweed, 1968), but he clearly recognizes Skarstrom's work in the full-length paper.

"Skarstrom (1959) has used pressure as the intensive variable in a two-column parapump type of system to remove moisture from air.".....Wilhelm et al., 1968.

This recognition of the kinship between these processes has been generally ignored, however, and pressure swing adsorption and thermal parametric pumping have remained separate processes in the minds of most chemical engineers.

The four basic stages for thermal parametric pumping are shown in Figure 4. For the recuperative mode, the stages are:

- I. Change of thermodynamic variable to desorptive level, i.e., high temperature & DOWNFLOW.
- II. DESORPTION at high temperature.
- III. Change of thermodynamic variable to adsorptive level, i.e., low temperature & UPFLOW.
- IV. ADSORPTION at low temperature.

Stages I→II→III→IV above are thus analogous to Stages II→III→IV→I for pressure swing adsorption on page 15. For

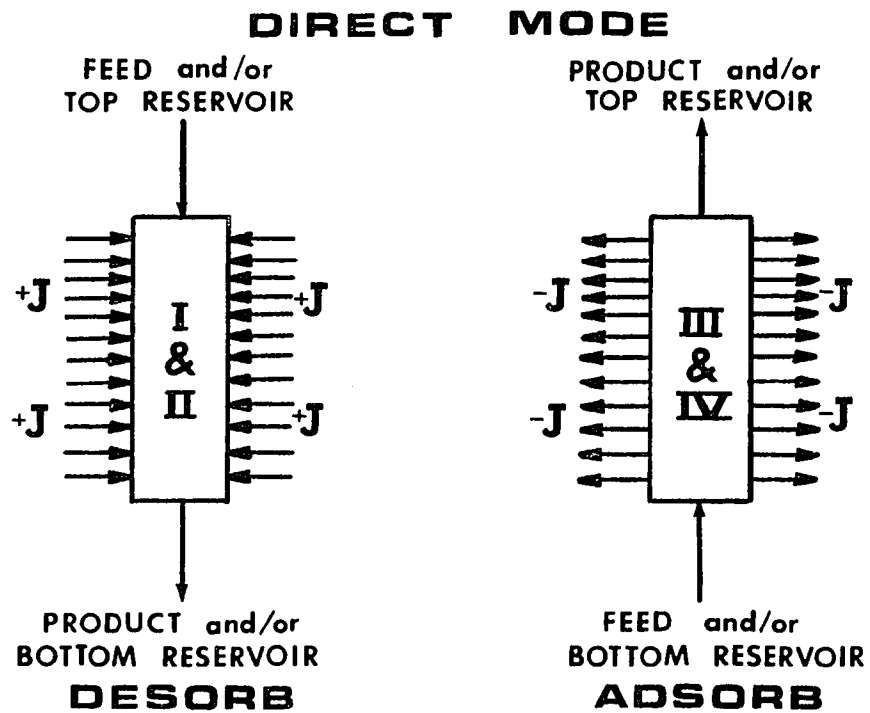
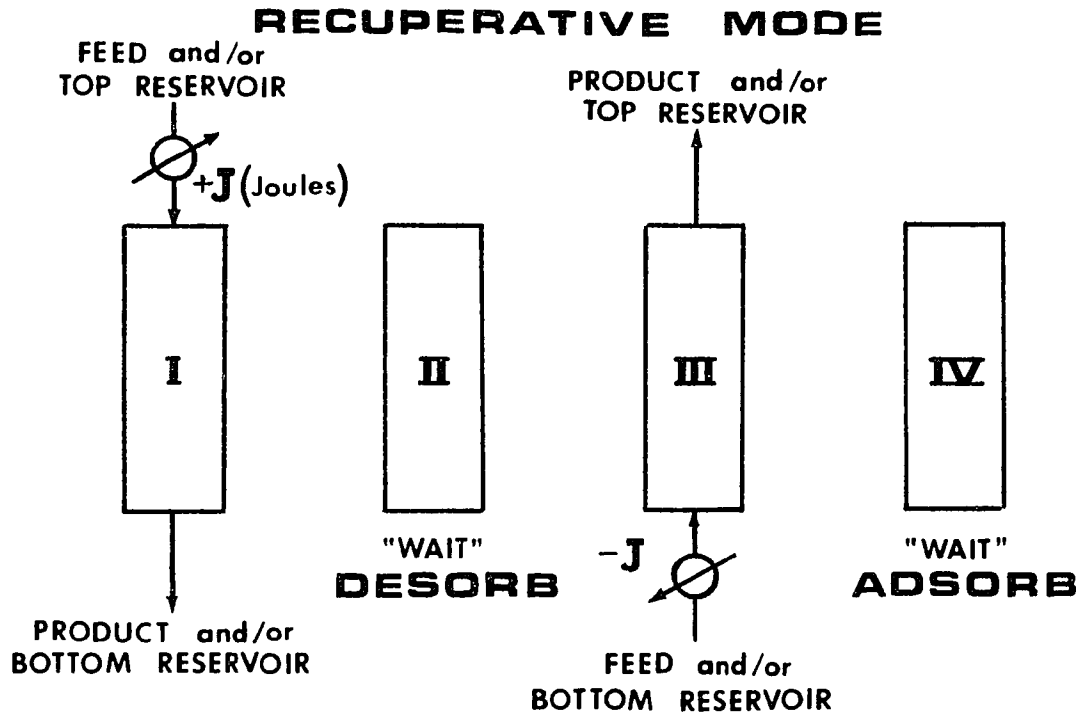


FIGURE 4. Thermal Parametric Pumping:
Process Diagrams for Recuperative and Direct Modes

direct mode operation (Figure 4), the temperature of the fluid to the cooling jacket and the flow direction of the fluid phase in the thermal parapump are changed simultaneously. In this case, Stages I & II and also Stages III & IV are combined.

Chen and Hill introduced the first completely continuous parametric pumping process in 1971. Five different versions of the thermal parapump (two continuous, two semi-continuous, and the batch pump) were analyzed in terms of the equilibrium theory and the appropriate mass transport equations. The mathematical model indicated that, under certain operating conditions, the batch pump and pumps with feed at the enriched end have the capacity for complete removal of a solute from one product fraction and the capacity for arbitrarily large enrichment of that solute in the other fraction. Separation factors and enrichment were predicted to be modest for pumps with feed at the depleted end. Experimental verifications of these models for the system toluene-n-heptane on silica gel have been subsequently presented by Chen and co-workers, 1972, 1973, & 1974a.

The problem of desalination of water is of universal interest and has been studied by Wilhelm et al., 1968; Rolke and Wilhelm, 1969; Chen et al., 1976; and Rice and Foo, 1981. Either mixed cation and anion exchange resins or a bifunctional resin may be used for this separation. Rolke and Wilhelm used a recuperative mode thermal parapumping process. Chen et al. examined this separation in a continuous direct-mode thermal parapump. Chen and co-workers used a non-equilibrium

model to correlate their experimental data. The criterion for approach to equilibrium operation was established for the cases where large separations were deemed possible. This separation has also been investigated by Sweed and Gregory, 1971, and Gregory and Sweed, 1972.

The separation in a parapump increases exponentially cycle-by-cycle up to some limit. Rice, 1973, and Foo and Rice, 1975 & 1977, have examined the ultimate separation in parametric pumps and correlated the limiting separations with axial dispersion. Thompson and Bowen, 1972, calculated that reservoir mixing limits the maximum separability of these processes. They theoretically examined three cases:

$$\begin{aligned} \text{ONE-COLUMN, MIXED RESERVOIRS:} & \quad [SF]_n \propto (\text{Constant})^n \\ \text{ONE-COLUMN, NONMIXED RESERVOIRS:} & \quad [SF]_n \propto (\text{Constant})^{2n-1} \\ \text{TWO-COLUMNS, NO RESERVOIRS:} & \quad [SF]_n \propto (\text{Constant})^{4n-1} \end{aligned}$$

The effect of the mixed reservoir dead volumes was included in the papers by Chen and co-workers from 1972 on. Rice and Foo, 1981, based their continuous direct-mode thermal parametric pumping process for the desalination of water on the two-column system without reservoirs as suggested by Thompson and Bowen. Rice and Foo correlated the separation factor as a function of production rate. Their mathematical model included the limiting effects of eddy diffusion and of finite mass transfer. Experimental verification of the model was presented.

Continuous thermal parametric pumping was extended to the separation of multicomponent mixtures by Chen et al., 1974b. The model system considered was toluene-aniline-

n-heptane on silica gel. A simple model for predicting multicomponent separations was developed. This method invoked the assumption that a multicomponent mixture contains a series of pseudo-binary systems. Each binary system consisted of one solute (toluene or aniline) plus the common inert solvent (n-heptane). Experimental data agreed reasonably well with the analytical predictions. The multicomponent mixture glucose-fructose-water on a cation exchange resin (Bio-Rad AG50W-X4, calcium form) was also studied by Chen and D'Emidio, 1975.

Cycling Zone Adsorption

Cycling zone adsorption may be operated in the STANDING WAVE MODE or the TRAVELING WAVE MODE. These modes are analogous, respectively, to the direct mode or the recuperative mode of parametric pumping. The adsorption/desorption principle is identical to the separation principle in parametric pumping, but there is no change in flow direction in cycling zone adsorption.

A typical cycling zone experiment is shown in Figure 5. The thermodynamic variable for this experiment is pH. The process would be exactly the same if temperature or any other intensive variable were used. The addition of the electric field is not germane at this point, but will be discussed in Chapter 3. Experimental results without the electric field are shown in Figure 6. The process in Figures 5 and 6 was operated in the traveling wave mode. The "WAIT" periods of time from the recuperative mode of parametric pumping

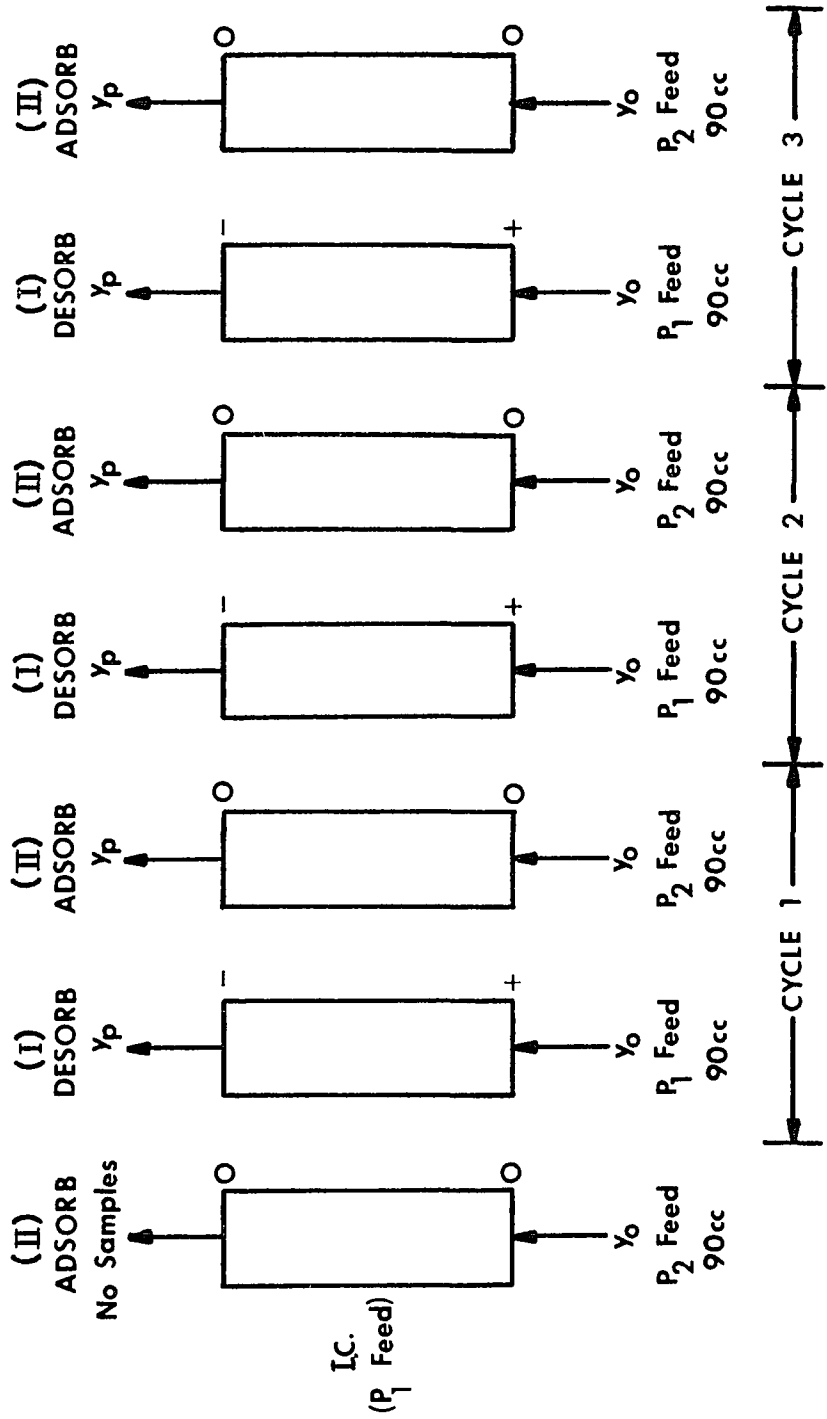


FIGURE 5. Cycling Zone Adsorption with Electric Field (Runs 9-12)

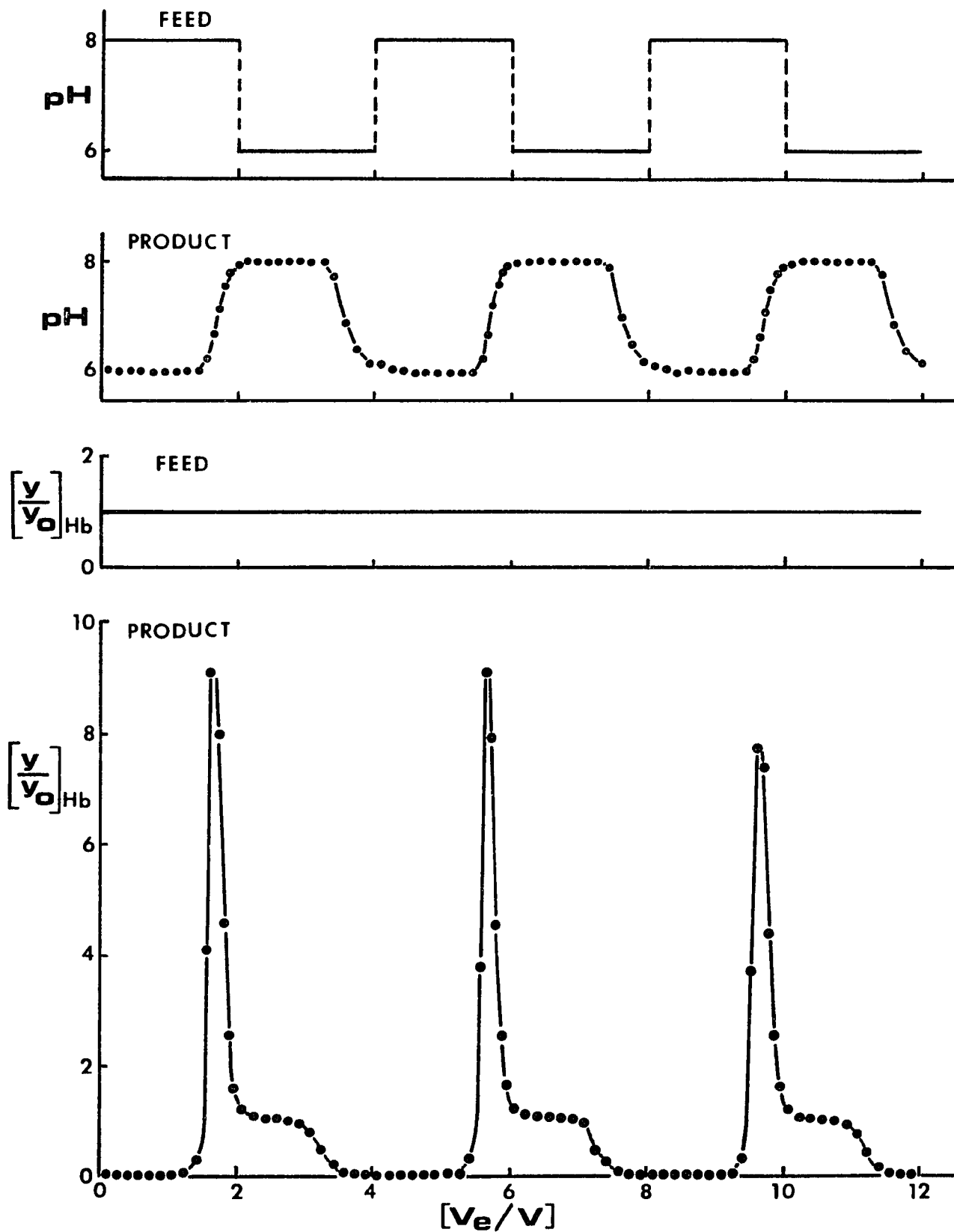


FIGURE 6. Experimental Results for Cycling Zone Adsorption (Run 9)

(Figure 4) are not necessary for the adsorption of some substances and have been eliminated in this case.

As seen in Figure 6, the peaks obtained in a cycling zone process may be quite high. The feed is concentrated up to nine times its initial value in Run 9. The conclusion reached by Chen et al., 1981b, and Ahmed, 1981, that better separation is achieved in parametric pumping than in cycling zone adsorption is based on the premise that the total effluent stream must go to products in both cases. If only the peaks are taken as products in cycling zone adsorption and the intermediate cuts are recycled and reprocessed, the separation will be substantially improved. The engineering cost, of course, is decreased production rate.

Recovery and separability are both important in protein processing. The third peak in Figure 6 is slightly less intense. The first two cycles were run one day, and the third cycle was run the following morning. The protein recovery in the third cycle has dropped from 97% to 90%. Proteins are subject to denaturization from a number of sources. The recovery is generally improved when the processing time is decreased. This further suggests that an increased production rate should result in increased recovery, or that an open-system (continuous or semi-continuous) should exhibit better recovery. Since the maximum separability is higher in a batch parapumping system, an optimization problem between separation and recovery arises. The experimental work by Chen and co-workers was run non-stop, whenever possible, in

order to obtain reliable data.

Wankat, 1978a, describes three possibilities for the traveling wave mode of operation, where ν is the velocity,

- (1) $\nu_{\text{variable}} > \nu_{\text{solute}}$
- (2) $\nu_{\text{solute,desorption}} > \nu_{\text{variable}} > \nu_{\text{solute,adsorption}}$
- (3) $\nu_{\text{solute,desorption}} > \nu_{\text{variable}}$
& $\nu_{\text{solute,adsorption}} > \nu_{\text{variable}}$.

The first case is the usual situation for thermal waves in liquid-solid adsorptive systems. Case (3) occurs in many gas systems and leads to poor separations. Busbice and Wankat, 1975, and Dore and Wankat, 1976, have observed the second case for pH waves in the cycling zone separation of glucose and fructose. The solutions of the transport equations for mass transfer in packed beds do not normally consider the relationship between the velocities of the thermodynamic variable and the solute, because the intensive variable is not changed, i.e., is not time-dependent, in conventional adsorption. For examples, see Bird et al., 1960, and Mickley et al., 1957. Mathematical methods for handling the time-dependency of the thermodynamic variable are suggested by Pigford et al., 1969b, and Wankat, 1974b.

The data in Figure 6 is typical of Case (2). In this case a sharp peak will be formed at the point where the thermodynamic variable (temperature or pH) changes.

$$\nu_{\text{Hb},P_1} > \nu_{\text{pH}} > \nu_{\text{Hb},P_2} \quad (1-1)$$

The solute is adsorbed in toto at P_2 and has a velocity of zero. If a pulse of feed is pumped through the packed bed

at P_1 without change of thermodynamic variable, its velocity is found to approximately equal the bulk fluid velocity (see Runs 20 & 22, Appendix B).

$$\nu_{\text{Hb}, P_1} \approx \nu_o \quad (1-2)$$

$$\nu_{\text{Hb}, P_2} \approx \text{ZERO} \quad (1-3)$$

The velocity of the pH wave is dependent on buffer concentration. In previous work, Chen et al., 1981a, used a high ionic strength buffer for desorption (0.2M phosphate buffer + 0.1M NaCl). At high ionic strength, the velocity of the pH wave approximately equals the bulk fluid velocity. The velocity of the pH wave for 0.1M phosphate buffer may be calculated from the data in Run 9 (Figure 6).

Consider the experimental data in Figure 6 in more detail. If the pH wave velocity were equal to the bulk fluid velocity, the pH of the product in each half cycle of the process as shown in Figure 5 would change after one void volume of upward displacement. Two times the column void volume or 90 cc of feed is introduced in each half cycle. Analysis of the data in Run 9 (Table 3) indicates that the pH changes from $P_2 = 6.0$ to $P_1 = 8.0$ at 1.64 bed volumes and from $P_1 = 8.0$ to $P_2 = 6.0$ at 1.56 bed volumes. The average pH velocity is,

$$\nu_{\text{pH}} = 0.62 \nu_o \text{ @ I.S.} = 0.1\text{M phosphate} \quad (1-4)$$

A pH wave lag occurs because of the fact that the fluid and the solid both have a finite pH capacity. Therefore, a finite amount of hydrogen ion concentration is removed from or added to the fluid as it moves across the packed bed in order to change the pH of the solid.

TABLE 3Calculation of pH Wave Velocity (Run 9)Feed: 0.01 weight % Hemoglobin ($P_2 = 6.0$ & $P_1 = 8.0$)Buffer: 0.1M NaH_2PO_4 + 0.1M Na_2HPO_4

Flow Rate: 2.5 cc/min

Power: None

		<u>v_e/v @ pH = 7.0</u>	<u>v_o/v_{pH}</u>	<u>B</u>	<u>δ_{exp}</u>
Cycle 1:	$P_2 \rightarrow P_1$	1.64	1.64	0.61	} 96.6
	$P_1 \rightarrow P_2$	3.56	1.56	0.64	
Cycle 2:	$P_2 \rightarrow P_1$	5.64	1.64	0.61	} 97.4
	$P_1 \rightarrow P_2$	7.58	1.58	0.63	
Cycle 3:	$P_2 \rightarrow P_1$	9.65	1.65	0.61	} 89.9
	$P_1 \rightarrow P_2$	11.56	1.56	0.64	

Now consider what happens in the situation described by Equations 1-1 to 1-4 and shown in Figure 6. The solute is adsorbed at P_2 and desorbed at P_1 . The faster moving P_1 solute will move ahead of the pH wave into the region where $pH = P_2$ and will be adsorbed. When the $pH = P_1$ wave catches up to the adsorbed solute, the solute will be desorbed at the point of pH change. This phenomenon creates a sharp concentration wave at the front of the pH wave as seen in Figure 6.

"We can expect large separations when the velocity of the cyclic variable lies between the solute wave velocities. This is caused by 'trapping' of the solute at the wave front of the cyclic variable.".....Wankat, 1978a.

The data on the cyclic adsorption of hemoglobin in Figure 6, therefore, indicates the potential for excellent separation in the hemoglobin-albumin-CM Sepharose model system. Either the traveling wave mode of cycling zone adsorption or the recuperative mode of parametric pumping has the potential for separation of proteins with different isoelectric points.

The original work on the standing wave and traveling wave modes of cycling zone adsorption was done by Pigford et al., 1969b; Baker and Pigford, 1971; and Gupta and Sweed, 1971. Either mode may be adapted to multicomponent separations. For a mixture of n components, $n+1$ operating levels of the thermodynamic variable are required. For the hemoglobin-albumin system, three pH levels such that $P_1 > I_A > P_2 > I_B > P_3$ where I_A and I_B are the isoelectric points of hemoglobin and albumin, respectively, will give concentrated peaks for both proteins. Additional information on multicomponent separations

is given by Wankat, 1975 & 1977; Wankat and Ross, 1976; Nelson et al., 1978; and Foo et al., 1980.

Electrochemical Parametric Pumping and Electropolarization Chromatography

Prior to the current investigation, only two studies have been reported that used electric field as the thermodynamic variable in parapumping processes. The intensive variable is normally cycled in order to stimulate adsorption/desorption of solute molecules between the fluid and the solid phases. This was the function of the electric potential in the cyclic desalination processes by Thompson and Bass, 1974a,b, and Oren and Soffer, 1978. The experimental study by Hollein et al., 1982, however, used electric potential for electrophoretic separation of the solutes in the fluid phase in combination with pH cyclic operation for adsorption/desorption. The separation principle associated with the electric field in the latter case is analogous to electropolarization chromatography.

The electric potential as a thermodynamic variable can be applied to the entire column at the same time without axial flow, so this variable operates in the so-called direct mode. Oren and Soffer, 1978, developed a four-stage batch process for electrochemical parametric pumping. The process scheme is identical to the direct thermal mode except that the flow functions are separated self-contained stages.

- I. Change of thermodynamic variable to adsorptive level, i.e., positive electric potential, plus ELECTROADSORPTION at positive field.

- II. UPFLOW of NaCl depleted stream at zero voltage to top reservoir.
- III. Change of thermodynamic variable to desorptive level, i.e., negative electric potential, plus ELECTRODESORPTION at negative field.
- IV. DOWNFLOW of NaCl enriched stream as zero voltage to bottom reservoir.

The parapumping process, whether pressure-driven, temperature-driven, or electric potential-driven, therefore, always consists of three sets of coupled cyclic functions. These are (1) change of thermodynamic variable, increase or decrease; (2) momentum transport, upflow or downflow; and (3) mass transport, adsorption or desorption. The pH-driven processes operate on the same principle.

Oren and Soffer, 1978, used an electrochemical column with two high surface porous carbon electrodes and a thin separator. The NaCl was adsorbed onto the carbon electrodes in the "double layer region". Details for the physical mechanism for this adsorption were given by Soffer and Folman, 1972. The batch process by Thompson and co-workers, 1974a,b, called "cyclic electrodialysis" also operated on the electro-adsorption/electrodesorption principle. The NaCl was adsorbed onto cation and anion selective membranes separated by a non-selective storage layer. Positive potential was applied during both adsorption and upflow (Stages I & II) and negative potential was applied during desorption and downflow (Stages III & IV). Separation factors in the range of 10^3 were obtained.

Reis and Lightfoot and co-workers have investigated the

separation of proteins including chymotrypsin, gamma globulin, hemoglobin, cyanmet-hemoglobin, human serum albumin, and lactic acid dehydrogenase via electropolarization chromatography. See Reis et al., 1974; Lee et al., 1974; Reis and Lightfoot, 1976; Chiang et al., 1979; and Shah et al., 1979. Chromatographic separations were carried out in small diameter tubes or ultrafiltration fibers without a stationary sorbent or solid phase. An axial electric field was applied. The effect of the electric field was to retard or decrease the velocity of the various proteins relative to the bulk fluid velocity. Separations were achieved due to the fact that different proteins exhibited different retardation velocities relative to the bulk fluid flow. The separation principle in electropolarization chromatography is the same as the effect of the electric field in the present investigation, except that an axial field is applied instead of a radial field.

Chapter 2

pH PARAMETRIC PUMPING

The direct-mode of operation is deemed to be possible for pH parametric pumping processes, but has never been demonstrated experimentally. For this mode of operation the walls of the column would consist of membranes which were permeable to hydrogen ions. The column would then be jacketed and the jacket periodically filled with acidic or basic solution. Table 4 outlines the pH cyclic processes which have been investigated to date, all of which were operated in the recuperative or traveling wave mode. Recuperative mode operation allows the use of a standard chromatographic column or packed bed. High and low pH streams are alternately introduced to the top and bottom of the column. The two oscillating streams are maintained at the correct pH by passage through dialyzers or by direct titration with small amounts of concentrated acid or base. Buffered solutions may be used in order to stabilize the pH of the process streams. Relatively little work has been done on either pH parametric pumping or pH cycling zone adsorption.

Sabadell and Sweed developed pH parametric pumping in 1970 for the separation of a mixture of Na^+ and K^+ in water on a cation exchange resin. Experimental runs of up to 500 cycles were made in a semi-continuous process with feed input and product withdrawal at one end of the system. Separations of 1.2 to 1.8 were reported.

TABLE 4

pH Cyclic Separation Processes

<u>Fluid Mixture</u>	<u>Solid Adsorbent</u>	<u>Process</u>	<u>Variable</u>	<u>Reference</u>
Na ⁺ , K ⁺ , Water	Cation Exchange Resin (IRC-84)	P.P.	pH	Sabadell & Sweed (1970)
α -Chromotrypsin, Trypsin, Water	Sepharose CVB CHOM	P.P.	pH & Affinity	Shaffer & Hamrin (1975)
Glucose, Fructose, in Water or Buffer	DBAE-cellulose	C.Z.A.	pH only Temperature pH & Temp.	Busbice & Wankat (1975), Dore & Wankat (1976)
Hemoglobin, Albumin, in Buffer Solution	SP Sephadex C-50	P.P.	pH	Chen et al. (1977)
Hemoglobin, Albumin, in Buffer Solution	CM Sepharose & DEAE Sepharose	P.P. C.Z.A.	pH & I.S.	Chen et al. (1979a, 1980a, 1980b, 1981a), S. Wu (1981), Yang (1981), Pancharoen (1982)
Hemoglobin, Albumin, in Buffer Solution	CM Sepharose	P.P.	pH only pH & Elec- tric Field	Chen et al. (1981c), Hollein et al. (1982)
Alkaline Phosphatase & Undesired Proteins in Buffer Solution	DEAE Sepharose	P.P. C.Z.A.	I.S. only pH only I.S. & pH	Chen et al. (1981b) Ahmed (1981)
Na ⁺ , K ⁺ , Water	Duolite C20	T.W.C.	pH	Bailly & Tondeur (1981)

NOTE: P.P. = Parametric Pumping, C.Z.A. = Cycling Zone Adsorption,
T.W.C. = Two-Way Chromatography, I.S. = Ionic Strength

Bailly and Tondeur, 1981, also examined the K^+/Na^+ separation in a similar process designated as "two-way chromatography." The process included a feed and product stream in each half cycle, but was still semi-continuous with flow between reservoirs in some stages. The focus of this work was on the propagation of the concentration wave through the chromatographic column. A detailed explanation of the movement of the Na^+ and K^+ concentration waves for one complete cycle was shown, and the potential for cyclic operation was suggested.

Chen and D'Emidio, 1975, used direct-mode, thermal parametric pumping to separate mixtures of glucose and fructose in aqueous solution on a cation exchange resin. Sugar mixtures are notoriously difficult to separate. Earlier studies by Chen et al., 1972, and Ahmed, 1974, used fuller's earth and activated carbon for this separation -- also in direct-mode thermal processes.

Busbice and Wankat, 1975, and Dore and Wankat, 1976, examined the glucose-fructose separation on a dihydroxyboryl-phenylsuccinamyl derivative of aminoethyl cellulose in cycling zone processes. Wankat and co-workers experimented with a thermal standing-wave mode, a pH traveling-wave mode, and a combination of the two modes. The pH traveling-wave mode gave much better separation than the direct or standing-wave thermal mode. The combined mode was only slightly better than the pH separation.

Both sugars are adsorbed at high pH, but fructose is

more strongly adsorbed than glucose. Little adsorption occurs at low pH for either sugar. Two pH levels were used in order to concentrate or enrich one sugar in a binary mixture, i.e., fructose-water or glucose-water. Three pH levels were used in order to separate the ternary mixture glucose-fructose-water. By selectively choosing the three pH levels, -- high pH (P_H), middle pH (P_M), and low pH (P_L) -- and then pumping in feed at P_H , P_M , and P_L in sequence, two peaks were formed. The less strongly adsorbed glucose was trapped at the P_M wave front and the more strongly fructose was trapped at the P_L wave front. Wankat and co-workers clearly described the dependence of the position and intensity of the concentration waves on the relationships between the velocities of the solutes at different pH levels and the velocity of the pH wave front. This idea was related to hemoglobin adsorption in Equations 1-1 to 1-4 of the present work and is included in the mathematical modeling in Chapter 6.

In 1975, Shaffer and Hamrin reported a process for enzyme separation based on a combination of affinity chromatography and pH parametric pumping. The removal of trypsin from solutions of trypsin in water and from a mixture of trypsin plus α -chymotrypsin in water was demonstrated experimentally and explained mathematically. Trypsin was adsorbed at high pH and desorbed at low pH. The batch parapump for this process was identical to the recuperative-mode pump shown in Figure 2, except that acidic or basic dialyzers were used in place of the heat exchangers at the two ends of the packed bed.

The remainder of the protein separation work in Table 4 was done by Hung Tsung Chen and co-workers at New Jersey Institute of Technology, 1977, 1979a, 1980a,b,c, and 1981a, b,c,d. The achievements in this research program are:

- (1) Proof that the pH parapump has the capability to separate arbitrary mixtures of proteins with different isoelectric points, Chen et al., 1977.
- (2) Development of the single-column enrichment process with finite mass transfer model, Wu, 1981.
- (3) Development of the multi-column splitting process with finite mass transfer model, Yang, 1981.
- (4) Modeling of the underlying separation principles using the graphical method and a local equilibrium model, Pancharoen, 1982.
- (5) Development of the single-column splitting process using electric field in conjunction with pH, Hollein et al., 1982.
- (6) Adaptation of pH parametric pumping to the purification of a real system (alkaline phosphatase plus undesired proteins) and development of the ionic strength parapump, Ahmed, 1981.
- (7) Development of multi-column affinity parametric pumping (without pH change) for the separation of lectins, Chao et al., 1981.

These processes demonstrate the feasibility of parametric pumping as a separation technique for proteins using the thermodynamic variables, pH, ionic strength, electric field, and molecular affinity. The experimentally demonstrated separation for the model system, human hemoglobin plus human serum albumin, has been dramatically improved from 6.5 in the original process by Chen et al., 1977, to 120 in the most recent process by Hollein et al., 1982.

The two separation problems of ENRICHMENT and SPLITTING need to be defined. Consider a mixture of proteins A and B

in water or buffer solution. The proteins may be separated in a parametric pumping process with top and bottom products in each cycle for a semi-continuous or continuous system or with products taken from the top and bottom reservoirs at the completion of the experiment for a batch system. If Protein A is enriched in the bottom product and stripped from the top product while Protein B concentrations remain unchanged, the parapumping process is labelled as an enrichment process. A splitting process is one in which Protein A is enriched in the bottom product and stripped from the top product while Protein B is enriched in the top product and stripped from the bottom product. In all of the investigations by Chen and co-workers on the separation of hemoglobin and albumin, hemoglobin is enriched in the bottom product and stripped from the top product. Hemoglobin is therefore related to Protein A and albumin to Protein B in this discussion.

The first protein separation process considered by Chen et al., 1977, for the separation of hemoglobin and albumin on Sephadex cation exchanger is shown in Figure 7. The semi-continuous pump was operated batchwise during upflow and continuously during downflow. A center feed was introduced during downflow between a stripping and an enriching column. Both products were taken during downflow with the top product being taken from the line between the top reservoir and the stripping column and the bottom product being taken from the line between the enriching column and the bottom reservoir. Low pH fluid was introduced at the top of the column and high

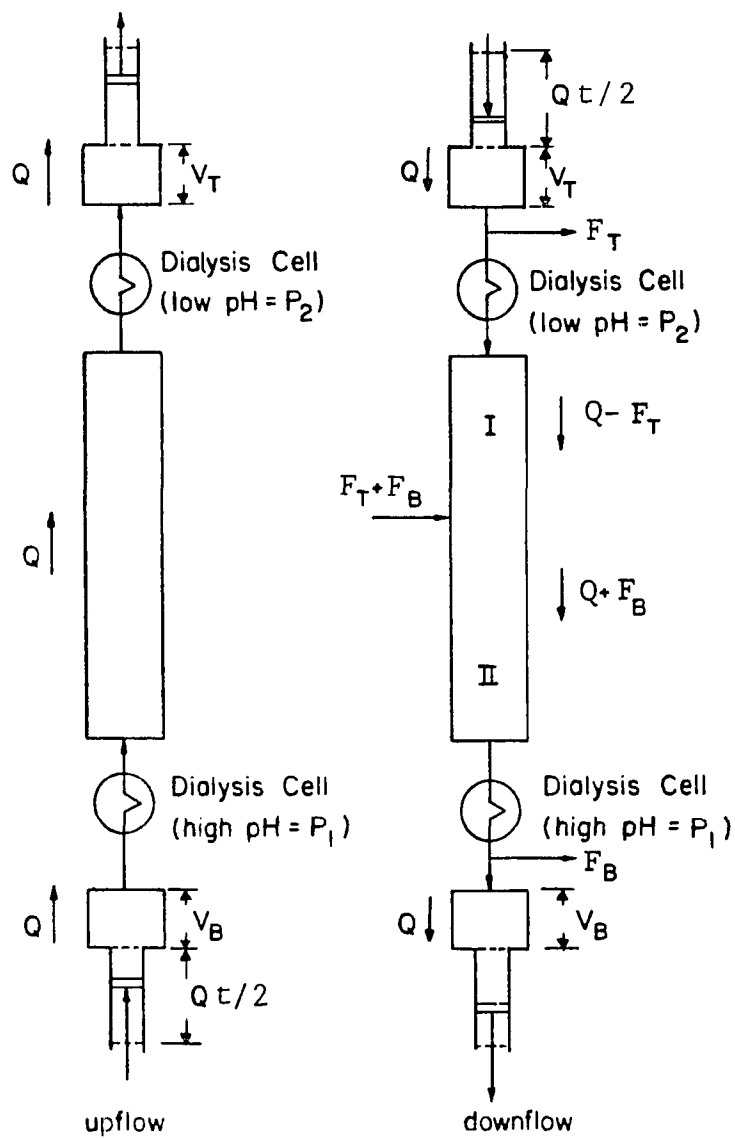


FIGURE 7. The Two-Stage Semicontinuous pH Parapump

Ref: Chen et al., AICHE J., 23, 697 (1977)

pH fluid was introduced at the bottom of the column. Various factors affecting the separation were examined, including the pH levels and the ionic strength of the protein solution, reservoir displacement, and product flow rate. The protein solution was buffered with monobasic and dibasic sodium phosphate with ionic strengths ranging from 0.035M to 0.2M. Hemoglobin was stripped from the top stream and enriched in the bottom stream while albumin concentrations remained unchanged. A hemoglobin separation factor of approximately 6.5 was achieved in the run with the greatest pH spread -- $P_1 = 8.9$ and $P_2 = 5.5$, where P_1 and P_2 are respectively above and below the isoelectric point of hemoglobin.

A "continuous" pH parametric pump was used to separate the model system hemoglobin-albumin on CM Sepharose cation exchanger by Chen et al., 1979a, and Wu, 1981. Note that this system is currently considered to be "semi-continuous" because each cycle contains two out of a total of four stages where product is not withdrawn. This process as shown in Figure 8 is the basis for the remainder of the protein separation research by Chen and co-workers. A single ion exchange column was used with a bottom feed at low pH in Stage II and a top feed at high pH in Stage IV. A top product which was stripped of hemoglobin and a bottom product which was enriched in hemoglobin were taken in Stages II and IV respectively. It was demonstrated that increasing the volume of the top product to some optimum level relative to the volume of the bottom product gave the pump the capacity for large enrichment of

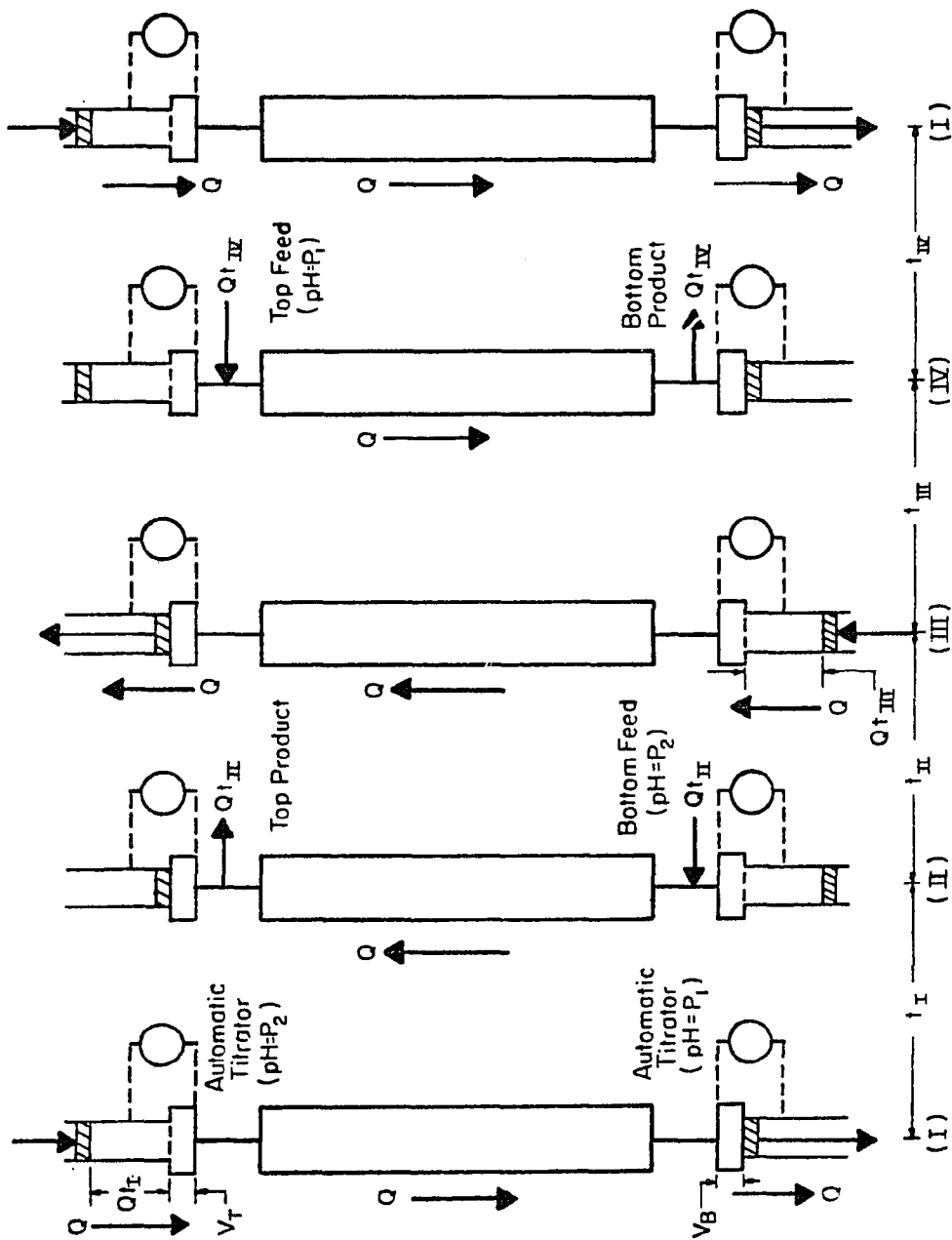


FIGURE 8. The Four-Stage pH Parapump

Ref: Chen et al., AICHE J., 25, 321 (1979b)

hemoglobin in the bottom product stream. Improved separation was obtained by using a high ionic strength buffer at high pH (0.2M phosphate + 0.1M NaCl @ $P_1 = 8.0$) for desorption and a low ionic strength buffer at low pH (0.05M phosphate @ $P_2 = 6.0$) for adsorption. Sodium is the counter ion for the CM Sepharose gel, so an increase in sodium ion concentration should enhance desorption as shown in Equations I-4 and I-5. and as experimentally demonstrated.

Splitting of multicomponent protein mixtures by multi-column pH parametric pumping was investigated theoretically and experimentally by Chen et al., 1980a, and Yang, 1981. The parapump consisted of a series of chromatographic columns packed alternately with cation and anion exchangers. The model system used for the experimental work was hemoglobin plus albumin on CM Sepharose cation exchanger and DEAE Sepharose anion exchanger. Separation of a mixture of n proteins requires a system of n columns and $n+2$ reservoirs. For the hemoglobin-albumin mixture, three pH levels were used such that $P_1 > I_A > P_2 > I_B > P_3$. Protein A or hemoglobin was adsorbed at P_2 and desorbed at P_1 on the cation exchanger while Protein B concentrations remained unchanged. Protein B or albumin was adsorbed at P_2 and desorbed at P_3 on the anion exchanger while Protein A concentrations remained unchanged. Optimization of the two-column pH parapump for the separation of the hemoglobin-albumin mixture has recently been completed by Varuntanya, 1982. A maximum separation factor of thirty-two was obtained in the batch process and twenty-one in the semi-continuous process.

The purification of the enzyme alkaline phosphatase was experimentally investigated by Chen et al., 1981b, and Ahmed, 1981, using a semi-continuous parametric pumping process with pH and ionic strength as the thermodynamic variables. Alkaline phosphatase, extracted from the human placenta and purified commercially, contains some undesired proteins which have approximately the same isoelectric point as the enzyme. An intensive variable other than pH is therefore required for the separation. Both cation and anion exchangers were investigated, but the DEAE anion exchanger resulted in a better separation. The enzyme and the impurities were both adsorbed at high pH and desorbed at low pH. The enzyme was less strongly adsorbed on the ion exchanger and, consequently, could be selectively desorbed at high pH by increasing the ionic strength of the buffer solution. The enzyme and the undesired proteins were adsorbed at $\text{pH} = 7.4$ and $\text{I.S.} = 0.1\text{M}$. The enzyme was desorbed and separated at $\text{pH} = 7.4$ by increasing the ionic strength from 0.1M to 0.6M . The undesired proteins were then desorbed at $\text{pH} = 4.0$ and $\text{I.S.} = 0.1\text{M}$. Experimental optimization of the enzyme purification indicated that a parapump with the proper combination of the two intensive variables, pH and ionic strength, was superior to a parapump based only on pH or ionic strength.

Separation of protein mixtures has also been investigated in multi-column cyclic processes based on affinity chromatography by Chao et al., 1981. Semi-continuous parametric pumping and continuous simulated moving bed operation were compared with batch operation for the separation of lectin

mixtures. The two systems both consisted of a series of columns packed alternately with Sephadex G-150 and Sepharose 4B gels. Concanavalin A was adsorbed on the Sephadex G-150 gel and eluted with d-glucose, while Ricinus Communis Agglutinin I (RCA₁₂₀) was adsorbed on the Sepharose 4B gel and eluted with β -lactose. The pH was kept constant at 7.3 and a 0.05M phosphate + 0.2M NaCl buffer was used for the entire process. The affinity parapump and the ionic strength parapump both provide potential methods for separating proteins with similar isoelectric points, and the pH parapump provides a separation technique for proteins with different isoelectric points.

Graphical Solution

Electrophoresis is one of the conventional methods of protein separation. Entire books have been written on the subject. For examples, see the recent review article by van Oss, 1979. The concept behind the present investigation was the development of a parametric pumping process based on conventional electrophoresis. Runs 1-3 in Appendix B were made by polyacrylamide gel electrophoresis in a LKB 7900 UNIPHOR apparatus. Electrophoresis is a very time-consuming process (low production rate). It was decided to combine electrophoresis with pH parametric pumping in order to improve the protein separation achieved in the single-column or the multi-column pH parapumps.

The final process was totally unknown and unprecedented at this point. The previous electrochemical parametric

pumping processes by Thompson and Bass, 1974a,b, and Oren and Soffer, 1978, used an electric field to institute adsorption and desorption. The assumption was made in the present case that the only effect of the electric field would be electrophoretic migration of the proteins in the fluid phase. It was conceivable, however, that the adsorptive capacity of a charged substance such as an ion exchange resin or a protein ion could be affected by the existence of an externally imposed electric field. The first step in this research adventure into the unknown was to prove qualitatively that the protein separation or hemoglobin enrichment achieved via the four-stage pH parapump (Figure 8) could be theoretically improved by the addition of an electric field to the process scheme. Qualitative proof was obtained by comparison of the maximum or equilibrium separations obtainable in the pH parapumps with and without electric field. This comparison was done by the graphical method.

The graphical method follows the model of Grevillot and Tondeur, 1976, 1977, and 1980, as developed for the direct mode of thermal parametric pumping. The graphical method was adapted to the recuperative mode of pH parametric pumping by Chen et al., 1980a,b, Pancharoen, 1982, and Yang, 1981. Grevillot and Tondeur considered the batch system with a single transfer step per half-cycle in 1976, the batch system with multiple transfer steps per half-cycle in 1977, and the open-system with multiple transfer steps per half-cycle in 1980. These graphs resemble McCabe-Thiele-type diagrams. Note that if the packed bed is taken as only one cell with

one transfer step per half-cycle, the graphical solutions for the recuperative mode and the direct mode are identical irrespective of the thermodynamic variable used.

Chen et al., 1980a,b, used the graphical method for the batch pH parapump in order to prove that there was a finite limit to the separation. Earlier solutions by the Method of Characteristics suggested that the separation increased infinitely with increasing number of cycles. For examples, see the equations by Thompson and Bowen, 1972, on page 21 of this dissertation, and also the original work by Pigford et al., 1969b. Chen and co-workers expanded the graphical method to handle the multi-column batch pH parapump with three or more operating levels for the thermodynamic variables. In the present work, the graphical method is adapted to the semi-continuous pH parapump in order to compare the limits for the protein separation or hemoglobin enrichment in the parapumps with and without electric field.

The graphical method is based on the LOCAL EQUILIBRIUM MODEL by Pigford et al., 1969b. This model recognizes the fact that a concentration gradient exists in the axial direction in a packed bed, but assumes "local equilibrium" between the fluid and solid phases.

"Despite the success of the computer calculations of separation factors reported by Wilhelm and his co-workers, one wonders what the origin of the separation is. The equations of transport used in the numerical work are so unwieldy that only qualitative explanations were offered. It was noticeable, however, in the computer results reported by Wilhelm et al. (1968), that, despite the large differences in composition at the ends of the column of adsorbent, the local differences between fluid composition were small.".....Pigford et al., 1969b.

The local equilibrium model calculates the limit of the separability in the parametric pump excluding the effects of axial diffusion and finite mass transfer. This model has been widely used in parapumping calculations because it clearly indicates the fundamental origin of the separability.

In mathematical terminology, consider the film theory in transport phenomena, where λ is the mass transfer coefficient through the film.

$$\frac{\partial C_S}{\partial t} = \lambda (C_L - C_L^*)$$

Or in dimensionless form,

$$\frac{\partial x}{\partial t} = \lambda (y - y^*) \quad (2-1)$$

Where, $x = \frac{C_S}{C_O}$

$$y = \frac{C_L}{C_O} \quad \text{or} \quad y^* = \frac{C_L^*}{C_O}$$

And C_O equals the concentration of the solute in the feed.

Assume a linear adsorption isotherm for the distribution of the solute between the fluid and the solid phases.

$$x = k_{pH} y^*, \quad \text{where } k_{pH} = f(pH) \quad (2-2)$$

The local equilibrium theory assumes that,

$$y \approx y^* \quad \text{or} \quad x \approx k_{pH} y \quad (2-3)$$

This assumption is only valid if the time t is relatively large or if λ is infinite. Chen et al., 1981a, have shown that λ is finite in the hemoglobin-albumin model system.

The eight-stage pH parapump (Figure 9) by Chen et al., 1981a, will be used for the graphical solution of the

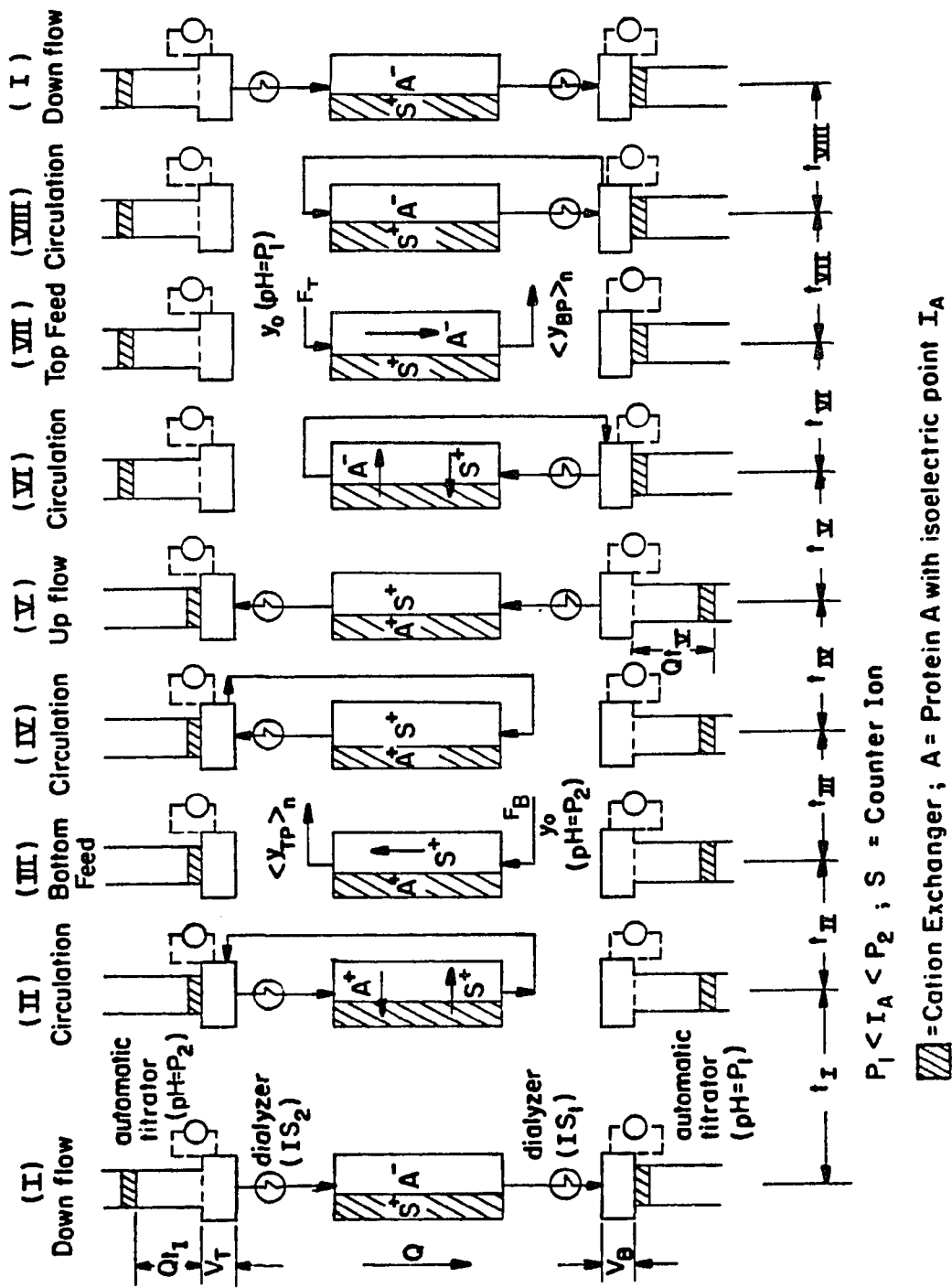


FIGURE 9. The Eight-Stage pH Parapump

Ref: Chen et al., Sep. Sci., 16, 45 (1981a)

semi-continuous processes with and without electric field. As the circulation time t_c becomes very large, the separation approaches the equilibrium values predicted by the local equilibrium model.

$$t_{II} = t_{IV} = t_{VI} = t_{VIII} = t_c = \infty \quad (2-4)$$

The eight-stage pH parapump is of interest because when the circulation time equals zero in Stages II, IV, VI, and VIII, the process is identical to the four-stage pH parapump in Figure 8. Thus, the eight-stage process with circulation predicts the limiting separation in the four-stage process. Infinite circulation times further simplify the graphical solution, because as t_c becomes very large the concentration gradient in the axial direction is eliminated. In this case, the entire column may be calculated as a single cell with one transfer step each for Stages I, III, V, and VII.

Following is a stage-by-stage description of the eight-stage pH parapump (Figure 9). The chromatographic column is filled with the cation exchanger CM Sepharose with counter ion S^+ or Na^+ . The volume of the solid phase is \bar{V} . The interstitial space or void volume is filled with feed at high pH = P_1 . The volume of the fluid phase is V . The bottom reservoir with dead volume V_B is also filled with P_1 feed. The top reservoir with dead volume V_T is filled with P_2 feed at a volume equal to $V + V_T$. The operating pH's are set so that $P_1 > I_A > P_2$ where I_A is the isoelectric point of the adsorbed protein or hemoglobin. The protein molecule becomes A^- at P_1 or A^+ at P_2 and is adsorbed at

P_2 and desorbed at P_1 . Dialyzers and automatic titrators are used in order to maintain the pH's at P_1 and P_2 in the bottom and top reservoirs, respectively, during the run.

The timing for the circulation stages is variable and will be given by Equation 2-4 for the present case. The durations for the other stages are as follows:

$$t_I = t_V = \frac{V}{Q} \quad (2-5)$$

$$t_{III} = \frac{F_B}{Q} \quad (2-6)$$

$$t_{VII} = \frac{F_T}{Q} \quad (2-7)$$

The bulk flow rates are equal to Q in all eight stages. One void volume will be displaced in Stages I, III, V, and VII for the present case or,

$$F_B = F_T = V \quad (2-8)$$

One complete cycle of operation contains eight distinct stages.

I. DOWNFLOW: The solution with $\text{pH} = P_2$ from the top reservoir enters the top of the column, and at the same time the solution in the column flows from the bottom of the column to the bottom reservoir, i.e., the variable is changed.

II. CIRCULATION: The fluid phase is circulated between the column and the top reservoir. This ensures that the pH in the column is completely changed from P_1 to P_2 , and that Protein A is adsorbed in the solid phase.

III. BOTTOM FEED: The feed with $\text{pH} = P_2$ enters the bottom of the column, and the solution depleted in Protein A

is pushed out from the top of the column as the top product.

IV. CIRCULATION: The fluid phase is circulated between the column and the top reservoir. This allows the concentration in the column and the top reservoir to become uniform, and ensures that Protein A from the fresh feed is adsorbed in the solid phase.

V. UPFLOW: The solution with $\text{pH} = P_1$ from the bottom reservoir enters the bottom of the column, and at the same time the solution in the column flows from the top of the column to the top reservoir, i.e., the variable is changed.

VI. CIRCULATION: The fluid phase is circulated between the column and the bottom reservoir. This ensures that the pH in the column is completely changed from P_2 to P_1 , and that Protein A is desorbed from the solid phase.

VII. TOP FEED: The feed with $\text{pH} = P_1$ enters the top of the column, and the solution enriched in Protein A is pushed out from the bottom of the column as the bottom product.

VIII. CIRCULATION: The fluid phase is circulated between the column and the bottom reservoir. This allows the concentration in the column and the bottom reservoir to become uniform. One cycle is now completed, and Stage I of the second cycle begins.

The separation for the batch pH parapump will be solved graphically first, since this is the simplest case to follow (Figures 10 and 11). Elimination of the feed stages from the eight-stage process, as well as the adjacent circulation stages, i.e., III, IV, VII, and VIII, results in the batch parapump in Figure 10. The batch pump is identical to the

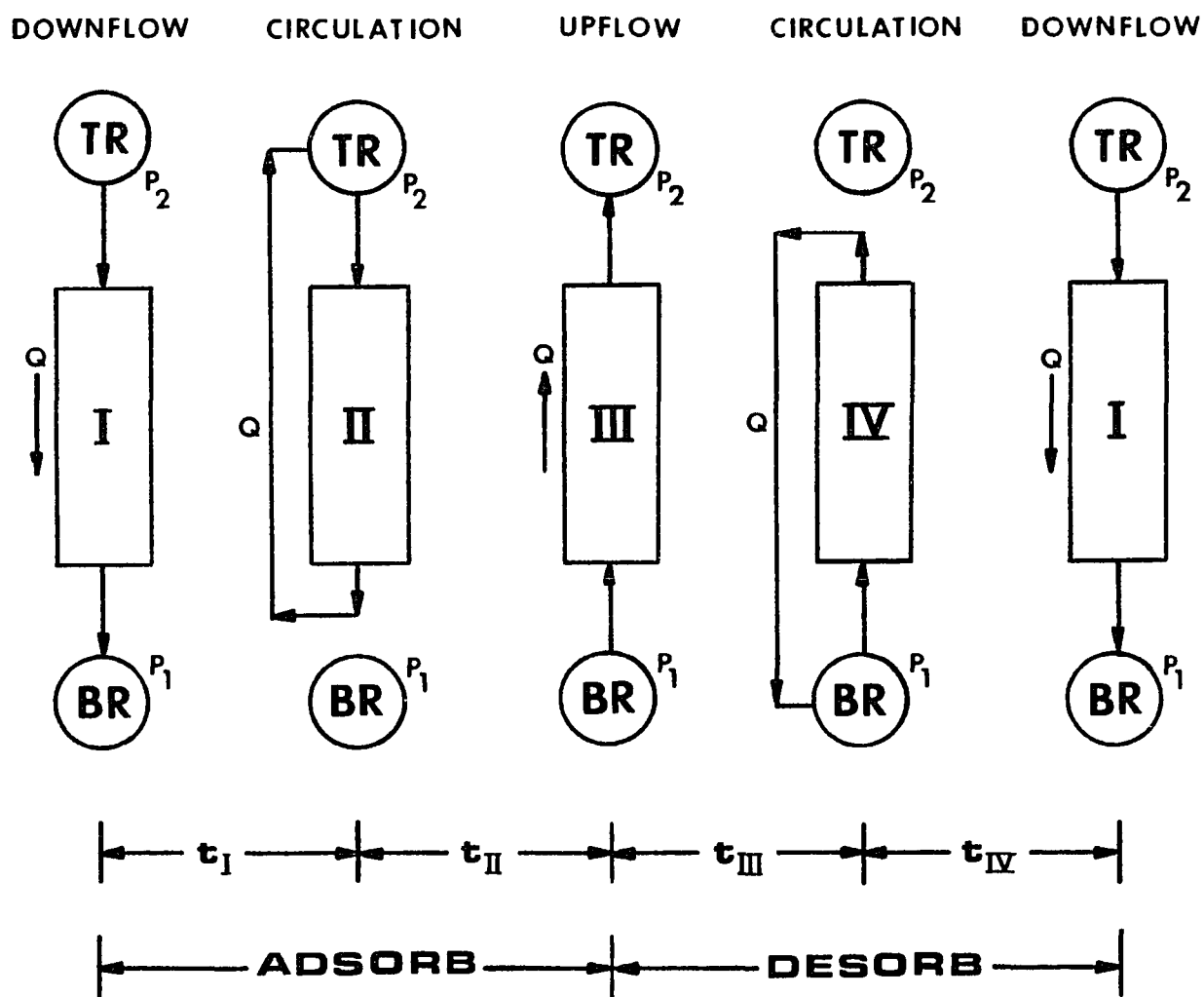


FIGURE 10. The Batch pH Parapump

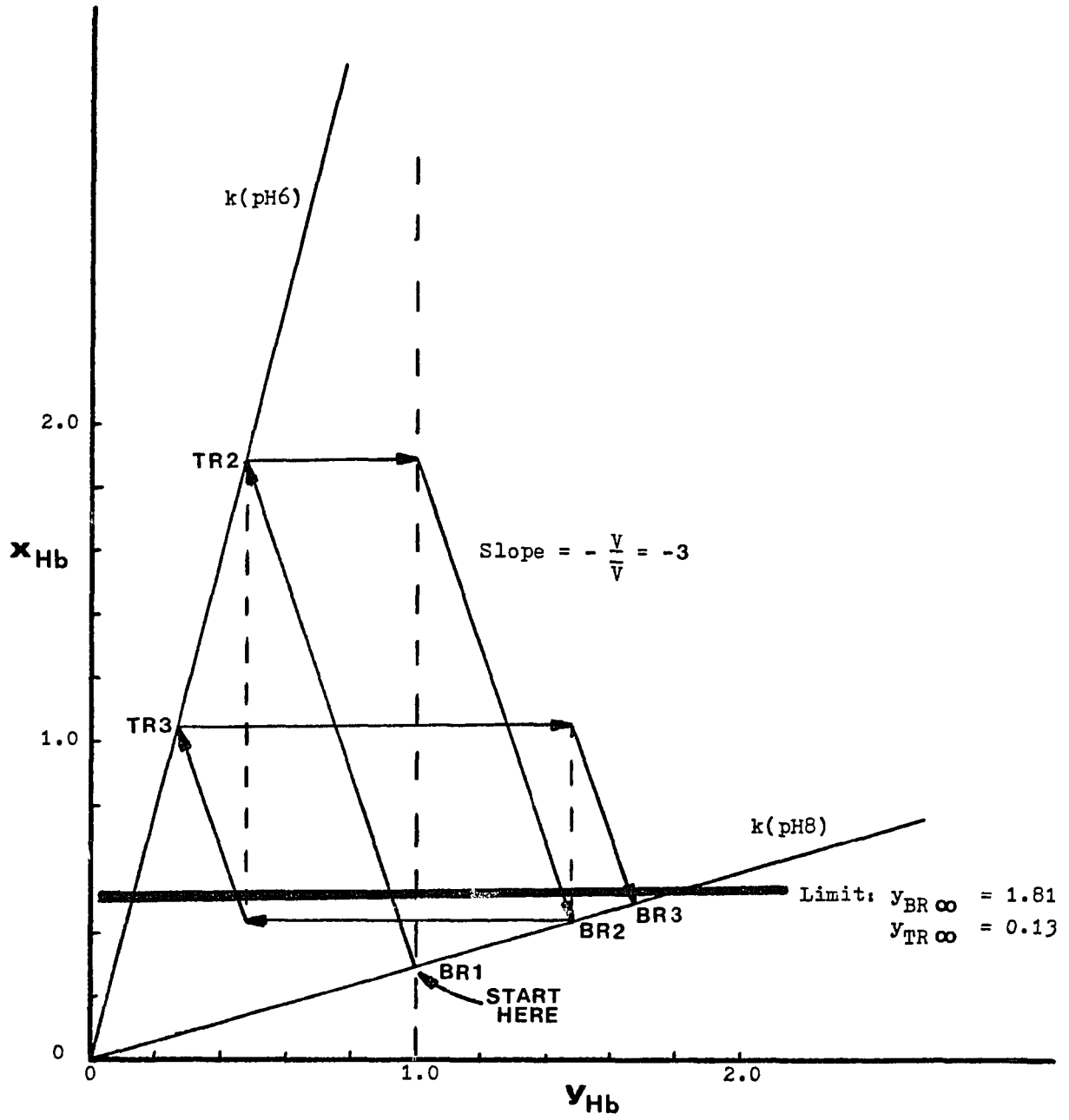


FIGURE 11. Graphical Solution for the Batch Process

thermal recuperative mode parapump previously considered (Figure 4), except that the "wait" stages are replaced by circulation stages. Adsorption and desorption take place in the downflow and upflow stages, respectively, and also in the adjacent circulation stages as shown in Figure 10.

The graphical solution for the batch parapump is shown in Figure 11. From Equation 2-3, the adsorption isotherms are plotted as lines passing through the origin for high pH, $k(\text{pH}8)$, and for low pH, $k(\text{pH}6)$. The starting conditions are indicated, and two complete cycles are graphed from START to TR2 to BR2 to TR3 to BR3. The limit for TR_∞ and BR_∞ is always a horizontal line for the batch parapump. The limit is shown as a boldfaced line.

The graphical solution is determined by a stage-by-stage equilibrium mass balance. The equilibrium relationships for the n'th cycle of operation in the batch parapump are,

$$\langle x_{C1} \rangle_n = k(\text{pH}@ P_1) \langle y_{BR} \rangle_n, P_1 = 8.0 \quad (2-9)$$

$$\langle x_{C2} \rangle_n = k(\text{pH}@ P_2) \langle y_{TR} \rangle_{n+1}, P_2 = 6.0 \quad (2-10)$$

At the beginning of Stage I of the n'th cycle,

$\langle y_{TR} \rangle_n$ = concentration of solute in the fluid in the top reservoir.

$\langle y_{BR} \rangle_n$ = concentration of solute in the fluid in the bottom reservoir and also in the fluid in the column.

$\langle x_{C1} \rangle_n$ = concentration of solute in the solid in the column.

In Stage I, fluid from the top reservoir is pumped into the column. In Stage II, the fluid in the column and the top reservoir is circulated until equilibrium of the solute

between the fluid and the solid phases is reached. The mass balance for Stages I and II becomes,

$$\begin{aligned}\bar{V}\langle x_{C1}\rangle_n + V\langle y_{TR}\rangle_n + V_T\langle y_{TR}\rangle_n \\ = \bar{V}\langle x_{C2}\rangle_n + (V + V_T)\langle y_{TR}\rangle_{n+1}\end{aligned}$$

Solving for $\langle x_{C2}\rangle_n$,

$$\langle x_{C2}\rangle_n = \langle x_{C1}\rangle_n - \frac{V + V_T}{\bar{V}} \{ \langle y_{TR}\rangle_{n+1} - \langle y_{TR}\rangle_n \} \quad (2-11)$$

At the beginning of Stage III of the n'th cycle,

$\langle y_{TR}\rangle_{n+1}$ = concentration of solute in the fluid in the top reservoir and also in the fluid in the column.

$\langle y_{BR}\rangle_n$ = concentration of solute in the fluid in the bottom reservoir.

$\langle x_{C2}\rangle_n$ = concentration of solute in the solid in the column.

In Stage III, fluid from the bottom reservoir is pumped into the column. In Stage IV, the fluid in the column and the bottom reservoir is circulated until equilibrium of the solute between the fluid and the solid phases is reached.

The mass balance for Stages III and IV becomes,

$$\begin{aligned}\bar{V}\langle x_{C2}\rangle_n + V\langle y_{BR}\rangle_n + V_B\langle y_{BR}\rangle_n \\ = \bar{V}\langle x_{C1}\rangle_{n+1} + (V + V_B)\langle y_{BR}\rangle_{n+1}\end{aligned}$$

Solving for $\langle x_{C1}\rangle_{n+1}$,

$$\langle x_{C1}\rangle_{n+1} = \langle x_{C2}\rangle_n - \frac{V + V_B}{\bar{V}} \{ \langle y_{BR}\rangle_{n+1} - \langle y_{BR}\rangle_n \} \quad (2-12)$$

From Equation 2-9,

$$\langle x_{C1}\rangle_{n+1} = k(\text{pH@ } P_1) \langle y_{BR}\rangle_{n+1} \quad (2-13)$$

The initial conditions for Stage I of the first cycle are known. The fluid concentrations, y_{TR} and y_{BR} , in the top reservoir, the bottom reservoir, and the column are all equal to the feed concentration y_0 or 1.0 on Figure 11. The initial solid concentration x_0 is found from Equation 2-9. The subsequent fluid and solid concentrations are found by solving Equations 2-10 & 2-11 simultaneously, then solving Equations 2-12 & 2-13 simultaneously, and repeating the calculations until steady-state values are obtained for $\langle y_{TR} \rangle_n$ and $\langle y_{BR} \rangle_n$.

The operating lines for adsorption have a slope of $-(V + V_T)/\bar{V}$ from Equation 2-11. The operating lines for desorption have a slope of $-(V + V_B)/\bar{V}$ from Equation 2-12. The graphical solution is further simplified by setting the reservoir dead volumes equal to zero, so that all the operating lines have the same slope or $-V/\bar{V}$.

$$V_T = V_B = \text{ZERO} \quad (2-14)$$

The STOP & GO algorithm developed by Sweed and Wilhelm, 1969, will be used in the graphical solution of Equations 2-9 to 2-14. Each operating stage is hypothetically divided into two separate steps:

GO - fluid displacement without interphase mass transfer.

STOP - interphase mass transfer without fluid displacement.

The GO lines are all horizontal arrows in Figure 11. The STOP lines are arrows slanting up or down with a negative slope, i.e., $-V/\bar{V}$.

Based on the equations above, follow the first two cycles for the batch pH parapump in Figure 11 step-by-step,

Cycle 1, Stages I & II:

START - at (x_o, y_o) .

STOP - Move along the operating line to equilibrium at low pH at (x_{TR2}, y_{TR2}) .

Cycle 1, Stages III & IV:

GO - to (x_{TR2}, y_{BR1}) . x_{TR2} is unchanged. Fluid from the bottom reservoir at y_{BR1} is added.

STOP - Move along the operating line to equilibrium at high pH at (x_{BR2}, y_{BR2}) .

Cycle 2, Stages I & II:

GO - to (x_{BR2}, y_{TR2}) . x_{BR2} is unchanged. Fluid from the top reservoir at y_{TR2} is added.

STOP - Move along the operating line to equilibrium at low pH at (x_{TR3}, y_{TR3}) .

Cycle 2, Stages III & IV:

GO - to (x_{TR3}, y_{BR2}) . x_{TR3} is unchanged. Fluid from the bottom reservoir at y_{BR2} is added.

STOP - Move along the operating line to equilibrium at high pH at (x_{BR3}, y_{BR3}) .

This procedure is continued cycle-to-cycle until there is no longer any change in the top and bottom reservoir concentrations.

The graphical solution for the semi-continuous pH parametric pumping process is shown in Figure 12. The graph is based on the eight-stage process in Figure 9. The graphical procedure is the same as that explained above, except that high and low pH feed stages are added. The first two cycles are outlined below.

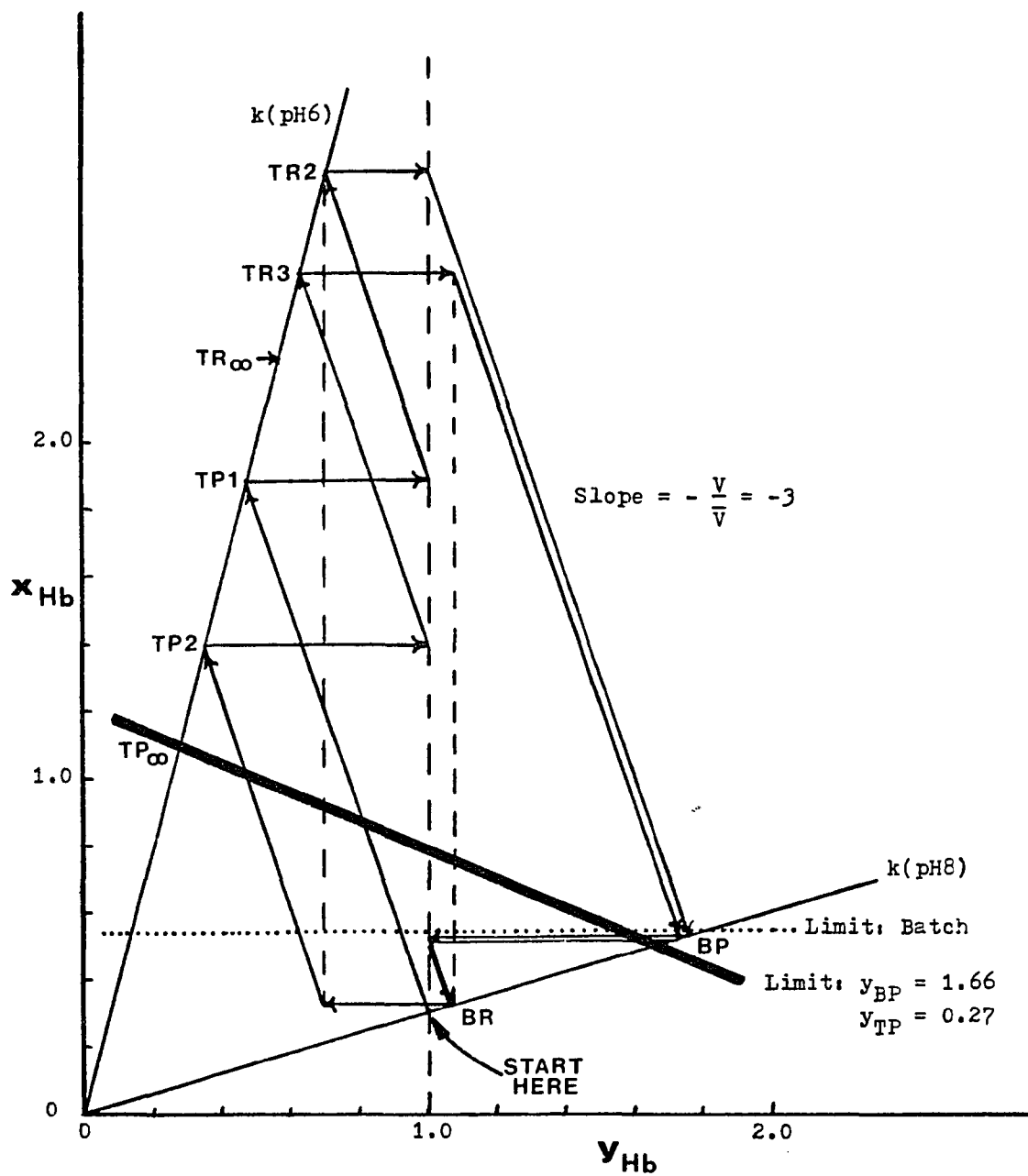


FIGURE 12. Graphical Solution for the Semicontinuous Process without Electric Field

Cycle 1, Stages I & II:

START - at (x_o, y_o) .

STOP - Move along the operating line to equilibrium at low pH at (x_{TP1}, y_{TP1}) .

Cycle 1, Stages III & IV:

GO - to (x_{TP1}, y_o) . x_{TP1} is unchanged. Fresh feed at y_o is added.

STOP - Move along the operating line to new equilibrium at low pH at (x_{TR2}, y_{TR2}) .

Cycle 1, Stages V & VI:

GO - to (x_{TR2}, y_{BR1}) . x_{TR2} is unchanged. Fluid from the bottom reservoir at y_{BR1} is added.

STOP - Move along the operating line to equilibrium at high pH at (x_{BP1}, y_{BP1}) .

Cycle 1, Stages VII & VIII:

GO - to (x_{BP1}, y_o) . x_{BP1} is unchanged. Fresh feed at y_o is added.

STOP - Move along the operating line to new equilibrium at high pH at (x_{BR2}, y_{BR2}) .

Cycle 2, Stages I & II:

GO - to (x_{BR2}, y_{TR2}) . x_{BR2} is unchanged. Fluid from the top reservoir at y_{TR2} is added.

STOP - Move along the operating line to equilibrium at low pH at (x_{TP2}, y_{TP2}) .

Cycle 2, Stages III & IV:

GO - to (x_{TP2}, y_o) . x_{TP2} is unchanged. Fresh feed at y_o is added.

STOP - Move along the operating line to new equilibrium at low pH at (x_{TR3}, y_{TR3}) .

Cycle 2, Stages V & VI:

GO - to (x_{TR3}, y_{BR2}) . x_{TR3} is unchanged. Fluid from the bottom reservoir at y_{BR2} is added.

STOP - Move along the operating line to equilibrium at high pH at (x_{BP2}, y_{BP2}) .

Cycle 2, Stages VII & VIII:

GO - to (x_{BP2}, y_0) . x_{BP2} is unchanged. Fresh feed at y_0 is added.

STOP - Move along the operating line to new equilibrium at high pH at (x_{BR3}, y_{BR3}) .

This procedure is continued cycle-to-cycle until there is no longer any change in the top and bottom product concentrations.

The batch limit is indicated by a dotted line in Figure 12. The semi-continuous limit is indicated by a boldfaced line. From comparison of the two lines, it can be seen that the batch separation is greater than the semi-continuous separation. The limit for TP_{∞} and BP_{∞} for the semi-continuous parapump is always a line of negative slope with the top product at higher y and the bottom product at lower y than the corresponding batch case. Therefore, the separation is always less for an open-system than a comparable closed-system.

The graphical solution for the semi-continuous process with electric field is shown in Figure 13. The eight-stage pH parapump is again the basis for the process, but an electric field is placed across the chromatographic column in Stages III and V. Comparison of the limiting separations in Figure 13 indicates that the separation can be improved by addition

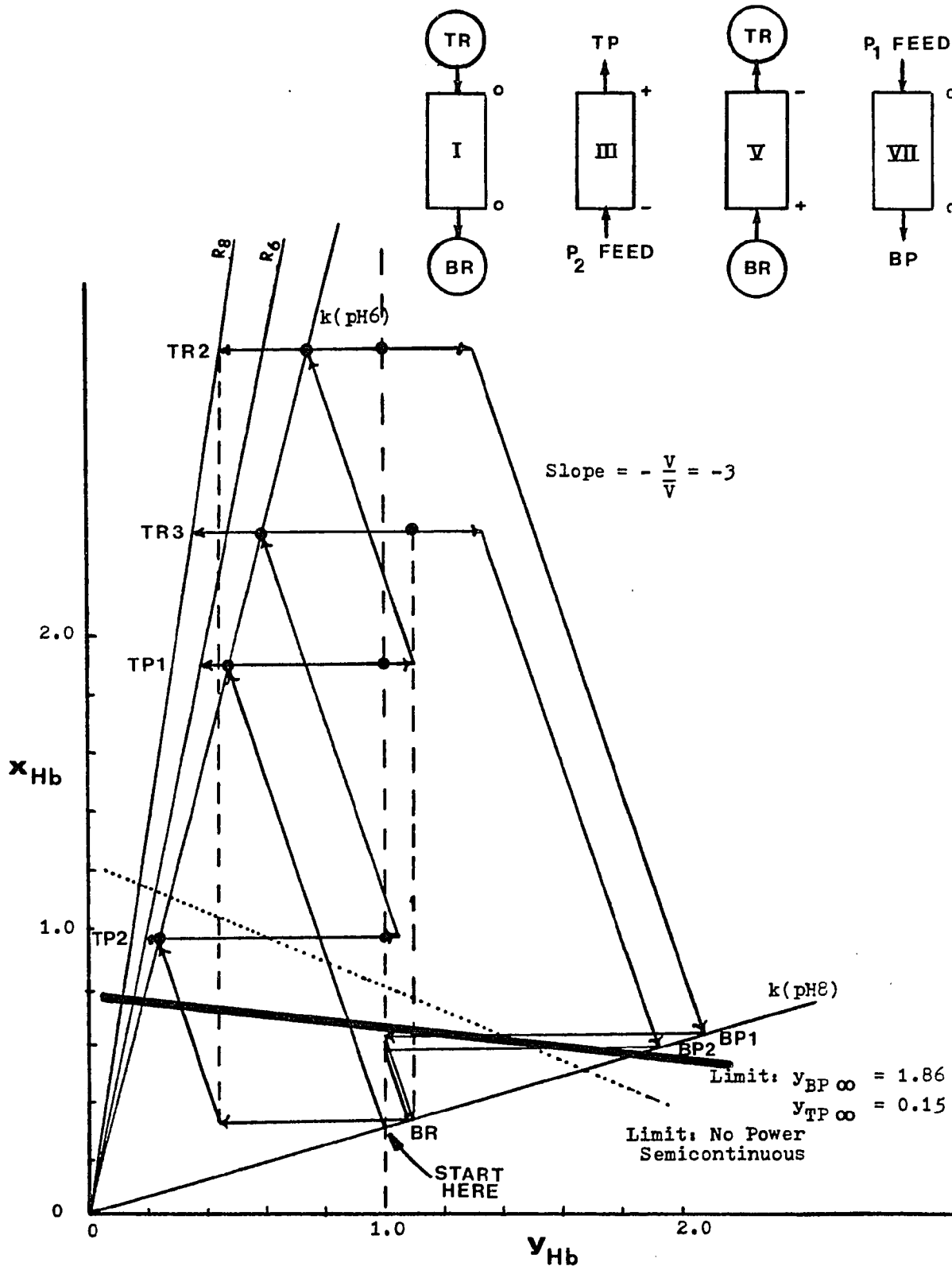


FIGURE 13. Graphical Solution for the Semicontinuous Process with Electric Field

of an electric field, if the assumptions used in calculating the effect of the electric field are correct. Experimental verification of the predictions in Figure 13 was obtained in some of the preliminary work which will be discussed briefly in Chapter 7. The new splitting process is of more interest and will be discussed in detail in Chapters 3 to 6. The graphical solution in Figure 13 is important at this point, because it proves the potential for improved separation due to the combination of pH and electric field in a para-pumping process.

At operating pH's higher than their isoelectric points, proteins carry a net negative charge and migrate toward the cathode with a velocity v_E which is directly proportional to the strength of the electric field E and the protein mobility μ . The mobility is a complex function of pH, protein concentration, buffer composition, buffer ionic strength, and particle diameter.

$$v_E = \mu E \quad , \quad \text{where } \mu = f(\text{pH}) \quad (2-15)$$

At pH's lower than their isoelectric points, the proteins carry a net positive charge and migrate toward the anode at a velocity v_E .

The retardation coefficient R_i as defined for protein separation by Shah et al., 1979, is a measure of the relative effectiveness of the electric field in a flow system.

$$R_i = \frac{\text{velocity of species } i \text{ at zero field}}{\text{velocity of species } i \text{ at finite field}} \quad (2-16)$$

When the electric field is applied in the axial direction in

a flow system, the net velocity ν_z in the axial direction is the sum of the migration velocity and the protein velocity at zero field. The migration velocity may be either positive or negative with respect to the protein velocity.

$$\nu_z = \nu_{Hb} \pm \nu_E \quad (2-17)$$

Equation 2-16 may be written in terms of these velocities.

$$R_i = \frac{\nu_{Hb}}{\nu_{Hb} \pm \nu_E}, \quad \text{where } \nu_E \leq \nu_{Hb} \quad (2-18)$$

The migration velocities are expected to be small compared to the protein velocities at zero field. Note that for $\nu_E \ll \nu_{Hb}$, the effect of the electric field becomes negligible. If ν_{Hb} equals zero, the migration velocity will also equal zero. The last statement simply says that if the protein molecule is totally adsorbed and doesn't move, the electric field cannot desorb the protein or otherwise move it.

In Stage III, feed at low pH is entering the bottom of the column. The charge on the protein is A^+ . A negative electrode is placed at the bottom of the column (Figure 13) in order to attract A^+ downwards and decrease its concentration in the top product stream. In Stage V, high pH fluid containing protein at A^- is entering the bottom of the column. The positive electrode is placed at the bottom of the column in order to attract A^- downwards and decrease its concentration in the fluid leaving the top of the column. The protein concentration in the fluid leaving the column in time Δt is:

$$y_{out} = \frac{\text{g mole protein out}}{\text{cc fluid out}}$$

$$y_{out} = \frac{(\nu_{Hb} - \nu_E) A \Delta t y_{column}}{\nu_{Hb} A \Delta t} \quad (2-19)$$

From Equations 2-18 and 2-19,

$$y_{out} = \frac{1}{R_i} y_{column} \quad , \quad \text{where } R_i = f(\text{pH}) \quad (2-20)$$

By mass balance, if Protein A is decreased in the top product streams; then Protein A must be increased in the bottom product streams. The electric field effect is only included in the GO step or fluid displacement step. The differences between Figures 12 and 13, consequently, only appear on the horizontal lines or GO steps for the top product (Stage III) and the top reservoir (Stage V). The mass balance for Stage III is,

$$\langle y_{TP} \rangle_n = \frac{1}{R_{pH6}} \langle y_{C,II} \rangle_n \quad (2-21)$$

$$\langle y_{C,III} \rangle_n = y_o + \left(1 - \frac{1}{R_{pH6}}\right) \langle y_{C,II} \rangle_n \quad (2-22)$$

Similarly for Stage V,

$$\langle y_{TR} \rangle_{n+1} = \frac{1}{R_{pH8}} \langle y_{C,IV} \rangle_n \quad (2-23)$$

$$\langle y_{C,V} \rangle_n = \langle y_{BR} \rangle_n + \left(1 - \frac{1}{R_{pH8}}\right) \langle y_{C,IV} \rangle_n \quad (2-24)$$

Where,

$\langle y_{C,II} \rangle_n$ = concentration of solute in the fluid in the column at the end of Stage II.

$\langle y_{C,III} \rangle_n$ = concentration of solute in the fluid in the column in Stage III after the GO step.

$\langle y_{C,IV} \rangle_n$ = concentration of solute in the fluid in the column at the end of Stage IV.

$\langle y_{C,V} \rangle_n$ = concentration of solute in the fluid in the column in Stage V after the GO step.

Based on Equations 2-9 to 2-14, 2-18, and 2-21 to 2-24, the first two cycles for the semi-continuous pH parapump with electric field are plotted in Figure 13. The procedure is the same as in Figure 12, except as noted below:

Cycle 1, Stages I & II:

STARTsame as without electric field.....

STOP - Move along the operating line to equilibrium at low pH at $(x_{TP1}, y_{C,III1})$.

Cycle 1, Stages III & IV:

GO - to $(x_{TP1}, y_{C,III1})$. x_{TP1} is unchanged. Fresh feed at y_0 is added. The fluid going from the top of the column to the top product is decreased in concentration by a finite amount. The fluid remaining in the column is increased in concentration by the same finite amount. This amount is represented by the segment of the horizontal line between the dot and the arrow head in Figure 13, with the finite decrease to the left and the finite increase to the right of the horizontal line. The top product leaves the top of the column at y_{TP1} .

STOP - Move along the operating line to new equilibrium at low pH at $(x_{TR2}, y_{C,IV1})$.

Cycle 1, Stages V & VI:

GO - to $(x_{TR2}, y_{C,V1})$. x_{TR2} is unchanged. Fluid from the bottom reservoir at y_{BR1} is added. Fluid flows from the top of the column to the top reservoir at a decreased concentration of y_{TR2} as indicated above.

STOPsame as without electric field.....

Cycle 1, Stages VII & VIII: same as without electric field.

Cycle 2, Stages I & II:

GOsame as without electric field.....

STOP - Move along the operating line to equilibrium at low pH at $(x_{TP2}, y_{C,II2})$.

Cycle 2, Stages III & IV:

GO - to $(x_{TP2}, y_{C,III2})$. x_{TP2} is unchanged. Fresh feed at y_0 is added. Fluid flows from the top of the column to the top product at a decreased concentration of y_{TP2} as indicated above.

STOP - Move along the operating line to new equilibrium at low pH at $(x_{TR3}, y_{C,IV2})$.

Cycle 2, Stages V & VI:

GO - to $(x_{TR3}, y_{C,V2})$. x_{TR3} is unchanged. Fluid from the bottom reservoir at y_{BR2} is added. Fluid flows from the top of the column to the top reservoir at a decreased concentration of y_{TR3} as indicated above.

STOPsame as without electric field.....

Cycle 2, Stages VII & VIII: same as without electric field. Again, the procedure is continued cycle-to-cycle until there is no longer any change in the top and bottom product concentrations.

The slope for the limiting separation in Figure 13 is less negative for the semi-continuous parapump with electric field than for the pump without electric field. As the retardation coefficients become very large, the boldfaced line in Figure 13 rotates counterclockwise and the separation approaches infinity. The pH parapump with electric field, therefore, also has the potential for better separation than the batch pump. The maximum separation is $y_{BP_\infty} = 2.00$ and y_{TP_∞} equal to zero.

Experimental Parameters

A typical parametric pumping experiment, based on the four-stage pH parapump by Chen et al., 1979b, is shown in Figure 14. A number of experimental parameters which affect the separation capability of the pump with and without an electric field were investigated, including the following:

- (1) Field strength.
- (2) Protein recovery.
- (3) Selection of buffer type.
- (4) Ionic strength of buffer.
- (5) Protein-protein interaction.
- (6) Optimum displacement.
- (7) Effect of adding circulation stages.

Additional parameters such as flow rate and the polarity of the electric field will be examined in the next chapter. The experimental results are compared with previous work on the four-stage pH parapump by Wu, 1981.

Steve Wu, 1981, optimized the flow rate for the hemoglobin-albumin-CM Sepharose system. Experiments were carried out in a 1.6 cm I.D. (2.0 cm^2 area) column at bulk volumetric flow rates Q of 0.5, 1.0, 2.0, 3.0, and 4.0 cc/min. Packed beds are normally scaled-up by setting a constant value for the volumetric flow rate per unit time per unit cross-sectional bed area. The column used in the current experiments is 2.6 cm I.D. (5.3 cm^2). The maximum separation was obtained at $Q = 1.0 \text{ cc/min}$ in the smaller column. By area scale-up, a volumetric flow rate of 2.6 cc/min is indicated for the

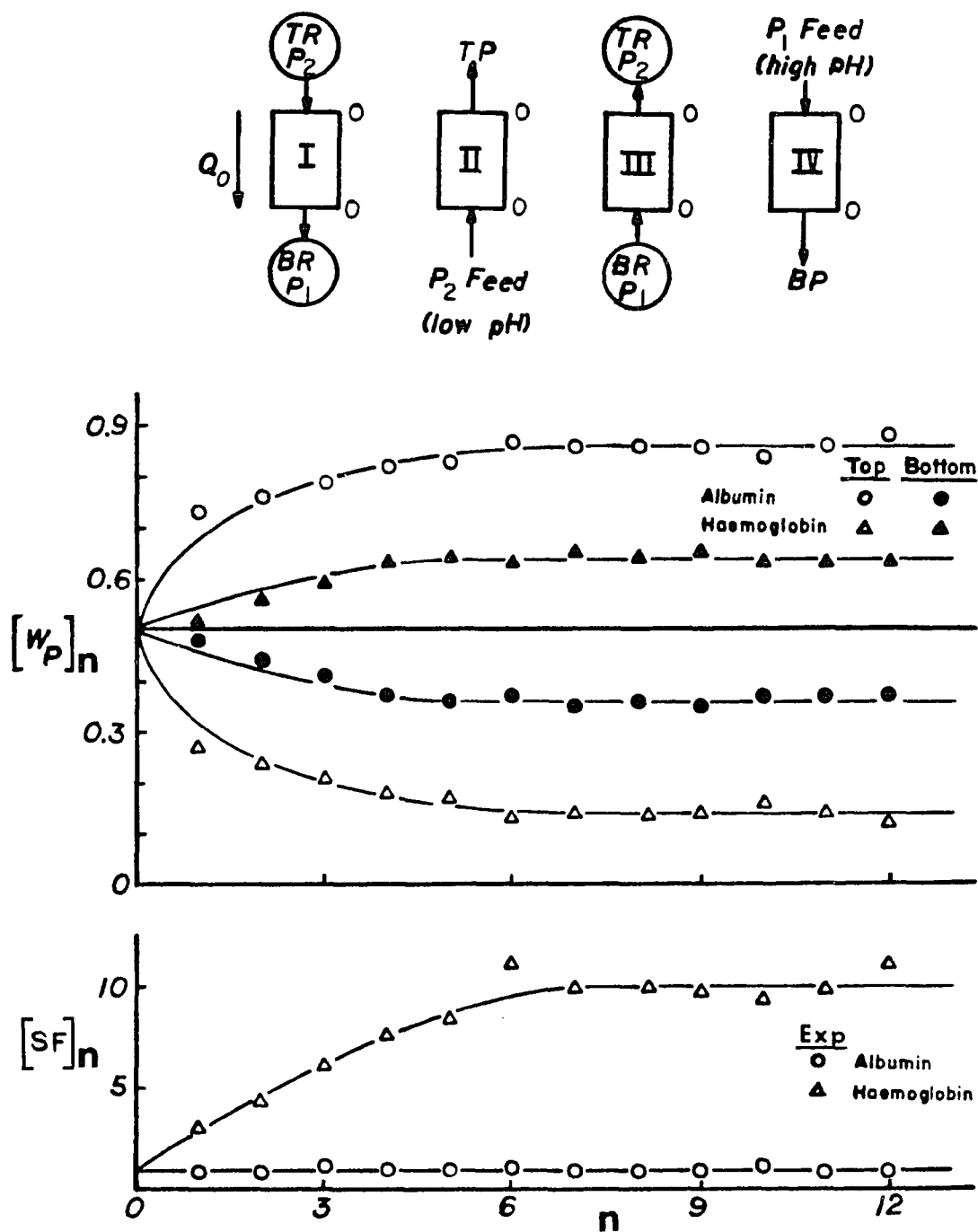


FIGURE 14. Experimental Results for the Four-Stage pH Parapump (Run 44)

larger column. A value of $Q = 2.5$ cc/min was used for the parapumping runs discussed below.

Figure 14 shows the separation of a mixture of hemoglobin and albumin in buffer solution (0.05M Tris-maleate + 0.05M NaOH). The cation exchanger CM Sepharose was used with pH's of $P_1 = 8.5$ and $P_2 = 6.0$, i.e., $P_1 > I_A > P_2$, leading to the expectation that hemoglobin would be removed from the top product stream and concentrated in the bottom product stream while albumin concentrations remained unchanged. The feed streams contained 0.01 weight percent of each protein. Since the protein concentrations are very low, the concentration ratio will be essentially identical to the weight percent ratio, i.e.,

$$y = \frac{C_L}{C_O} = \frac{Y}{Y_O} \quad (2-25)$$

If the hemoglobin is completely adsorbed, the limit for the albumin concentration in the top product stream is 100%. The maximum hemoglobin weight fraction in the bottom product stream is calculated as follows:

$$\left[\langle y_{BP} \rangle_{\infty} \right]_{Hb} = \frac{\left[\langle y_{BP} \rangle_{\infty} \right]_{Hb} [Y_O]_{Hb}}{\left[\langle y_{BP} \rangle_{\infty} \right]_{Hb} [Y_O]_{Hb} + \left[\langle y_{BP} \rangle_{\infty} \right]_{Al} [Y_O]_{Al}} \quad (2-26)$$

For the case of complete $F_B + F_T$ hemoglobin adsorption at P_2 ,

$$\left[\langle y_{BP} \rangle_{\infty} \right]_{Hb} = \frac{F_B + F_T}{F_T} \quad \& \quad \left[\langle y_{BP} \rangle_{\infty} \right]_{Al} = 1.0 \quad (2-27)$$

For Run 44 in Figure 14, $[Y_O]_{Hb} = [Y_O]_{Al}$ and $F_B = F_T$, so the

maximum possible weight fraction for hemoglobin in the bottom product stream should be 66.7%. The following separations were obtained in Run 44:

	<u>Weight Percent</u>			
	<u>Top Product</u>		<u>Bottom Product</u>	
	<u>Maximum Possible</u>	<u>Exp.</u>	<u>Maximum Possible</u>	<u>Exp.</u>
Hemoglobin	-	14	66.7	64
Albumin	100	86	-	36

As also seen in Figure 14, the separation increases cycle-to-cycle up to some limit, then remains constant ad infinitum with increasing cycle number. The separation factor SF for the n'th cycle of operation is defined as follows based on Equation I-1:

$$[\langle SF \rangle_n]_{Hb} = \frac{[\langle y_{BP} \rangle_n]}{[\langle y_{TP} \rangle_n]_{Hb}} \quad (2-28)$$

$$[\langle SF \rangle_n]_{Al} = \frac{[\langle y_{TP} \rangle_n]}{[\langle y_{BP} \rangle_n]_{Al}} \quad (2-29)$$

The separation factor for albumin is unity for the enrichment case being considered, but Equation 2-29 as well as the equations for the overall separation factor α will be needed for the splitting process. The overall separation factor for the mixture is the product of the individual separation factors:

$$\alpha_n = [\langle SF \rangle_n]_{Hb} [\langle SF \rangle_n]_{Al} = \frac{[\langle y_{BP} \rangle_n]}{[\langle y_{TP} \rangle_n]_{HB}} \frac{[\langle y_{TP} \rangle_n]}{[\langle y_{BP} \rangle_n]_{Al}} \quad (2-30)$$

The overall separation factor may also be expressed in terms of the weight fractions of the individual proteins. Divide the numerator and the denominator of Equation 2-30 by

$$\left\{ \left[\langle y_{BP} \rangle_n \right]_{Hb} + \left[\langle y_{BP} \rangle_n \right]_{Al} \right\} \text{ and also by } \left\{ \left[\langle y_{TP} \rangle_n \right]_{Hb} + \left[\langle y_{TP} \rangle_n \right]_{Al} \right\}.$$

$$\alpha_n = \frac{\left[\langle w_{BP} \rangle_n \right]_{Hb} \left[\langle w_{TP} \rangle_n \right]_{Al}}{\left[\langle w_{TP} \rangle_n \right]_{Hb} \left[\langle w_{BP} \rangle_n \right]_{Al}} \quad (2-31)$$

Both the hemoglobin separation factor and the overall separation factor are ten for Run 44, Figure 14.

In order to achieve a maximum effect when adding an electric field to the pH parapumping process, it is desirable to maximize the field strength (volts) in order to maximize the migration velocity ν_E -- Equation 2-15. Unfortunately an increase in voltage leads to increased heat generation, and proteins may be denatured by high temperatures.

$$\text{Heat} = \text{Volts} \times \text{Current} \times \text{Time}$$

The electrophoresis column was jacketed and cooled, but the mechanical cooling capacity of the apparatus is finite. In order to maximize the field strength with minimum heat generation, the current must be minimized. This is done by the use of an organic buffer or partially ionized buffer at low ionic strength. The most common buffers for electrophoresis are 0.025M to 0.075M barbital solutions or 0.03M to 0.12M Tris solutions (Chin, 1970). A low ionic strength organic buffer, 0.05M Tris-maleate + NaOH, was used in Run 44. The previous work by Chen et al., 1979b, used a high ionic strength phosphate buffer (0.2M + 0.1M NaCl) for desorption and 0.05M

phosphate buffer for adsorption.

Table 5 examines the protein recoveries from pulse experiments on the mixture at three different buffer concentrations, 0.05M, 0.10M, and 0.20M. The operating conditions for these experiments are given in Appendix B. As the ionic strength of the buffer increases at constant voltage, the power and heat generation increase while the protein recovery drops. The protein recovery is acceptable when the power is below 10 watts.

Three different buffers were examined in the experimental work: (a) phosphate, $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$, (b) Tris-maleate plus NaOH, and (c) Tris plus glycine. These buffer solutions are made up of: (a) two inorganic components, (b) an organic plus an inorganic component, and (c) two organic components. The Tris/glycine buffer allowed the highest voltage to be used, i.e., approximately 320 volts at 10 watts, but the pH control was poor. The buffer components were evidently separated by the electric field. Figure 15 shows the voltage and wattage for the first two buffer systems at various ionic strengths. At 10 watts, the voltage increases as the concentration of the phosphate buffer decreases from 0.20M to 0.05M. pH Control was poor for 0.025M phosphate buffer. The voltage is also increased by using 0.05M Tris-maleate/NaOH instead of 0.05M phosphate buffer. The number of ions in solution at the same concentration, i.e., 0.05M, is obviously less for the Tris-maleate/NaOH buffer than the phosphate buffer. Depending on the pH or on the relative amounts of the two components in

TABLE 5

Protein Recovery in Pulse Experiments

Feed: 0.01 weight % Hemoglobin + 0.01 weight % Albumin

Buffer: Tris-maleate + NaOH

Electric Field: Either 210 volts or ZERO volts

<u>Run</u>	<u>I.S.</u>	<u>Watts</u>	<u>Q</u>	<u>δ_{Hb}</u>	<u>δ_{Al}</u>	<u>$\delta_{Avg.}$</u>
5	0.05M	0	0.5	86.5	100.5	96
13	"	"	2.5	95.6	102.-	
20	"	"	0.5	97.5	93.0	
6	"	9	0.5	85.0	95.9	90
7	"	"	0.5	87.3	91.0	
8	"	"	0.5	90.6	97.1	
21	"	"	0.5	91.7	84.5	
19	0.10M	17	0.5	59.3	82.1	71
17	0.20M	0	2.5	83.5	84.7	84
18	"	34	0.5	54.7	79.2	67

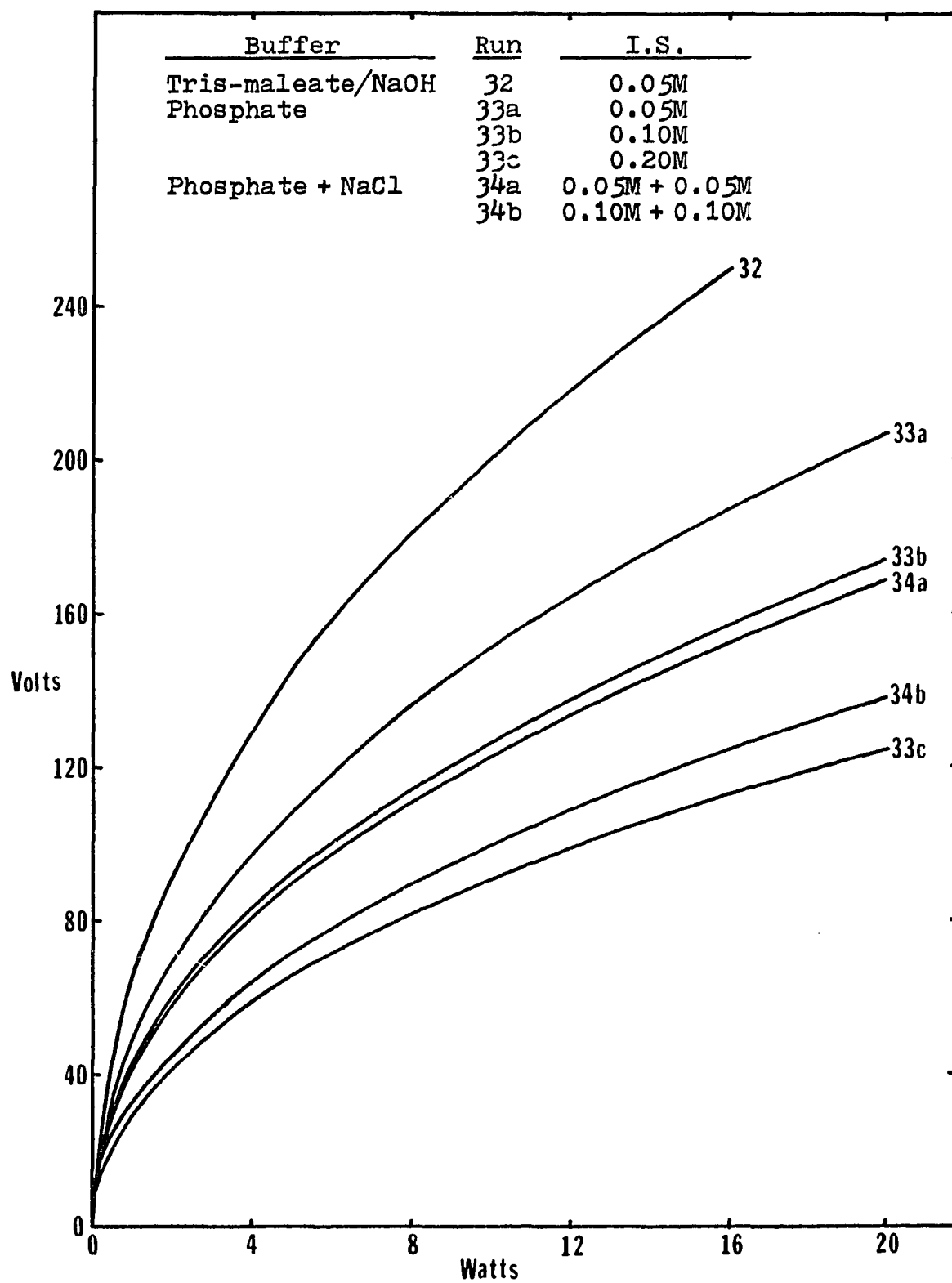


FIGURE 15. Field Strength as a Function of Buffer Concentration and Power Generation

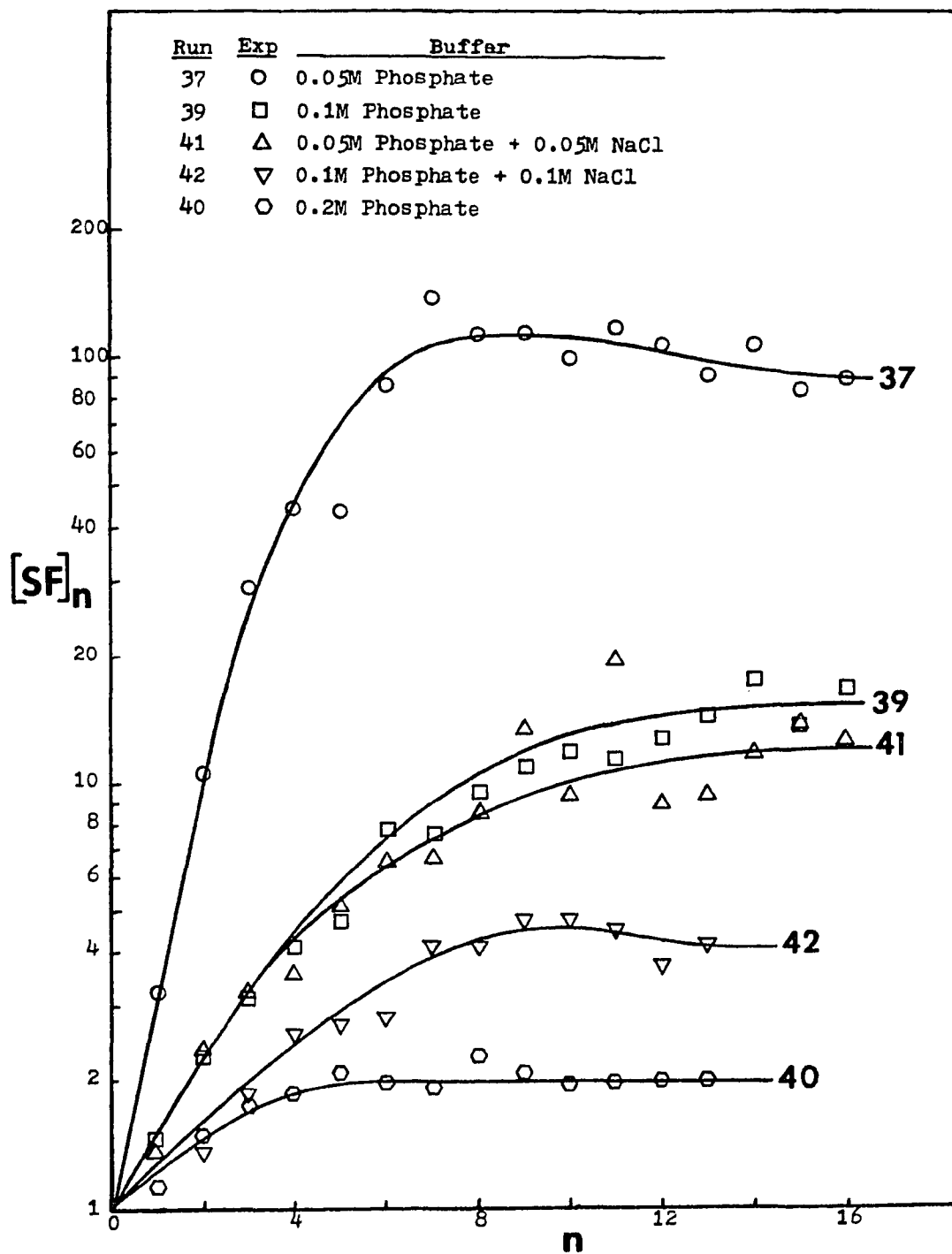


FIGURE 16. Effect of Buffer Concentration on Hemoglobin Separation (Experimental)

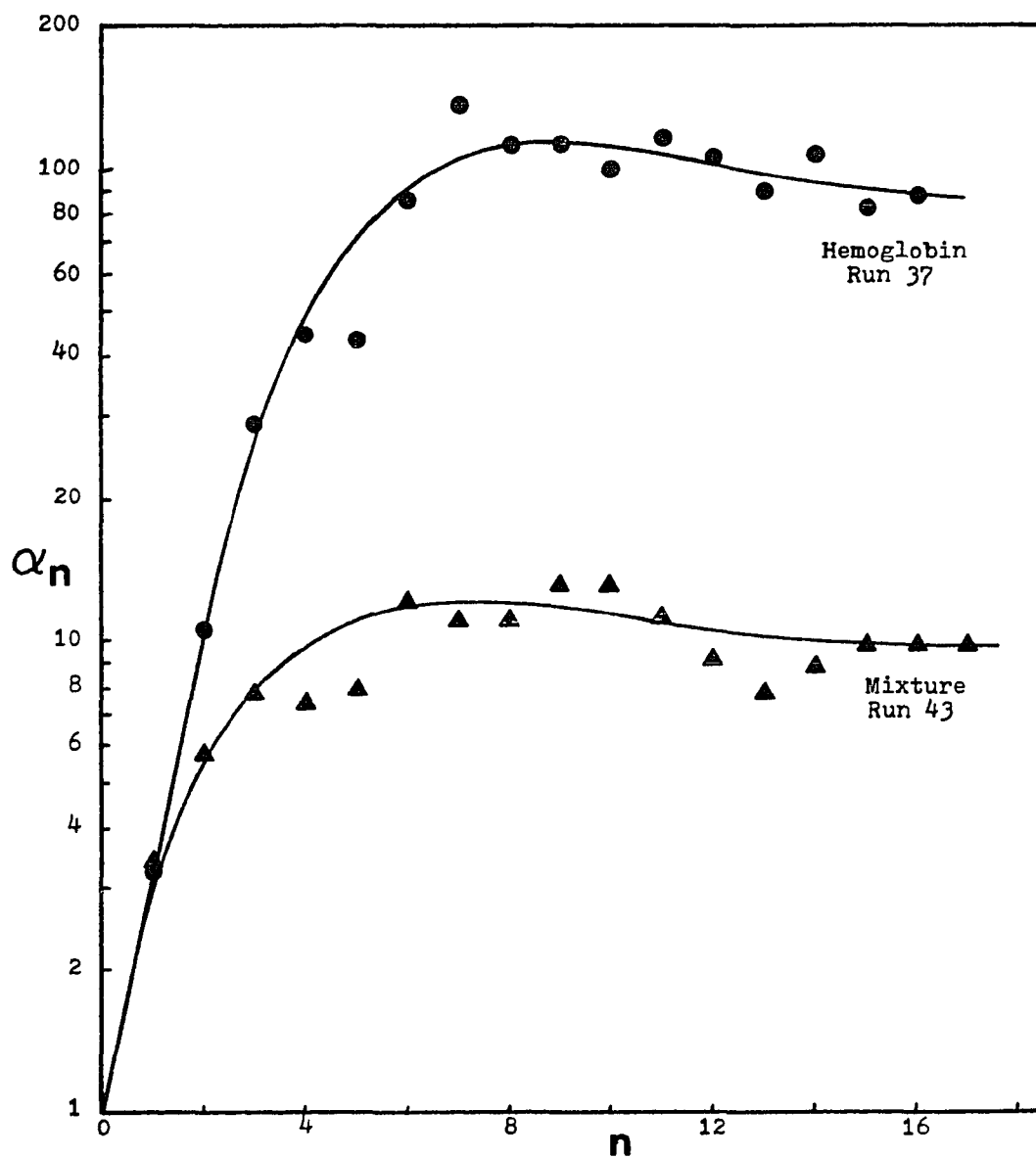


FIGURE 17. Separation of Mixture vs. Single Component
(Experimental Results)

the buffer solution, the Tris-maleate/NaOH buffer contains an average of zero to two ions per molecule while the phosphate buffer contains an average of two to three ions per molecule.

The parapumping enrichment of a hemoglobin-buffer solution via the four-stage pH parapump is shown in Figure 16. If the optimum displacement is used, the separation increases as the ionic strength decreases. The reservoir displacements for Stages I and III are shown in Table 6, and the optimum displacement will be examined more thoroughly in Figures 18 to 20. The best case shown experimentally in Figure 16 was the 0.05M phosphate buffer solution.

The enrichment in the hemoglobin-buffer system is compared to the enrichment for the hemoglobin-albumin-buffer mixture in Figure 17. The separation is decreased from approximately 100 to ten by the presence of the second protein. Since albumin is not adsorbed at P_1 or P_2 , competition for adsorption sites cannot be the reason for the observed decrease in separation. Steiner, 1953, reported that at pH's between the isoelectric points of serum albumin (\sim pH 5) and lysozyme (\sim pH 11), an association complex of the two proteins was formed. In the present case, the adsorption takes place at pH = 6.0, which is between the isoelectric points of hemoglobin and albumin. Evidently, some fraction of the positively charged hemoglobin is attracted to the negatively charged albumin, i.e., protein-protein interaction. The Hb^+Al^- complex neutralizes a portion of the hemoglobin and prevents it from being attracted to the ion exchange resin. The constants for the transport equations, in the present case,

must be determined from experimental data on the mixture rather than on hemoglobin and/or albumin alone.

The optimum displacement for the 0.05M phosphate buffer for Runs 37 and 43 in Figures 16 and 17 was 72.5 cc or 1.61 bed volumes as determined from Run 4, Figure 18. The displacement should be large enough that the concentration wave travels the length of the column, but small enough that the adsorbed protein does not break through to the top product. Also, the displacement must be set so that the pH change in Stages I or III has been accomplished. In Stage I, the pH changes from 8.0 to 6.0 at 73.8 cc or 1.64 bed volumes. In Stage III, the pH changes from 6.0 to 8.0 at 89.5 cc or 1.99 bed volumes. The pH wave velocities indicate that a slightly larger displacement might be desirable, but a larger displacement in Stage III would push hemoglobin into the top product.

As shown in Figure 19, the experimental separation for the mixture with the 0.05M phosphate buffer is much better than data previously obtained by Wu, 1981. In fact, the separations for the 0.05M phosphate buffer, the 0.05M Tris-maleate/NaOH buffer, and the high ionic strength phosphate buffer are all around ten. The displacements, however, are drastically different as shown in Table 6. Poor separation was previously obtained for the low ionic strength phosphate buffer, because the displacement was too small -- 1.07 instead of 1.61 times the bed volume.

The separation is also poor if the displacement is too high as shown in Figure 20. The pH and concentration waves

travel faster in the 0.10M phosphate buffer than the 0.05M phosphate buffer, so the displacement in Figure 20 had to be decreased from 72.5 cc to 65 cc in order to obtain maximum enrichment for the hemoglobin-buffer system in Runs 38 and 39.

Figure 21 shows the experimental results for the six-stage pH parapump. A comparison of the actual and the maximum possible separations shows that the operation of the parapump with circulation is better than the four-stage system.

	<u>Weight Percent</u>			
	<u>Top Product</u>		<u>Bottom Product</u>	
	<u>Maximum Possible</u>	<u>Exp.</u>	<u>Maximum Possible</u>	<u>Exp.</u>
Hemoglobin	-	8	66.7	63
Albumin	100	92	-	37

The separation is improved from ten in Run 44 to twenty in Run 45 by adding the circulation stages as shown in Figure 22. The improved separation is due to a decrease in the weight percent of hemoglobin in the top product from 14% in Run 44 to 8% in Run 45.

TABLE 6

Displacement in Parapumping ExperimentsFlow Rate: $Q = 2.5$ cc/min

Power: None

Void Volume: $V = 45.0$ cc, Present work.
 $V = 22.5$ cc, Wu, 1981.

<u>Run</u>	<u>Buffer</u>	<u>I.S.</u>	<u>$Q_{t_I} = Q_{t_{III}}$</u>	<u>Q_{t_I}/V</u>
Feed:	0.01 weight % Hemoglobin.....			
37	Phosphate	0.05M	72.5	1.61
38	"	0.10M	"	"
39	"	"	65.0	1.44
40	"	0.20M	"	"
41	"	0.05M + 0.05M NaCl	70.0	1.56
42	"	0.10M + 0.10M NaCl	65.0	1.44
Feed:	0.01 weight % Hemoglobin + 0.01 weight % Albumin.....			
43	Phosphate	0.05M	72.5	1.61
44	Tris-maleate/NaOH	"	90.0	2.00
Feed:	0.02 weight % Hemoglobin + 0.02 weight % Albumin.....			
Wu #4	Phosphate	0.05M	24.0	1.07
Wu #6	Phosphate/NaCl	0.20M + 0.10M NaCl also 0.05M	"	"

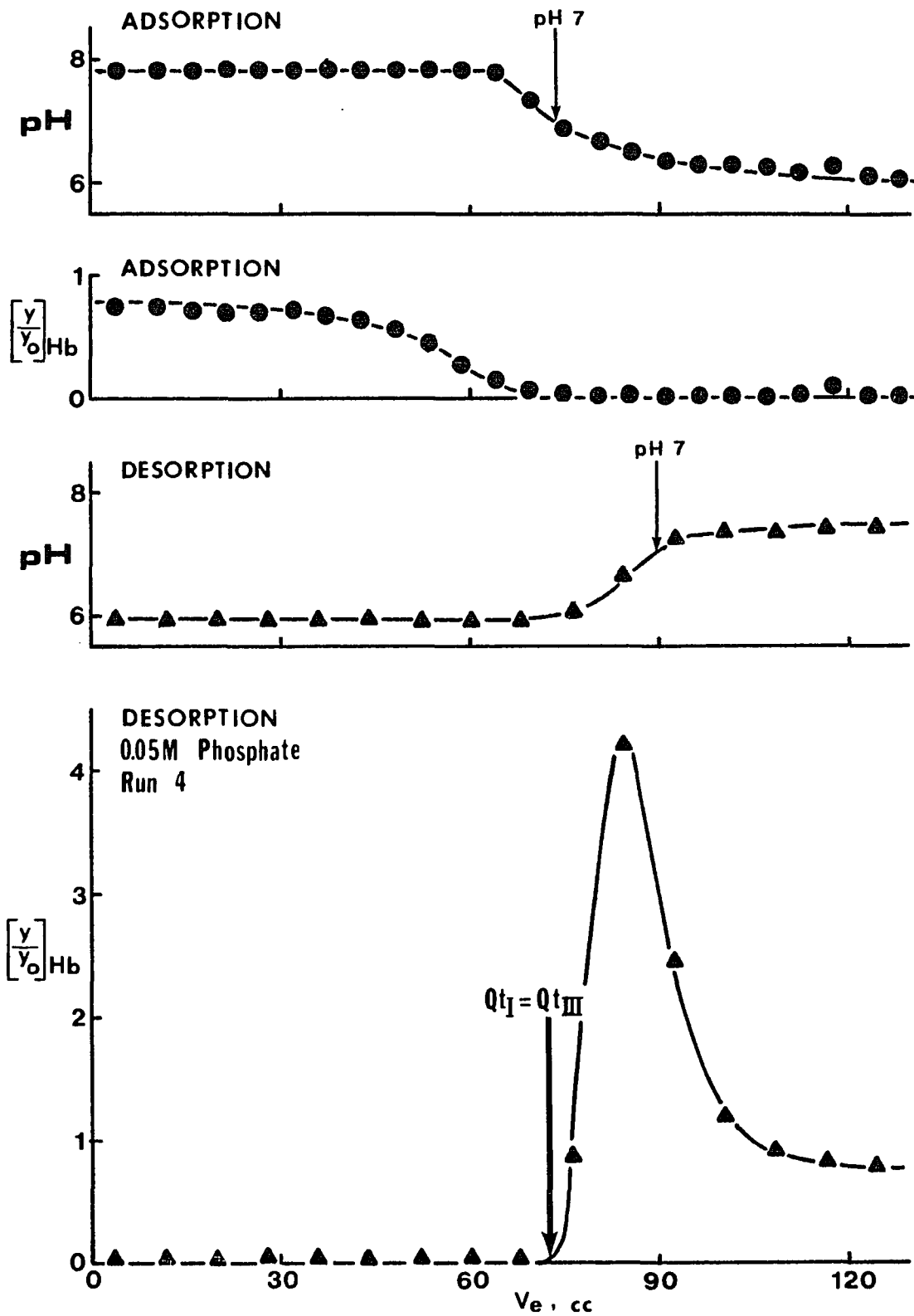


FIGURE 18. Optimum Displacement from Pulse Experiments

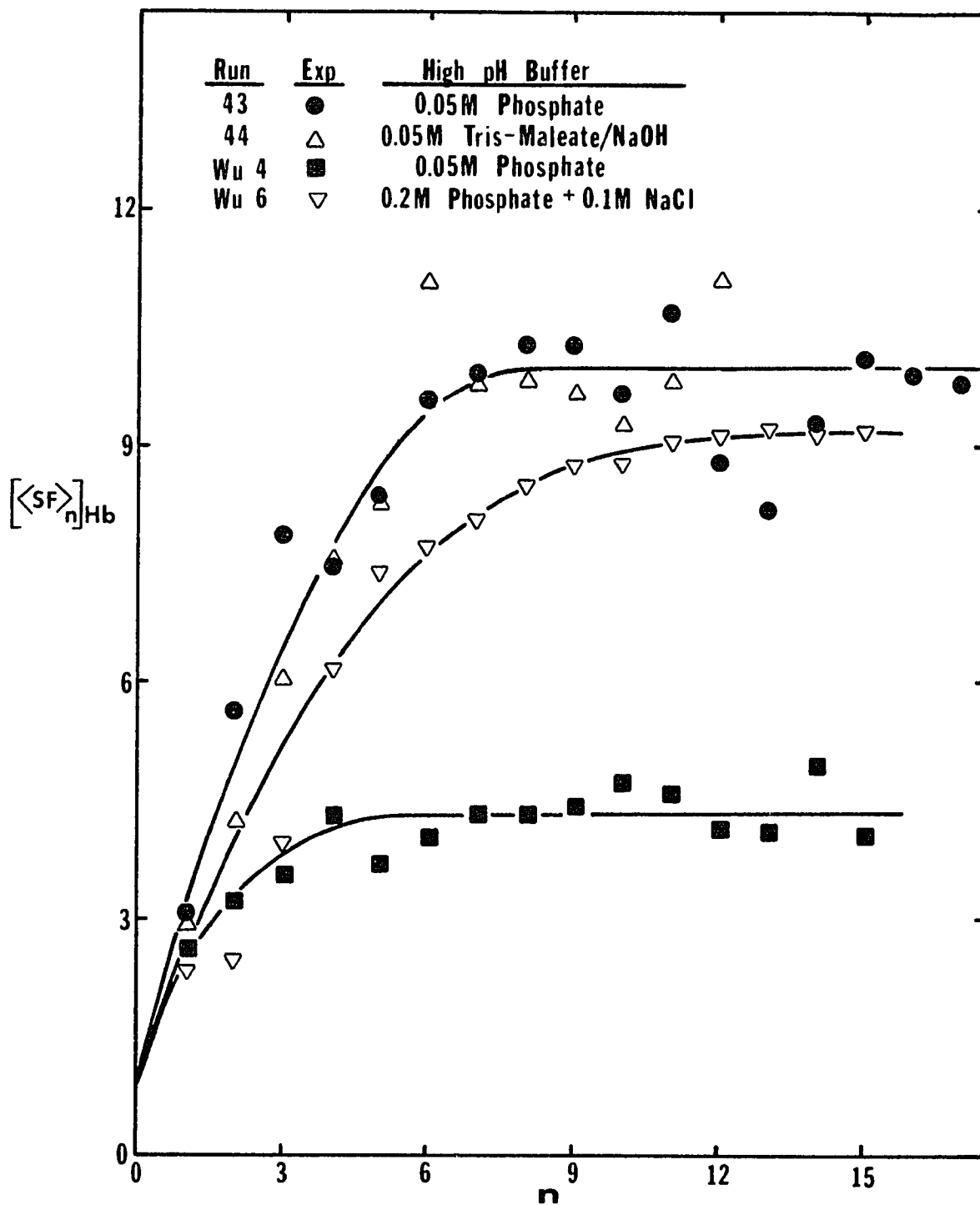


FIGURE 19. Comparison with Previous Results at Low Displacement Volumes (Experimental)

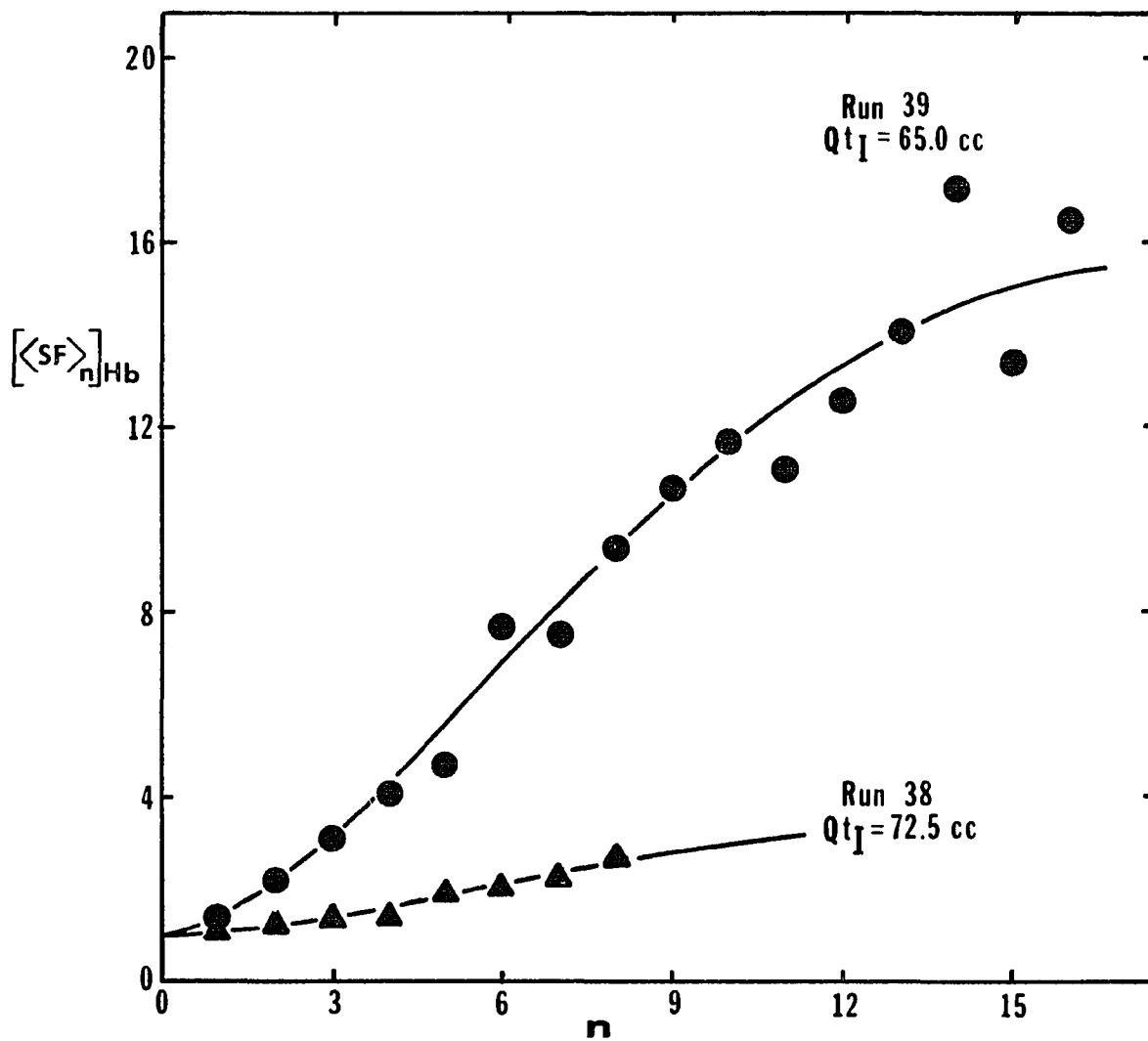


FIGURE 20. Comparison of Separations at Optimum and High Displacement Volumes (Experimental)

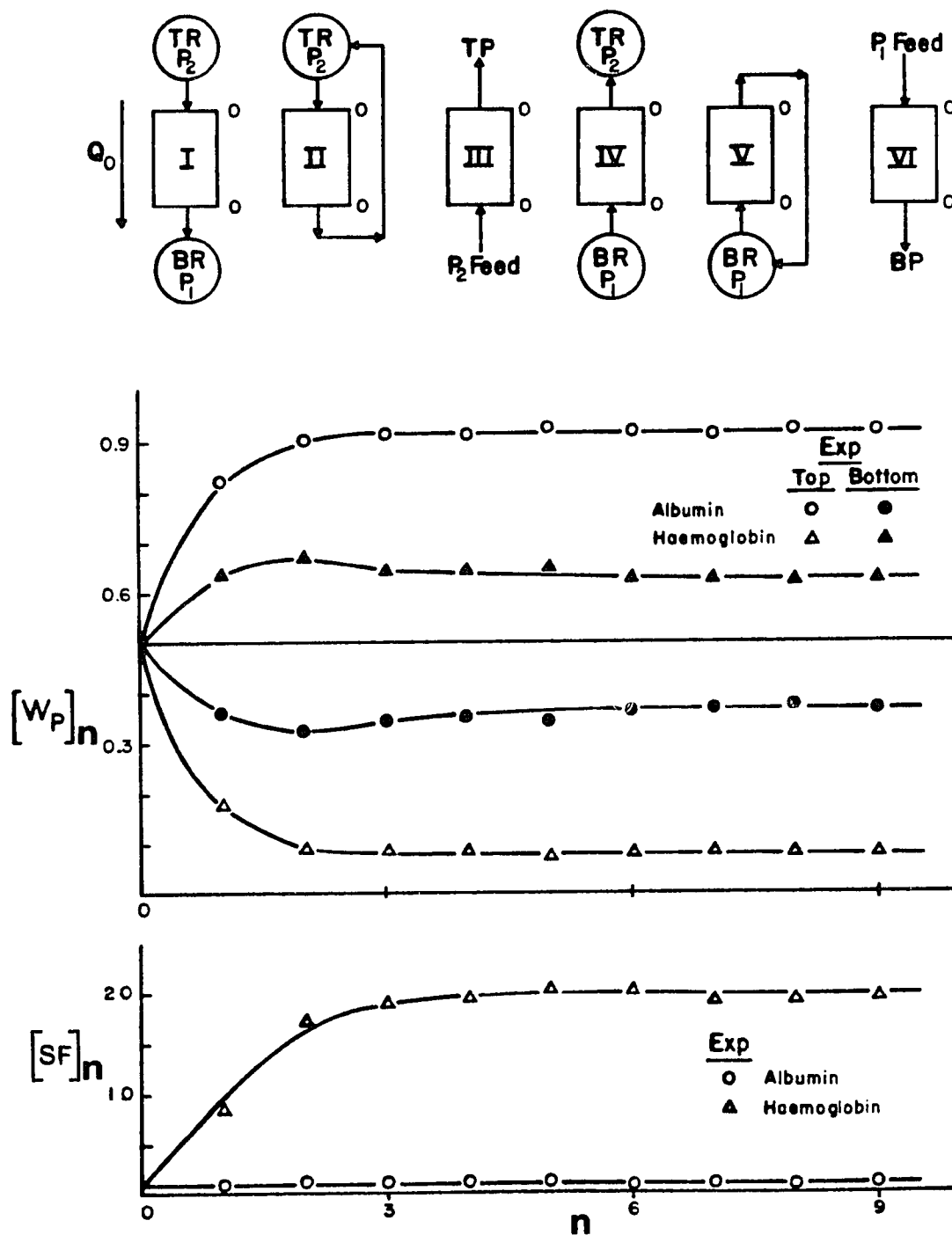


FIGURE 21. Experimental Results for the Six-Stage pH Parapump (Run 45)

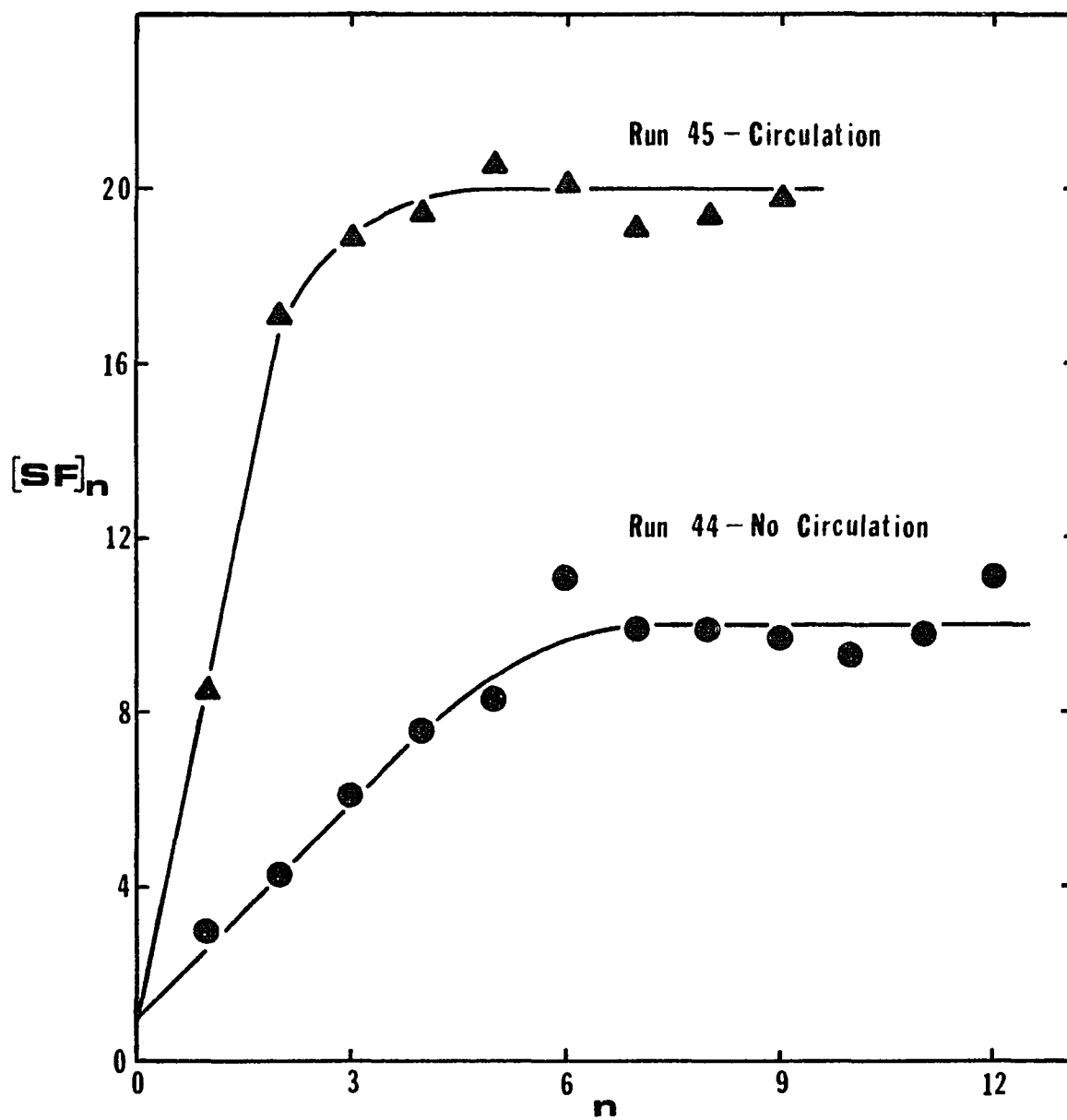


FIGURE 22. Effect of Circulation on Hemoglobin Separation

Chapter 3

ELECTROPOLARIZATION CHROMATOGRAPHY

The protein separation achieved in the six-stage pH parametric pumping process (Figure 21) can be improved by applying an electric field across the chromatographic column during some stages of the process. Net upward movement of Protein B or albumin relative to Protein A or hemoglobin is instituted by positive/negative or on/off cycling of the electric field coupled with cyclic variation of pH and flow direction. Breakthrough curves or pulse experiments, simulating the various pH conditions existing during the process, were run in order to determine the most effective stages for application of the electric field and the optimum placement of the positive and negative electrodes relative to the flow direction in these stages. Duplicate experiments were made with finite voltage and with zero voltage in order to distinguish the effect of the electric field from the effect of the pH change on the concentration waves for Proteins A and B.

Four experiments (Runs 9 - 12) were made by cycling zone adsorption with three cycles of pH change, $P_2 \rightarrow P_1 \rightarrow P_2$, per experiment. The remainder of the data to be discussed in this chapter involves only one cycle per experiment with either a step or pulse change in that cycle. The cycling zone data without electric field has already been discussed in detail (Figures 5 and 6). Figure 23 shows that the effect of the electric field on the cycling zone data is both real and reproducible. In each cycle, both the pH and concentration

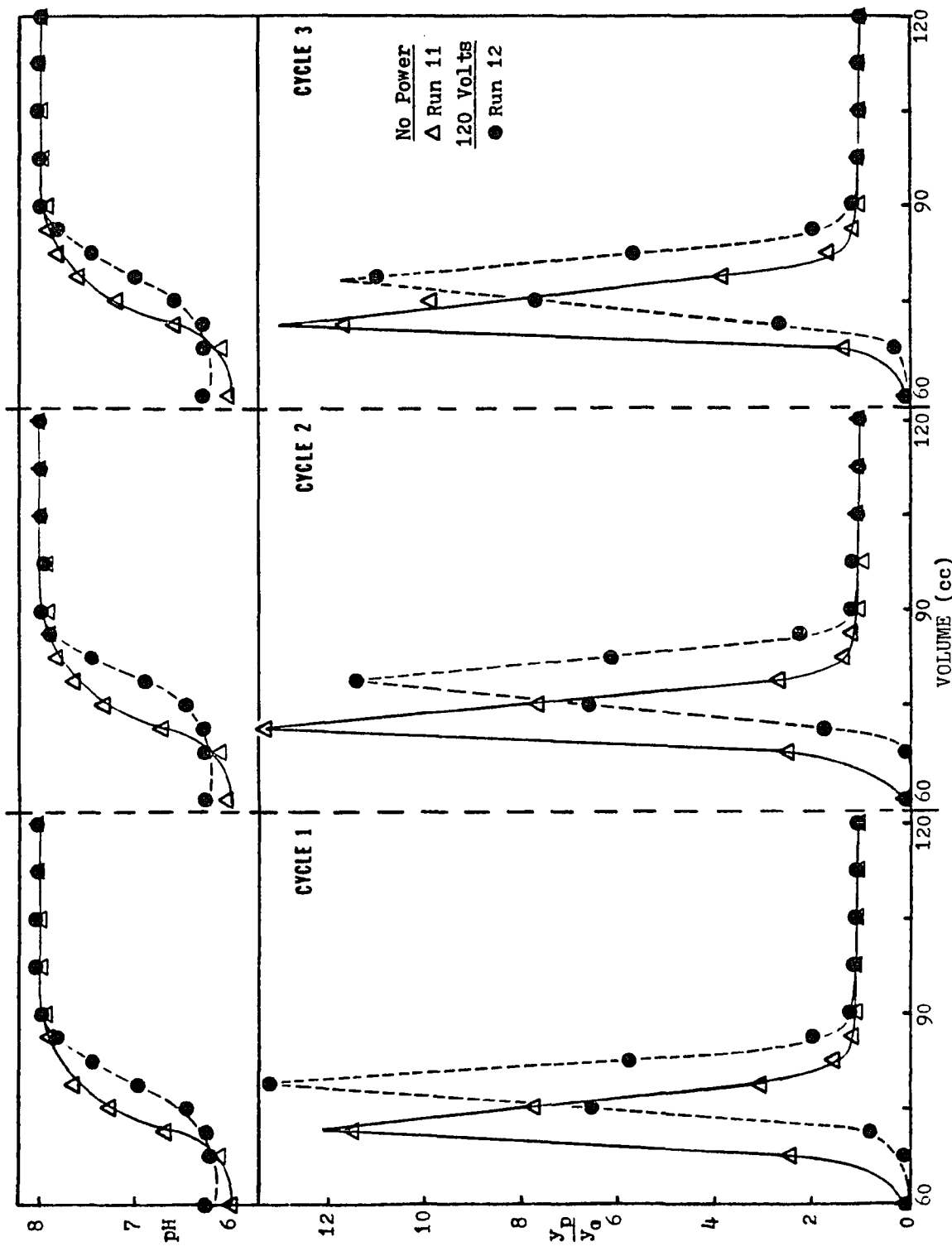


FIGURE 23. Effect of Electric Field in Cycling Zone Adsorption

waves are delayed by placement of the cathode at the entrance of the chromatographic column. The hemoglobin concentration wave appears as a sharp peak at the point of pH change from P_2 to P_1 in each cycle, irregardless of whether or not the electric field is applied.

The three cycles in Runs 9 - 12 were superimposed on each other in the separate runs to give average values for one complete cycle in each case. The results are shown in Figure 24. The concentration wave is delayed in both Runs 10 and 12, but the effect of the electric field is more pronounced in Run 12 where the bulk flow rate is lower. This may be expected from Equations 1-2 and 2-18 or,

$$R_{\text{Hb}} = \frac{\nu_0}{\nu_0 \pm \nu_{\text{E,Hb}}} \quad (3-1)$$

Also note,

$$\begin{aligned} \nu_{\text{Al,P}_1} &\approx \nu_0 \\ \nu_{\text{Al,P}_2} &\approx \nu_0 \\ R_{\text{Al}} &= \frac{\nu_0}{\nu_0 \pm \nu_{\text{E,Al}}} \end{aligned} \quad (3-2)$$

For a given set of operating conditions, the migration velocity ν_{E} is fixed for a given protein. The effect of the electric field, as indicated by the magnitude of the retardation coefficients, becomes significant when the bulk fluid velocity ν_0 is low enough to be of the same order of magnitude as the migration velocities $\nu_{\text{E,Hb}}$ and $\nu_{\text{E,Al}}$. If the bulk fluid velocity is too low, molecular diffusion and axial dispersion become important and negate any increase in the

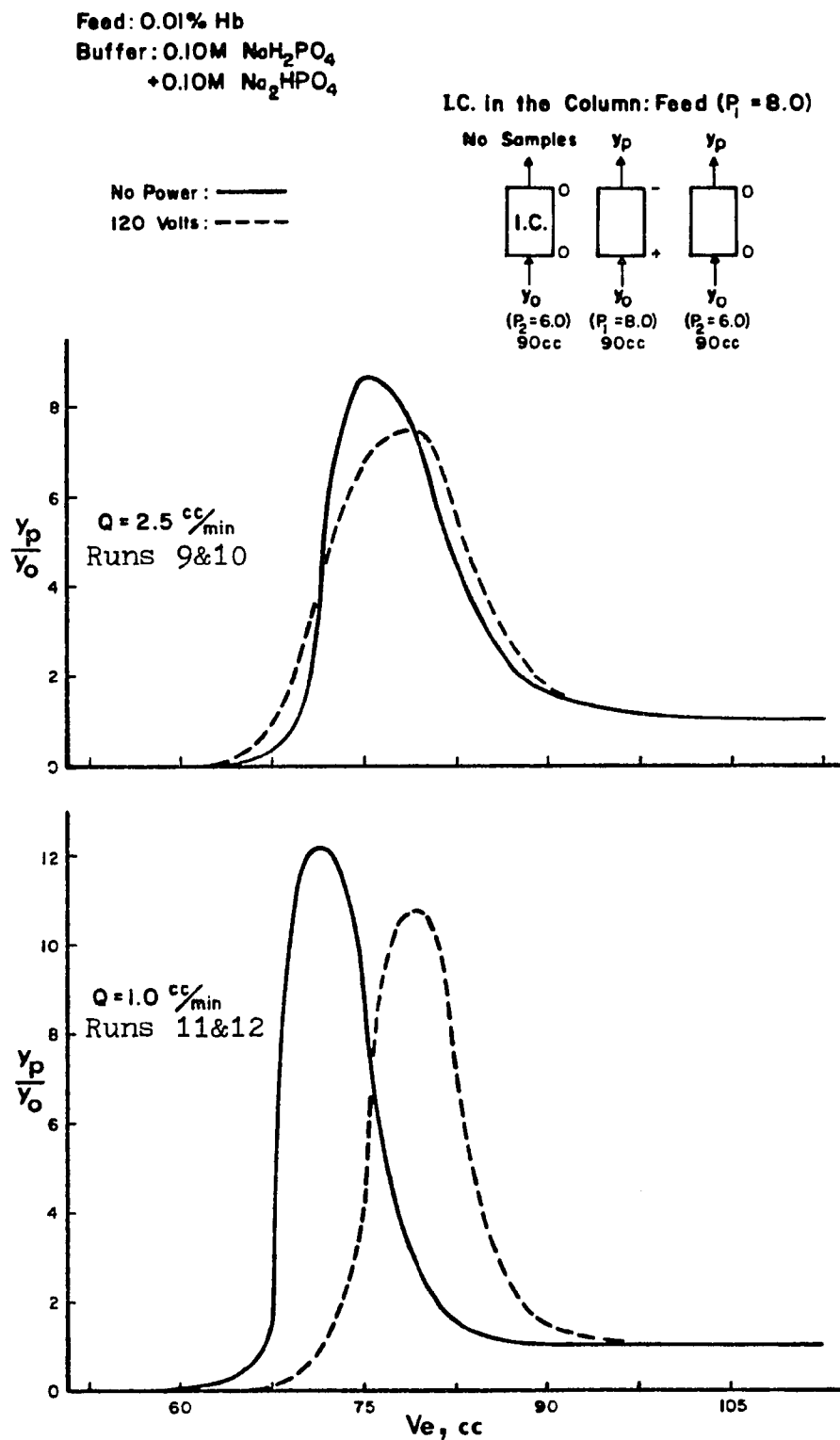


FIGURE 24. Effect of Bulk Flow Rate on the Concentration Wave in Electropolarization Chromatography

protein separation due to the electric field. On the other hand, the electric field will become significant if the operating conditions are such that the protein mobilities and migration velocities are relatively high.

A bulk flow rate of 1.0 cc/min gave significant results for the hemoglobin retardation coefficient in Figure 24 as compared with 2.5 cc/min. A bulk flow rate of 0.5 cc/min was used for the remainder of the chromatographic separations to be considered below. Also, 0.05M Tris-maleate/NaOH buffer was used instead of the phosphate buffer in order to increase the field strength from 120 volts to 210 volts without excessive heat generation. As shown in the previous chapter, good pH parapumping separations can be obtained with the Tris-maleate/NaOH buffer by increasing the displacements in Stages I and IV (Figure 21) to approximately two bed volumes. The optimum displacements for the Tris-maleate/NaOH buffer will be examined further in Chapter 4.

The optimum placement of the electrodes in each stage of the six-stage pH parapump (Figure 21) depends on the relative magnitude and direction of the migration velocities of the two proteins at P_1 and P_2 . Protein B is negatively charged at both P_1 and P_2 and can be enriched in the top streams by placing the cathode at the top of the column throughout the process. At P_2 , Protein A is adsorbed by the cation exchanger and the equilibrium concentration in the fluid phase is nearly zero. Under these conditions, no effect of the electric field is expected. Negatively charged

Protein A is present in the column in Stages I, IV, V, and VI, and net downward movement of Protein A dictates placement of the cathode at the bottom of the column in these stages. There is, therefore, a conflict in the optimum electrode placement for Stages I, IV, V, and VI based on the directions of the migration velocities of the two proteins, so the relative movement of the centers of mass of the two proteins must be examined. Effects of the electric field on the various stages of parapumping are experimentally demonstrated in Figures 26 to 29, and the experimental migration velocities are calculated in Table 7.

The mobility of human hemoglobin is linear with pH, i.e., zero at the isoelectric point and increasing in either positive or negative magnitude as the pH departs from the isoelectric point (Pauling et al., 1949).

$$\mu_{\text{Hb}} \propto I_A - \text{pH}$$

If hemoglobin and albumin are similar except for their isoelectric points, albumin would be expected to have a greater mobility and migration velocity at $P_1 > I_A > I_B$ because:

$$|P_1 - I_B| > |P_1 - I_A|$$

The mobility of serum albumin is given for a number of conditions by Mysels and Mysels, 1961 (human serum albumin), and by Møller et al., 1961 (bovine serum albumin). The electrophoretic migration of hemoglobin and albumin through polyacrylamide gel at $P_1 = 8.6$ is shown in Figure 25. As expected, the albumin peak exits the gel in a shorter period of time indicating that the migration velocity is greater for albumin

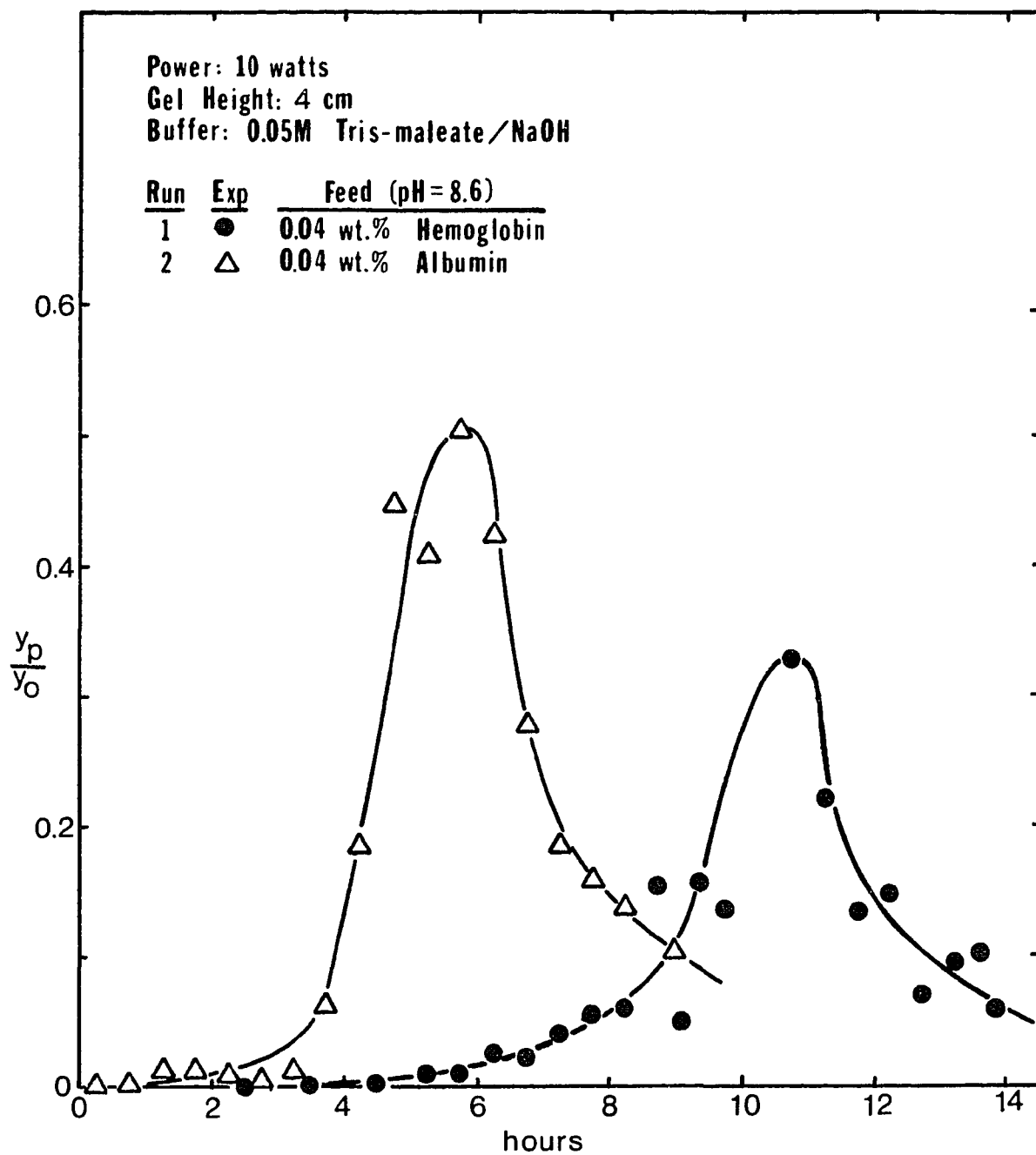


FIGURE 25. Separation of Hemoglobin and Albumin via Polyacrylamide Gel Electrophoresis

than hemoglobin at P_1 . Similar results are shown for the separation of a mixture of ovalbumin, bovine serum albumin, and hemoglobin on polyacrylamide gel by Öhman, 1973.

Since the greatest migration velocities are expected at P_1 , first consider Stages V and VI as shown in Figure 26. Both proteins are negatively charged and the entire stage is operated at P_1 . If the cathode is placed at the bottom of the chromatographic column, movement of both proteins occurs in the direction of the cathode with a peak developing in the forward direction. Since $|P_1 - I_B| > |P_1 - I_A|$, Protein B is more affected by the field as evidenced by its more distinct peak in Figure 26 and its greater migration velocity in Table 7. Since Protein B is more affected, the protein split will be favored by placement of the cathode at the top rather than the bottom of the chromatographic column in Stages V and VI. However, the effect of the electric field is not significant under these conditions, so no power is necessary for Stages V or VI.

Next consider Stages II and III which are operated at P_2 and are examined experimentally in Figure 27. Protein A is positive and Protein B is negative in these stages, so the cathode should be placed at the top of the column. The concentration of Protein A in the fluid phase is nearly zero and will be little affected by the presence of a finite field. Since $|P_2 - I_B| < |P_1 - I_B|$, the migration velocity for Protein B in Table 7 is even smaller than observed in the previous case. The only effect of the electric field observed for Stages II

and III in Figure 27 is a decrease in recovery of albumin from 98.2% in Run 26 to 92.2% in Run 27. The effect of the electric field on the protein separation in Stages II and III is negligible.

The relative migration velocities of Proteins A and B in Stages I and IV are dependent on position and time, because the pH is changed from P_2 to P_1 and vice versa during these stages. The protein mobility and the resulting migration velocity are strong functions of pH. The average effect of the electric field over the P_1 to P_2 pH range is shown in Figures 28 and 29. Based on the relative movement of hemoglobin (Protein A) and albumin (Protein B) in these pulse experiments, a number of generalizations for the process can be made.

In Stage I, negatively charged Protein A is leaving the bottom of the column while positively charged Protein A is entering the top of the column. The A^+ is immediately adsorbed by the cation exchanger, so the cathode should be placed at the bottom of the chromatographic column in order to favor downward movement of A^- . As seen in Figure 28, a peak develops for both A and B in the forward direction in Case Ia, which is detrimental to the split of A and B. If the cathode is placed at the top of the chromatographic column to favor upward movement of B^- as demonstrated in Case Ic, both proteins are retarded by the electric field. Since the total Protein A removed from the column, as seen from comparison of the areas under the hemoglobin curves in

Figure 28, is approximately equal in all three cases at $Q_0 t_I$ equal to or greater than 67.5 cc, no relative advantage is achieved for the two-component split by addition of an electric field in Stage I.

In Stage IV, the fluid leaving the top of the column contains A^+ at very low concentration and B^- at feed concentration. The fluid entering the bottom of the column from Cycle 2 ad infinitum contains A^- at enriched concentrations and B^- at feed concentration. Placement of the cathode at the top of the chromatographic column in this stage should move both Protein A and Protein B from the entering P_1 fluid forward, relative to the concentration waves at zero field. The Protein A which moves into the P_2 fluid in the upper part of the column will stick to the gel and be desorbed in the trailing P_1 fluid giving a sharp peak behind $pH = I_A$ as shown for Cases IVa and IVb in Figure 29. Conversely, placement of the cathode at the bottom of the chromatographic column in Stage IV will move both Protein A and Protein B downward, relative to the mass flow at zero field as shown in Case IVc in Figure 29. For positive, negative, or zero fields, the hemoglobin desorption wave always falls immediately behind $pH = I_A$, but the hemoglobin peak is much broader in Case IVc due to the electrophoretic migration of Protein A or hemoglobin into the lower part of the column or the trailing P_1 fluid. Also, the net mass movement due to the electric field is much greater for the adsorbed protein, hemoglobin in the present investigation, as seen in Table 7 and Figure 29. This phenom-

phenomenon was unexpected, but is the key to enhanced separation in the pH parapumping process from the electric field effect.

It is also interesting to note the effect of the electric field on the pH waves in Figures 28 and 29. Soffer and Folman, 1972, studied the pH changes of a solution in contact with positive and negative carbon electrodes. The pH of the solution increases (becomes more basic) at the negative electrode, but the pH decreases (becomes more acidic) at the positive electrode. In the electropolarization chromatographic experiments in Figures 28 and 29, the fluid passes near the anode once with an expected pH increase, and near the cathode once with an expected pH decrease. A net pH change is observed as follows:

Cathode at Entrance Relative to Flow Direction.

Case Ic - pH Wave is delayed, i.e., P_1 changes to P_2 later.....net pH increase.

Case IVc - pH Wave is delayed, i.e., P_2 changes to P_1 later.....net pH decrease.

Anode at Entrance Relative to Flow Direction.

Case Ia - pH Wave moves faster, i.e., P_1 changes to P_2 earlier.....net pH decrease.

Case IVa - pH Wave moves faster, i.e., P_2 changes to P_1 earlier.....net pH increase.

On cyclic positive and negative charging, Soffer and Folman, 1972, reported partially irreversible pH changes in the first cycle, followed by reproducible hysteresis loops in the second and third cycles. The irreversible pH changes in the present investigation are well-documented, but not

well-understood. The pH changes are identical for pure buffer solutions and are related to movement of the buffer ions in the electric field plus consumption of hydrogen and hydroxyl ions at the electrodes. Evolution of electrode gases (presumable H_2 and O_2) was noted in all experiments.

Comparison of the migration velocities for Proteins A and B in Table 7 for all the cases considered above, indicates that Protein A has the highest velocity due to the electric field in Cases IVa and IVc. The greatest difference between the centers of mass of Proteins A and B occurs in Case IVc. This best case is plotted in Figure 30 in order to study the separation possible via electropolarization chromatography. Complete separation is theoretically possible, but is not experimentally observed. For comparison with pH parapumping, the hemoglobin rich product will be considered to be the bottom product and the albumin rich product will be considered to be the top product. The break point is taken at the point where the hemoglobin and albumin concentration curves cross in Figure 30, and all effluent is included in either the top or the bottom product.

	<u>Weight Percent</u>			
	<u>Top Product</u>		<u>Bottom Product</u>	
	<u>Maximum Possible</u>	<u>Exp.</u>	<u>Maximum Possible</u>	<u>Exp.</u>
Hemoglobin	-	17	100	80
Albumin	100	83	-	20

The overall separation factor α for this experiment is twenty,

which is the same as the separation achieved experimentally for the six-stage pH parapump in Figure 21. A separation factor of twenty was also obtained for polyacrylamide gel electrophoresis by J.F. Chao (unpublished work). The fundamental criterion in the development of the pH parapump with electric field must be successful experimental demonstration of an overall separation factor significantly better than twenty.

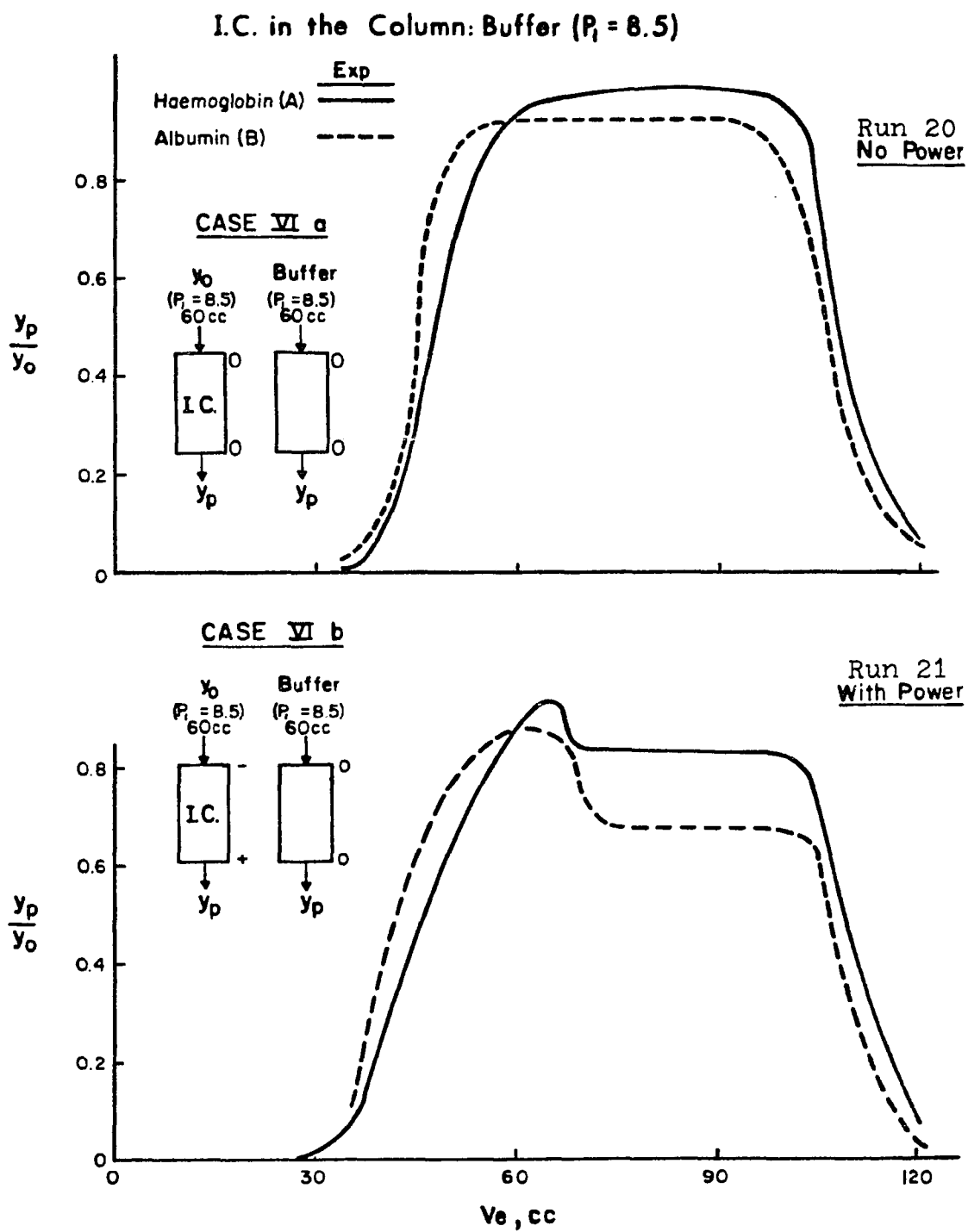


FIGURE 26. Effect of Electric Field in Stage VI

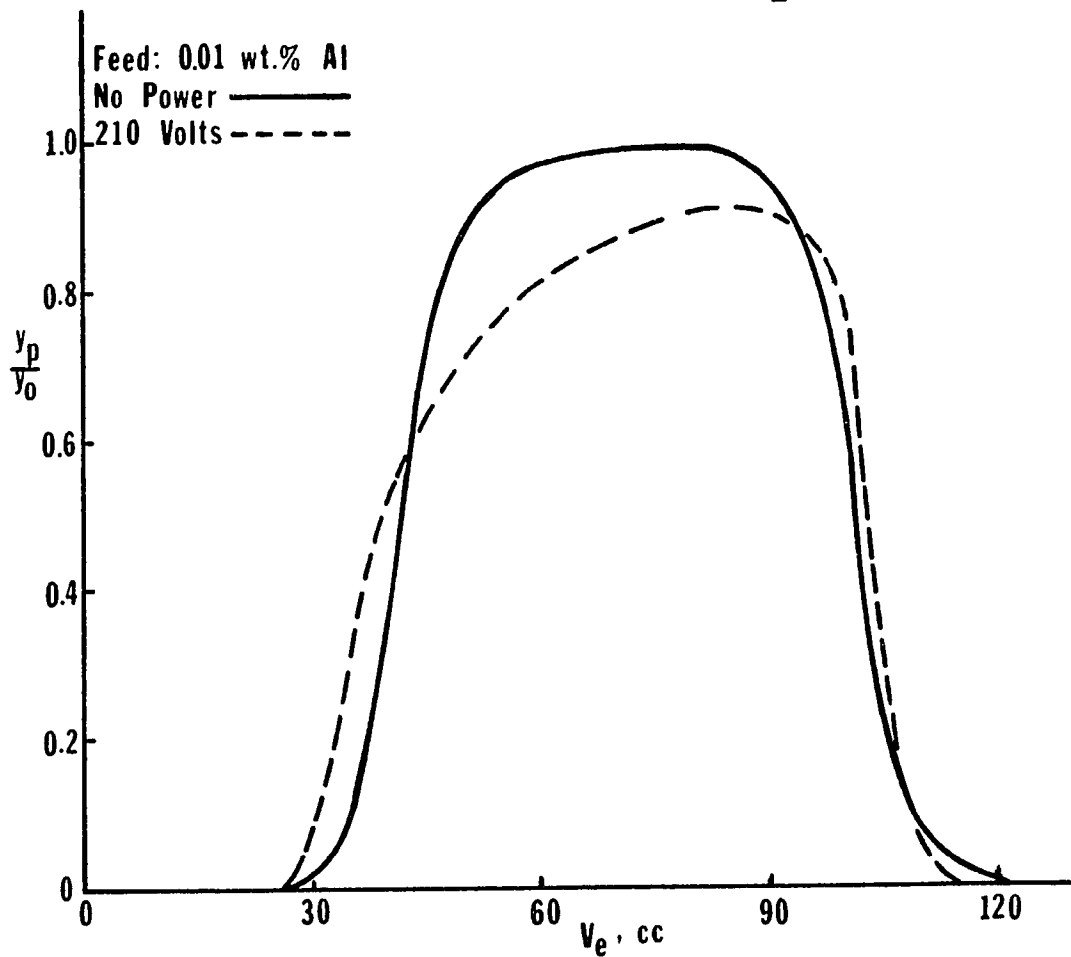
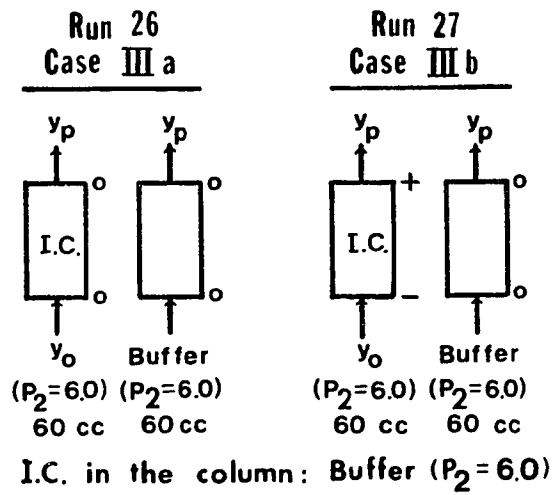


FIGURE 27. Effect of Electric Field in Stage III

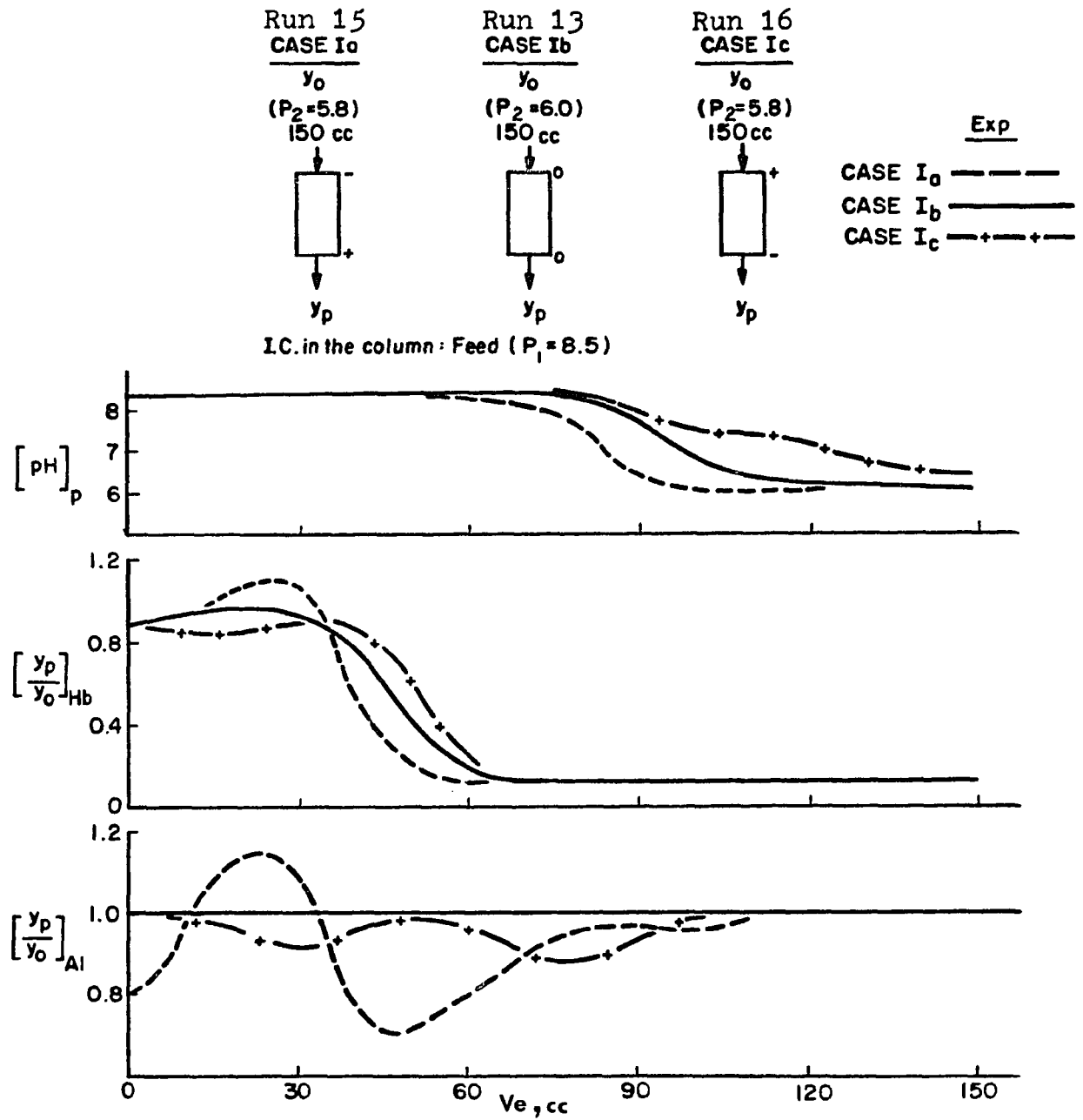


FIGURE 28. Effect of Electric Field in Stage I

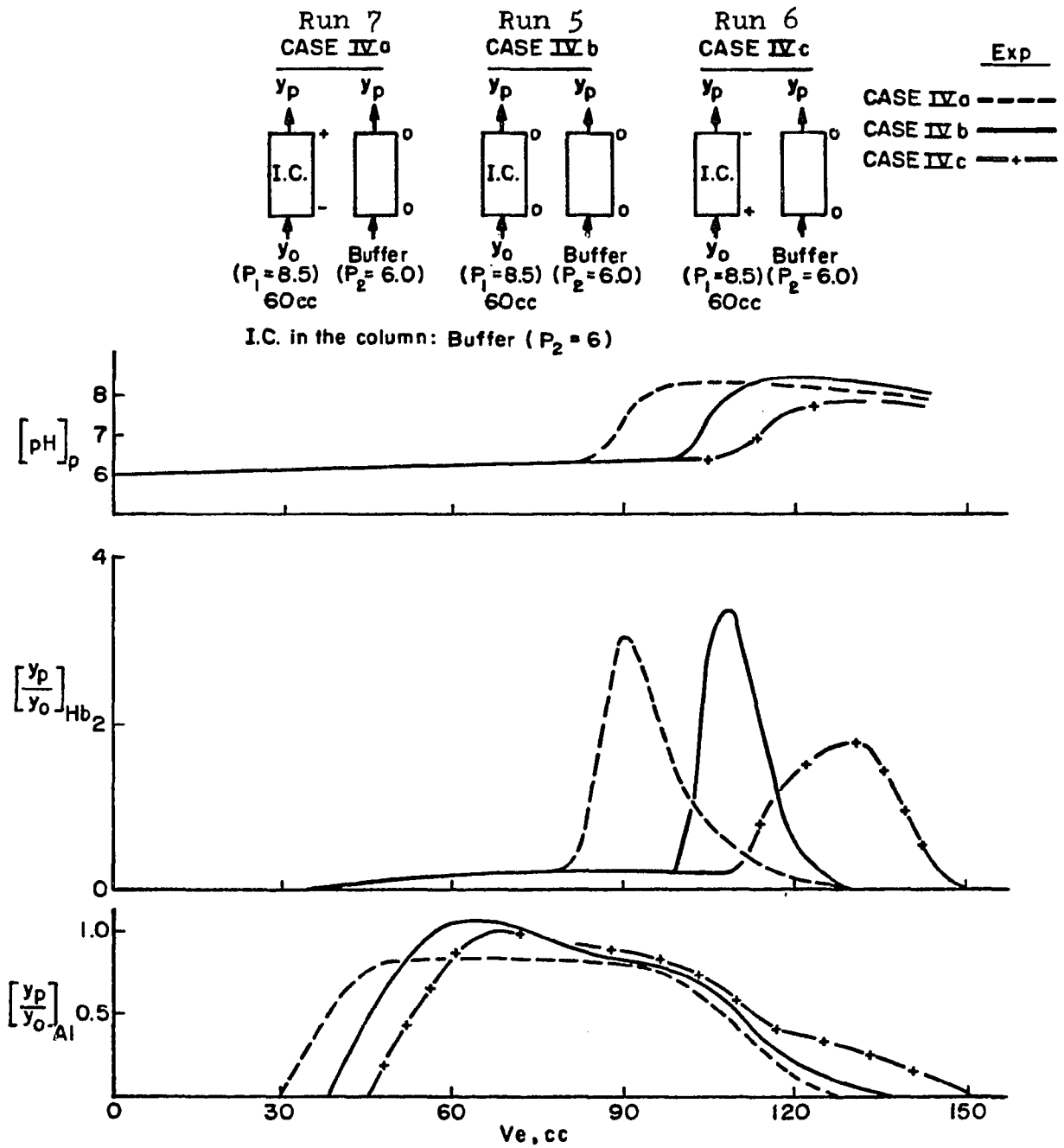


FIGURE 29. Effect of Electric Field in Stage IV

TABLE 7. Migration Velocities

Feed: 0.01 weight % Hemoglobin + 0.01 weight % Albumin
(except Case III - Albumin only)

Buffer: 0.05M Tris-maleate + 0.05M NaOH

$Q_o = Q_p = 0.5$ cc/min, $V = 45$ cc, $V_o = +0.167$ cm/min

Figure	Case	Volts	\bar{t}_p (min)	\bar{M}_{Hb} (cc)	\bar{M}_{Al} (cc)	$ \bar{M}_{Hb} - \bar{M}_{Al} $	$\nu_{E,Hb}$ ($\frac{cm}{min}$)	$\nu_{E,Al}$ ($\frac{cm}{min}$)
28	Ia	210	45.0	21.0	29.3	8.3	+0.019	+0.033
28	Ib	0	0	23.6	33.8	10.2	0	0
28	Ic	210	45.0	25.9	33.6	7.7	-0.017	+0.001
27	IIIa	0	0	-	71.9	-	-	0
27	IIIb	210	120.0	-	73.0	-	-	-0.003
29	IVa	210	120.0	89.7	69.8	19.9	+0.046	+0.015
29	IVb	0	0	106.2	75.1	31.1	0	0
29	IVc	210	120.0	123.3	83.3	40.0	-0.048	-0.023
26	VIa	0	0	75.7	73.2	2.5	0	0
26	VIb	210	120.0	74.8	69.6	5.2	+0.003	+0.010

Note: Vector direction of migration velocity ν_E is positive or negative with respect to V_o being positive. Also, Case I centers of mass are based on $V_e = 67.5$ cc.

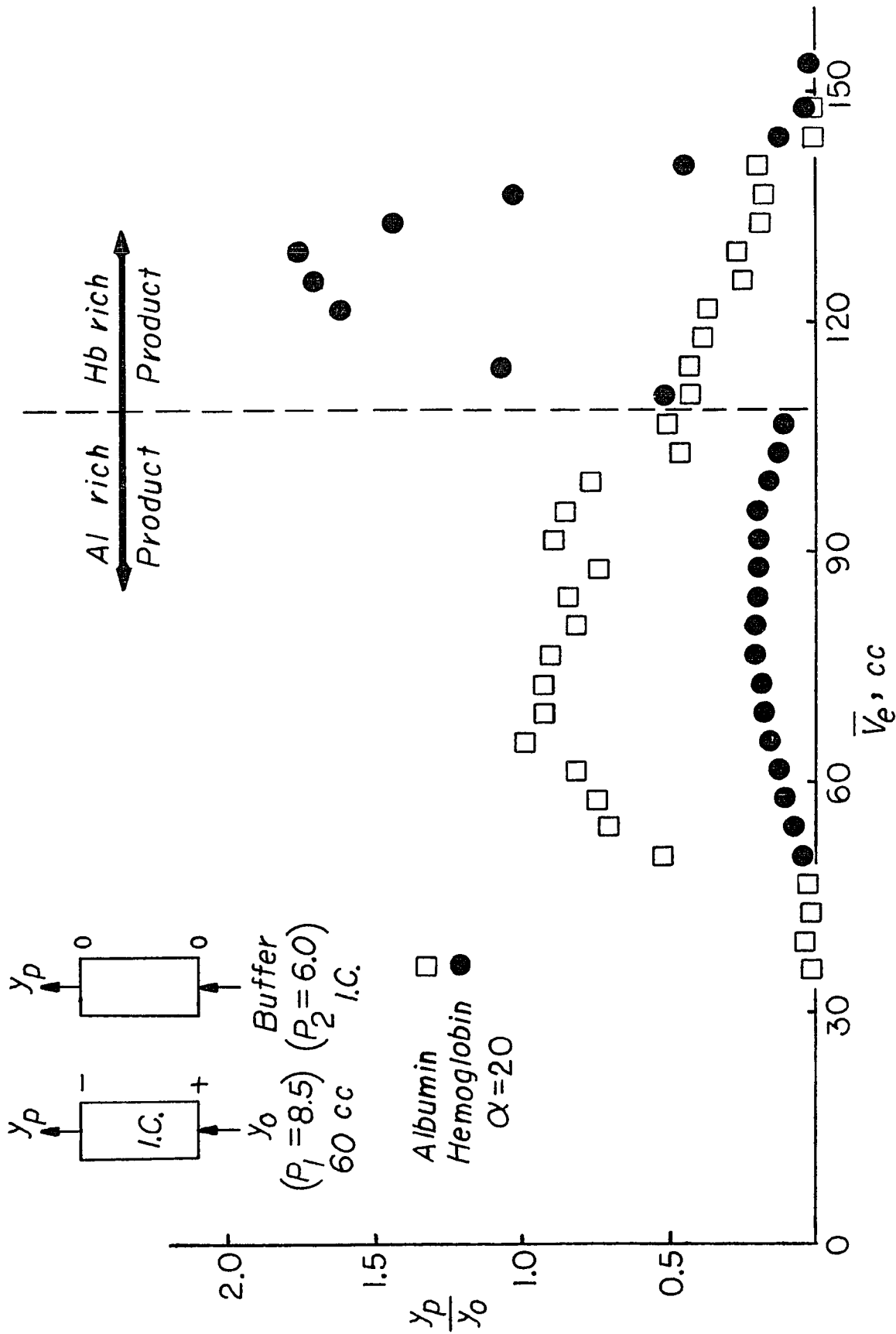


FIGURE 30. Batch Separation Via Electropolarization Chromatography (Run 6)

Chapter 4

PARAMETRIC PUMPING WITH pH AND ELECTRIC FIELD

Based on the preceding discussion, a new separation process was developed as shown in Figure 31. The goal in this process is to maintain the separation of Protein A achieved in the six-stage pH parametric pumping process while selectively removing Protein B from the bottom product via addition of an electric field in certain stages of the pH parapumping process. This will enhance the purity of the bottom product stream and, thus, the overall separation. More specifically, a hemoglobin separation factor of at least twenty must be achieved while the albumin separation factor is increased above one. In this way, the product or overall separation factor α will be greater than twenty.

The highest migration velocity for either protein was observed for hemoglobin in Stage IV, so the electric field will only be applied in Stage IV. The greatest difference between the centers of mass of Proteins A and B occurs in Case IVc, so the cathode will be placed at the bottom of the chromatographic column during Stage IV. An on/off cycling of the electric field was used in the new process, with a high flow rate Q_0 in the zero-field stages and a low flow rate Q_p coupled to a finite field in Stage IV. A low flow rate is necessary in Stage IV in order to make the electric field effective. The use of a high flow rate in the other stages minimizes cycle time and consequently maximizes production rate (g protein/min).

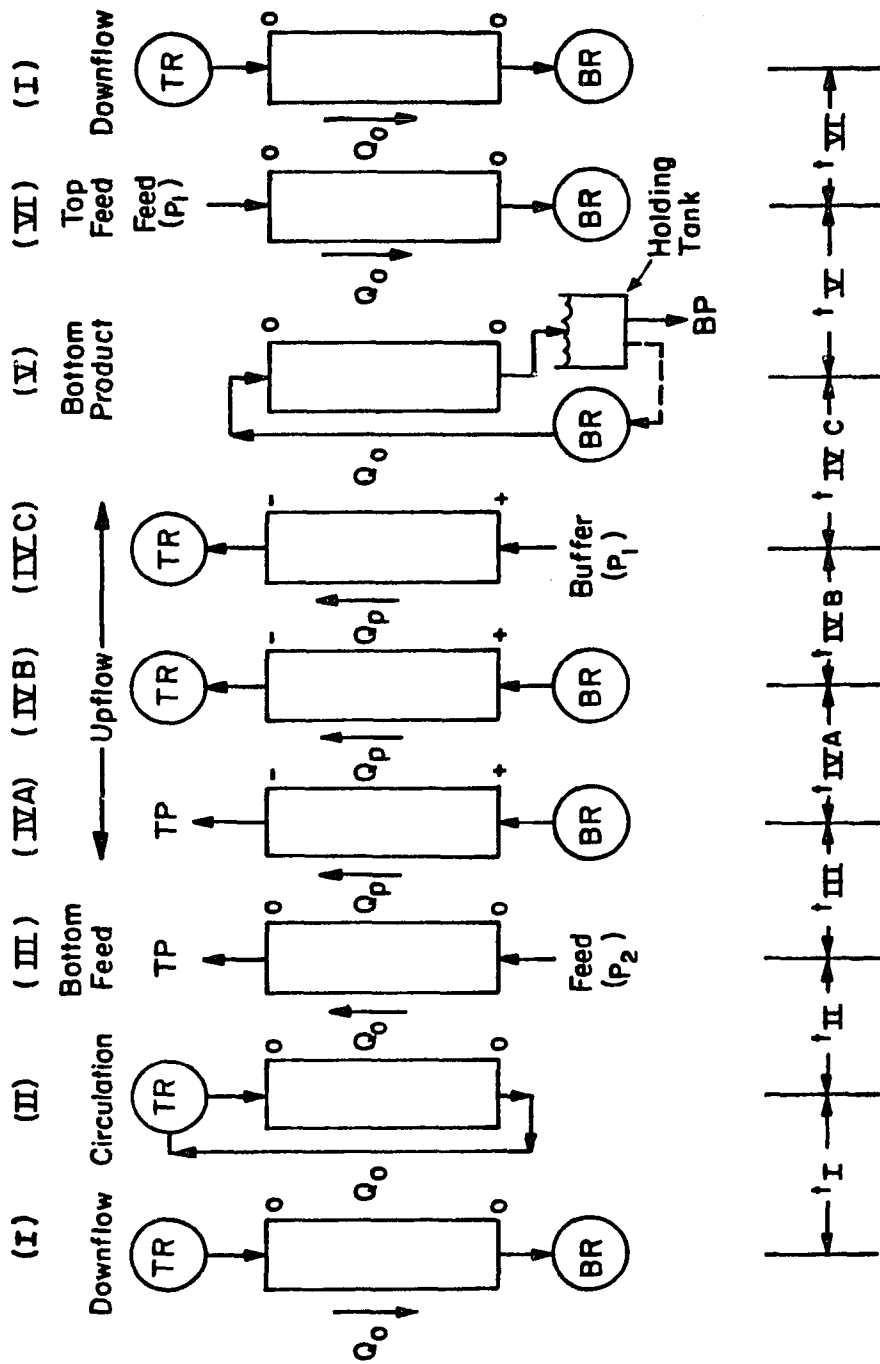


FIGURE 31. Process Diagram for the pH Parapump with Electric Field (Mode 7)

In the previous pH parametric pumping experiments by Chen and co-workers, the downflow or displacement from the top reservoir to the column to the bottom reservoir was equal to the upflow or displacement from the bottom reservoir to the column to the top reservoir. In other words, the displacements were equal for Stages I and III in the four-stage process (Figure 8), Stages I and V in the eight-stage process (Figure 9), Stages I and III in the batch process (Figure 10), and also Stages I and IV in the six-stage process (Figure 21). In the present case (Figure 31), the displacements in the upflow and downflow stages are not equal.

$$Q_o t_I < Q_p t_{IV} \quad (4-1)$$

The optimum displacements for Stages I, II, and IV in the new process were determined experimentally as shown in Figures 32 and 33.

The operation of Stage I is simulated in Figure 32. After one void volume of downward displacement (45 cc), the hemoglobin-rich solution originally in the column has been pumped to the bottom reservoir, but the pH of the solution in the column does not change from P_1 to P_2 until after approximately two void volumes of downward displacement (93.75 cc). As seen in Figure 32, the solution leaving the bottom of the column between $V_e = 45$ cc and $V_e = 93.75$ cc is very low in hemoglobin concentration. This indicates that the pH at the inlet of the column changes immediately to P_2 so that the incoming hemoglobin is adsorbed. The P_1 fluid originally in the column moves downward at the bulk velocity,

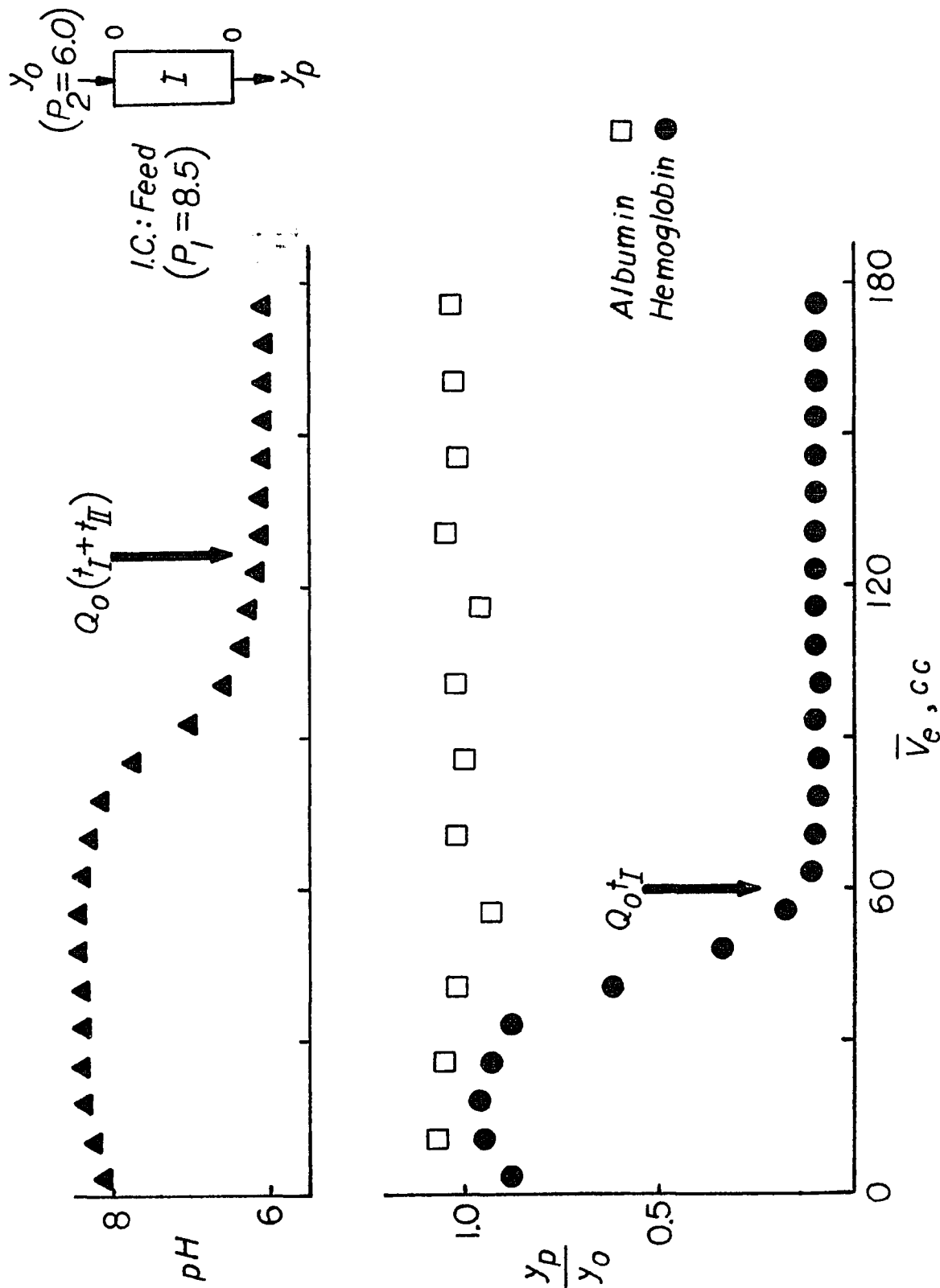


FIGURE 32. Optimum Displacements for Stages I & II (Run 13)

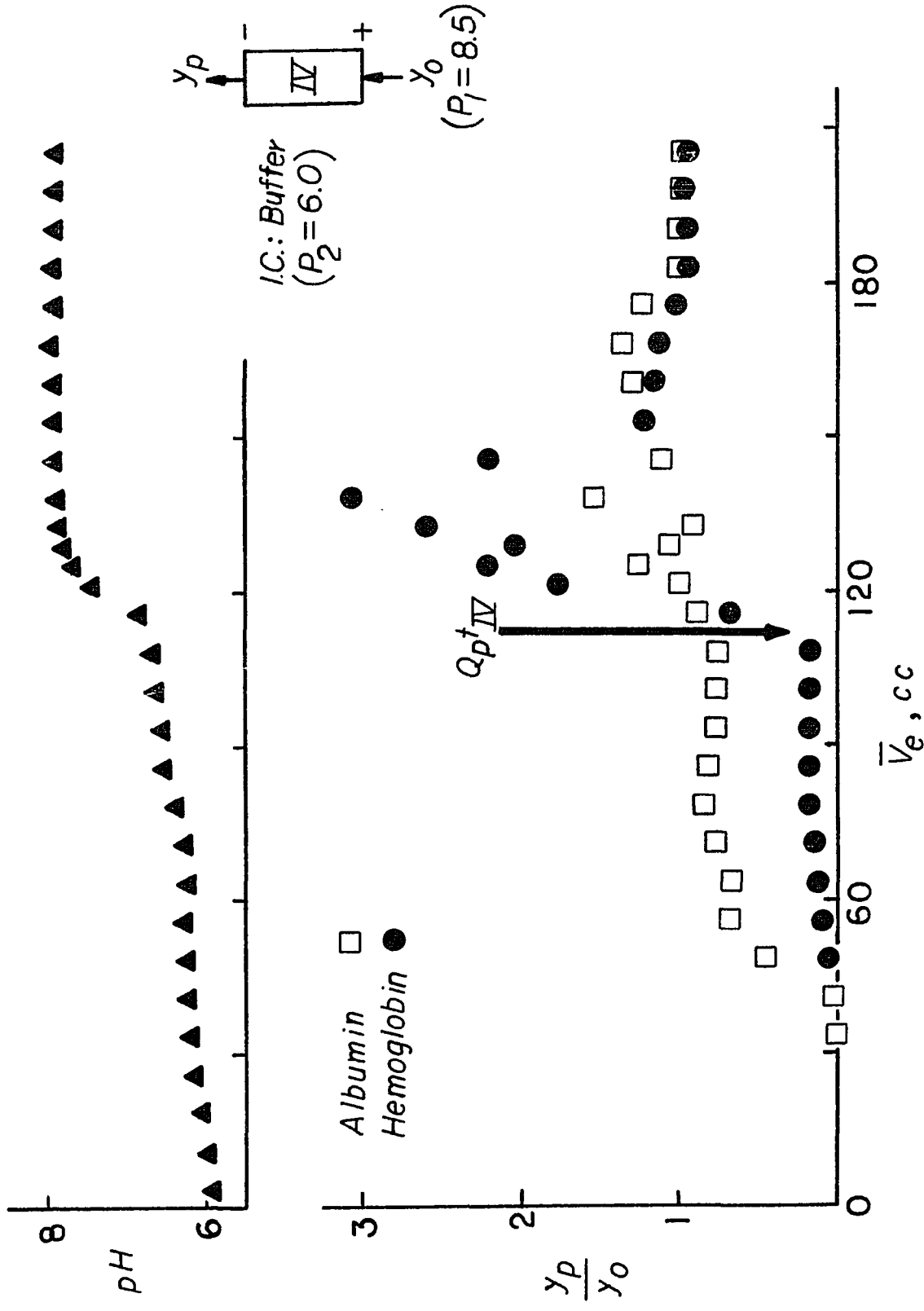


FIGURE 33. Maximum Upflow for Stage IV of the New Process (Run 8)

but the pH change lags behind the concentration wave, i.e., the pH wave velocity is less than the bulk velocity. The downward displacement is stopped at $Q_0 t_I$ after the wave of concentrated hemoglobin has exited the column (Figure 32). A value of $Q_0 t_I = 67.5$ cc was used in the following experiments, but this displacement could probably be reduced to one void volume or 45 cc. The pH of the fluid in the column is corrected by circulation to the top reservoir and titration of the contents of the top reservoir in Stage II, so that the fluid exiting the column is at $\text{pH} = P_2$. A total time of $Q_0 (t_I + t_{II}) = 135.0$ cc was used as indicated in Figure 32.

The operation of Stage IV of the new process is simulated in Figure 33. After one void volume of upward displacement, the hemoglobin concentration wave is still in the bottom portion of the column and albumin-rich solution is in the top portion of the column. High-pH buffer is pumped into the bottom of the column in order to push the hemoglobin wave to the top of the column while pushing additional albumin into the top streams. A total displacement of $Q_p t_{IV} = 107.5-112.5$ cc was used in the following experiments. Since $Q_p t_{IV} > Q_0 t_I$, additional top product was taken at the beginning of Stage IV and extraneous fluid, i.e., fluid not from the bottom reservoir, was added at the end of Stage IV as indicated in Figure 31 in order to balance the mass flows between the reservoirs.

The time durations for the various stages are:

$$t_I \approx t_{II} \approx t_V \geq \frac{V}{Q_0} \quad (4-2)$$

$$t_{III} = \frac{F_B}{Q_O} \quad (4-3)$$

$$t_{IVA} = t_{IVC} = \frac{F_O}{Q_P} \quad (4-4)$$

$$t_{IVA} + t_{IVB} = t_{IVB} + t_{IVC} = \frac{Q_O}{Q_P} t_I \quad (4-5)$$

$$t_{VI} = \frac{F_T}{Q_O} \quad (4-6)$$

Also note:

$$P_T = F_B + F_O \quad (4-7)$$

$$P_B = F_T \quad (4-8)$$

Description of the New Splitting Process

Following is a stage-by-stage description for Mode 7 operation of the pH parapump with electric field (Figure 31). This modus operandi was arrived at after much trial-and-error experimentation and numerous mathematical forays, some of which will be discussed in later chapters. Modes 6 and 7 are the basis for the papers by Chen et al., 1981c, and Hollein et al., 1982.

At $t=0$, the column is saturated with P_1 feed. The bottom reservoir (V_B ; $V_B > V$) is filled with P_1 feed and the top reservoir ($V + V_T$) is filled with P_2 feed. Automatic titrators are used to maintain the pH's at P_1 and P_2 in the various reservoirs as shown in Figure 35. One complete cycle contains six distinct stages as outlined below. Stage IV is divided into three parts.

I. DOWNFLOW: The solution with $\text{pH} = P_2$ from the top

reservoir enters the top of the column, and at the same time the solution in the column flows from the bottom of the column to the bottom reservoir. Total downflow equals 1.5V.

II. CIRCULATION: The fluid phase is circulated between the column and the top reservoir. This ensures that the pH in the column is completely changed from P_1 to P_2 , and that Protein A is adsorbed in the solid phase. As seen in Figure 32, at the end of Stage I the pH in the lower half of the column is above I_A . It is crucial that this pH be corrected.

III. BOTTOM FEED: The feed with pH equal to P_2 enters the bottom of the column, and the solution rich in Protein B is pushed out from the top of the column as the top product.

IV. UPFLOW: The optimum volume for upflow was experimentally determined in Figure 33 and was found to be 2.4V for the operating conditions considered, i.e., 0.05M Tris-maleate/NaOH buffer at 210 volts and 0.5 cc/min. The cathode is placed at the bottom of the column in order to delay the movement of Protein A relative to Protein B. The electric field is maintained at constant voltage and the bulk flow rate is reduced from Q_0 to Q_p . Stage IV is divided into three parts in order to specify the mass flow balance between the reservoirs.

In STAGE IVA, the solution from the bottom reservoir is pumped into the bottom of the column, and additional top product is taken. The total product volume for this stage is 0.9V.

In STAGE IVB, the remainder of the solution from the

bottom reservoir, to a total of $Q_0 t_I = 1.5V$, is pumped into the column and part of the fluid in the column is pumped to the top reservoir.

In STAGE IVC, the high pH buffer with a volume equal to $0.9V$ is pumped into the bottom of the column in order to push the protein B rich solution, originally in the top of the column, to the top reservoir. High pH feed may be used in Stage IVC instead of high pH buffer, but this has the undesirable feature of adding Protein B to the bottom streams.

V. BOTTOM PRODUCT: The bulk volumetric flow rate is returned to Q_0 . At the beginning of Stage V, a strong concentration gradient exists across the column with both proteins richer near the top of the column and the fluid in the bottom of the column close to buffer concentration. Dead volume solution from the bottom reservoir is pumped into the top of the column, and the fluid in the column is pushed out of the bottom of the column and mixed in the holding tank. The bottom product is taken from the tank, and the remainder of the solution in the tank is then returned to the bottom reservoir.

VI. TOP FEED: The high pH feed with a volume equal to P_B (the bottom product volume taken during the previous stage) is pumped into the top of the column, and the fluid in the column is pushed to the bottom reservoir. One cycle is now completed and Stage I of the second cycle begins.

The separation principle in the new process is shown schematically in Figure 34. The movement of Proteins A and B

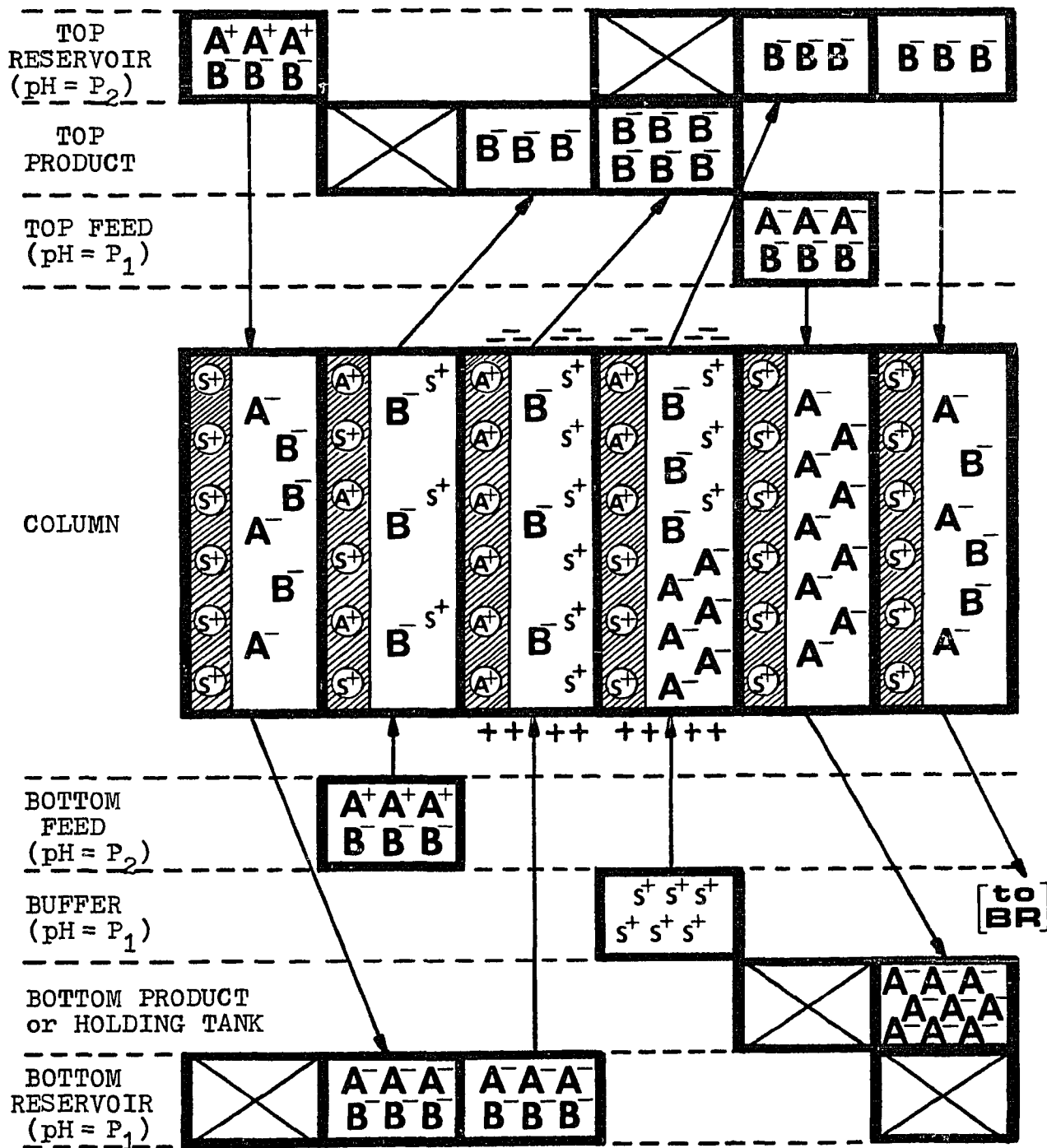


FIGURE 34. Schematic Description of the Separation Principle in the New Process (Modes 5-8)

is followed stage-by-stage through one complete cycle. A complete split of Proteins A and B is hypothetically achieved in one cycle in Figure 34. In reality, the procedure must be repeated for six or more cycles to reach the final separation and a 100% split is not observed. The conditions used for the schematic description are similar to the simplifications used for the graphical method. Both cases indicate that the maximum possible separation is infinite in the presence of the electric field.

$$t_{III} = \infty \quad (2-4)$$

$$t_I = t_V = \frac{V}{Q_0} \quad (2-5)$$

$$F_T = F_B = V \quad (2-8)$$

$$V_T = V_B = \text{ZERO} \quad (2-14)$$

Also Note:

$$t_{IVA} = t_{IVC} = \frac{V}{Q_p} \quad \text{or} \quad F_0 = V \quad (4-9)$$

$$t_{IVB} = \text{ZERO} \quad (4-10)$$

Therefore, Stage IVB does not appear in Figure 34. Also from Equations 4-8, 2-5, and 2-8, $P_B = Q_0 t_V$, so the entire contents of the holding tank go to the bottom product in Figure 34.

In Stages I, II, and III, Protein A^+ from the top reservoir or the bottom feed at $\text{pH} = P_2$ enters the packed bed and is exchanged for the counter ion S^+ from the cation exchanger. Protein B^- is unaffected and moves into the top streams. In Stages IV, V, and VI, the pH is changed to P_1 , so that A^+ is changed to A^- and desorbed from the ion exchanger.

Thus, the bottom streams are enriched in Protein A⁻ and the ion exchanger is regenerated. Also in Stage IV, A⁻ is retained by the electric field while B⁻ is unaffected. Actually, part of the B⁻ will also be retained by the electric field, but in smaller quantities than A⁻. All of the process fluids contain the counter ion S⁺ in excess, but S⁺ is only indicated in the fluid in the column for simplicity. Figure 34 suggests a limit of 100% albumin in the top product and 100% hemoglobin in the bottom product. The best split achieved experimentally was Run 55 (Appendix B) with 92% albumin in the top product and 89% hemoglobin in the bottom product.

Experimental Set-Up

The experimental apparatus is shown in Figure 35. A LKB 7900 UNIPHOR electrophoresis system was modified for continuous operation by the addition of a second elution stopper. The UNIPHOR system is in the center of Figure 35. The process reservoirs and process pump are drawn on the left-hand side of the column, and the external buffer reservoirs and buffer pump are drawn on the right-hand side. The UNIPHOR column has an internal cylindrical shape similar to a standard chromatographic column. The column (0.026 m I.D. and 0.15 m height) was packed with CM Sepharose cation exchanger. The UNIPHOR system consists of three parts -- the central chamber which becomes an ion exchange column in the present investigation, and two buffer chambers which are separated from the central chamber at each end by a filter

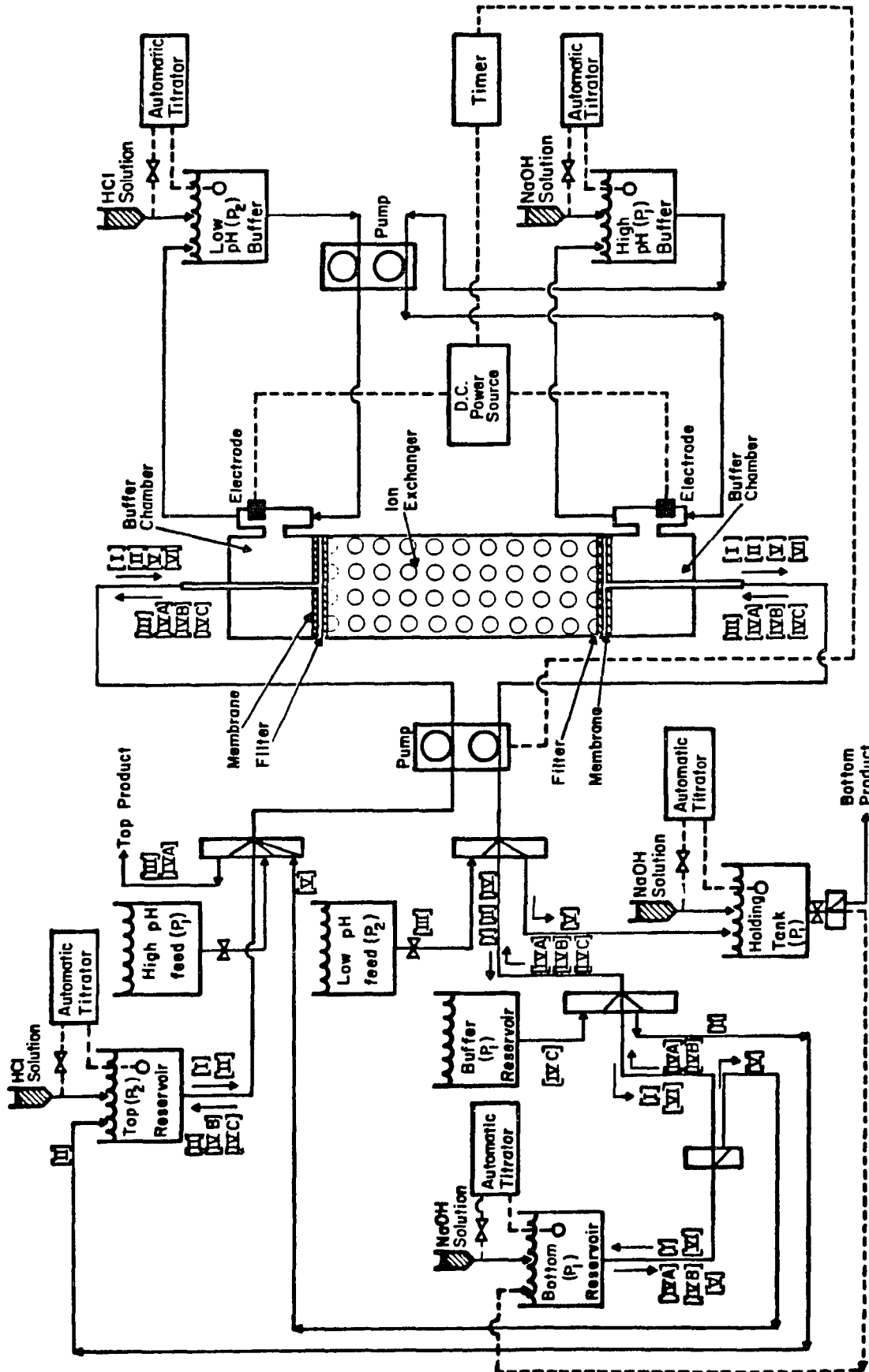


FIGURE 35. Experimental Apparatus (Mode 7)

PH. 100. 100

plus a semipermeable membrane. The system was maintained at 278°K by circulation of cooling water through the jacket of the UNIPHOR column and the jackets of the UNIPHOR buffer chambers. The external buffer reservoirs (2 liter volume) were kept at 288°K.

Reciprocating flow through the system was obtained by use of a reversible peristaltic pump (manufactured by Pharmacia Fine Chemicals). A higher capacity peristaltic pump (manufactured by BIO-RAD laboratories) was used for external buffer circulation. A Buchler 3-1500 power supply was used for a direct current source to maintain constant voltage across the column. The feed pump and the power supply were connected to a timer for precise control of the reservoir displacements and feed volumes. Valves were placed on each inlet channel of the feed pump in order to introduce the reservoir liquids, to introduce the high or low pH feeds, and to take product samples.

One of the initial accomplishments in this investigation (after some five months of frustrating failures) was being able to make a parametric pumping run with electric field of six cycles duration!!! Earlier runs were aborted after one or two cycles due to one of the following problems:

- (1) The ion exchange bed was plugged by degraded proteins when the power was set too high for adequate heat removal.
- (2) The LKB filter allowed passage of fine particles which plugged the exit tubing. Also, the LKB filter became impacted with some substances including visible needle-like crystals of hemoglobin.
- (3) The LKB membrane ruptured many times allowing mixing

of the process fluids with the external buffer. This problem was initially attributed to pressure build-up from either of the first two problems. In the final analysis, the LKB membrane was found to be fragile and likely to rupture for no reason.

The power limitations were discussed in Chapter 2. The LKB filter was replaced with a Pharmacia filter specifically designed for use with ion exchange resins. Finally, the LKB membrane was replaced with SPECTRAPHOR membrane tubing (M.W. cutoff approximately 3500).

Minor modifications were made on the elution stoppers in order to supply adequate support for the solid phase. Plastic grids were placed at the top of the elution stoppers, and lucite rings were added inside the stoppers to contain the grids. The membrane is immediately above the plastic grid, and the filter is immediately above the membrane. If undesirable operating conditions cause a high pressure drop across the packed bed, buffer solution from the process streams will flow through the membranes to the external buffer streams. Under normal operating conditions, however, bulk flow through the membranes should not occur.

Each product stream was analyzed at the end of every cycle using a Bausch and Lomb spectrophotometer. Hemoglobin concentration was determined by absorbance at a wavelength of 403 μm , and the hemoglobin readings were corrected for pH as explained in Appendix A. Total protein was determined at a wavelength of 595 μm using BIO-RAD protein assay. Albumin concentrations were then determined by difference. Total protein concentration was checked at 280 μm in the initial work.

Experimental Results

Eight runs (Runs 53-60) were made via the new splitting process. The operating conditions are listed in Table 8. The base case for comparison purposes is the six-stage pH parapumping process without electric field (Run 45). In order to compare the separation in the various operating modes (Modes 5-8), a series of runs were made with $F_B = F_T = 35$ cc as indicated in Table 8. Mode 6 operation is diagrammed in Figure 36. The difference between Modes 6 and 7 is in the operation of Stages V and VI. Mode 5 (Run 53, Appendix B) is identical to Mode 6 except that Stage V in Figure 36 is eliminated, i.e., $t_V = 0$. The separation in Run 53 was greater than in the base case, but the data was very unstable ($\alpha = \dots 34, 23, 60, 28, 56$) so Mode 5 operation was rejected.

Examination of Figures 21 and 36 shows that the base case is identical to Mode 6 if $F_0 = 0$ and the field strength in Stage IV is zero. In order to avoid duplication, only a few selected runs are discussed in this chapter -- the remainder are included in the mathematical modeling in Chapters 5 and 6. Experimental results for Modes 6 and 7 are compared to the base case in Figures 37 and 38.

As seen in Figure 37, the top product was identical in Runs 45, 55, and 56. The top product was less pure for Run 54 because the total upflow in Stage IV (112.5 cc) was too large, leading to a fractional breakthrough of the hemoglobin desorption wave to the top product streams. The total upflow for Stage IV in Runs 55-60 was reduced to 107.5 cc.

TABLE 8. Operating Conditions for the New Process

Feed: 0.01 weight % Hemoglobin + 0.01 weight % Albumin

Buffer: 0.05M Tris-maleate + 0.05M NaOH

$Q_0 = 2.5$ cc/min, $Q_p = 0.5$ cc/min, $V = 45$ cc, $E = 210$ volts

<u>Run</u>	<u>Mode</u>	<u>F_B</u>	<u>F_T</u>	<u>F₀</u>	<u>t_I</u>	<u>t_{II}</u>	<u>t_{IV}</u>	<u>t_V</u>	<u>V_T</u>	<u>V_B</u>
45	-	35.0	35.0	0.0	36	24	36	48	105.0	60.0
53	5	35.0	35.0	45.0	27	27	225	0	82.5	60.0
54	6	35.0	35.0	"	"	"	"	54	"	"
55	7	52.5	17.5	40.0	"	"	215	24	112.5	90.0
56	"	35.0	35.0	"	"	"	"	"	"	"
57	"	17.5	17.5	"	"	"	"	"	"	"
58	"	17.5	52.5	"	"	"	"	"	"	"
59	"	52.5	52.5	"	"	"	"	"	"	"
60	8	35.0	35.0	"	"	"	"	45	"	"

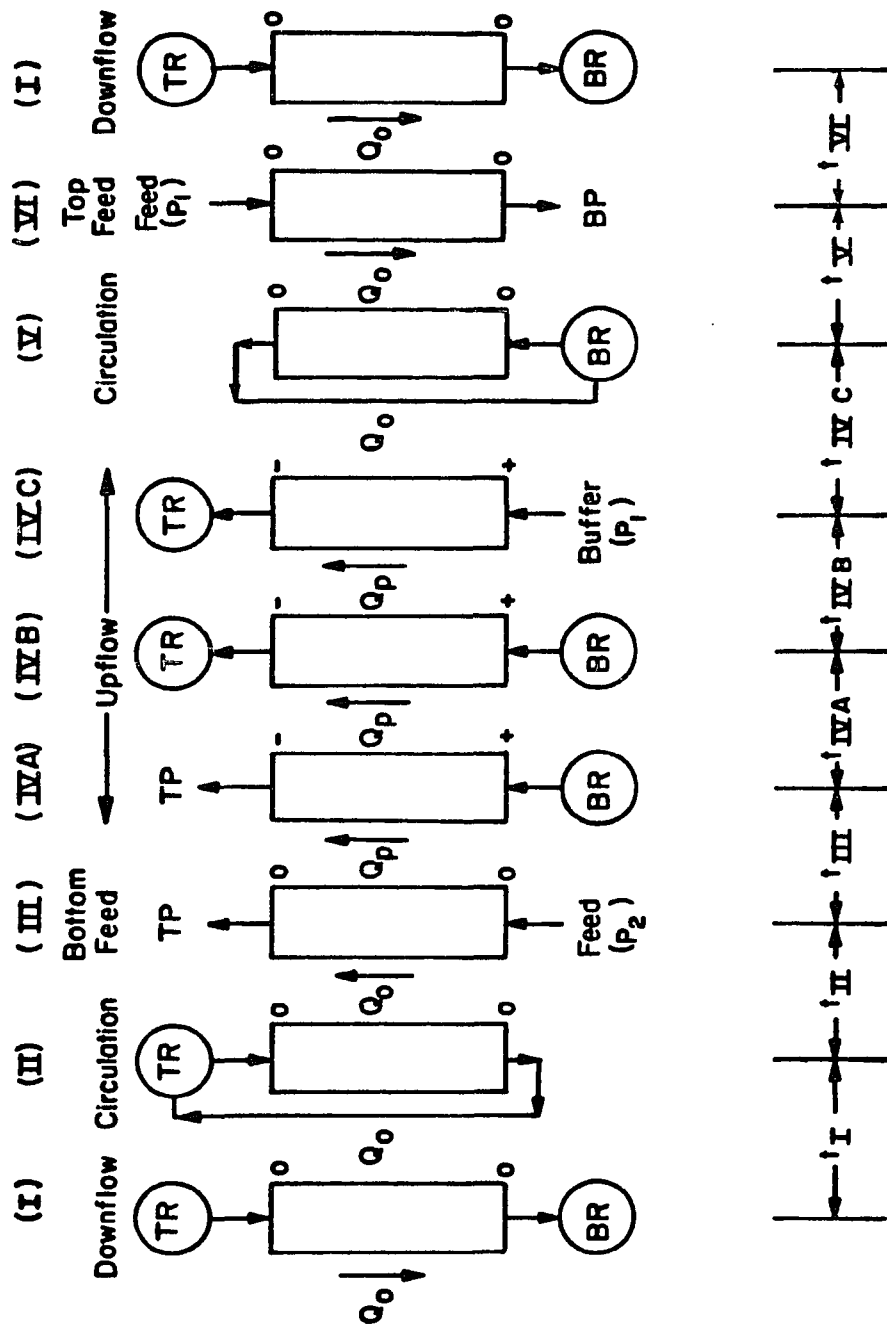


FIGURE 36. Process Diagram for the pH Parapump with Electric Field (Mode 6)

		EXP			
Run		Haemoglobin	Albumin	F_B	F_T
45	Pump without power	△	▲	35	35
54	Mode 6	○	●	35	35
56	Mode 7	□	■	35	35
55	Mode 7	○	●	52.5	17.5

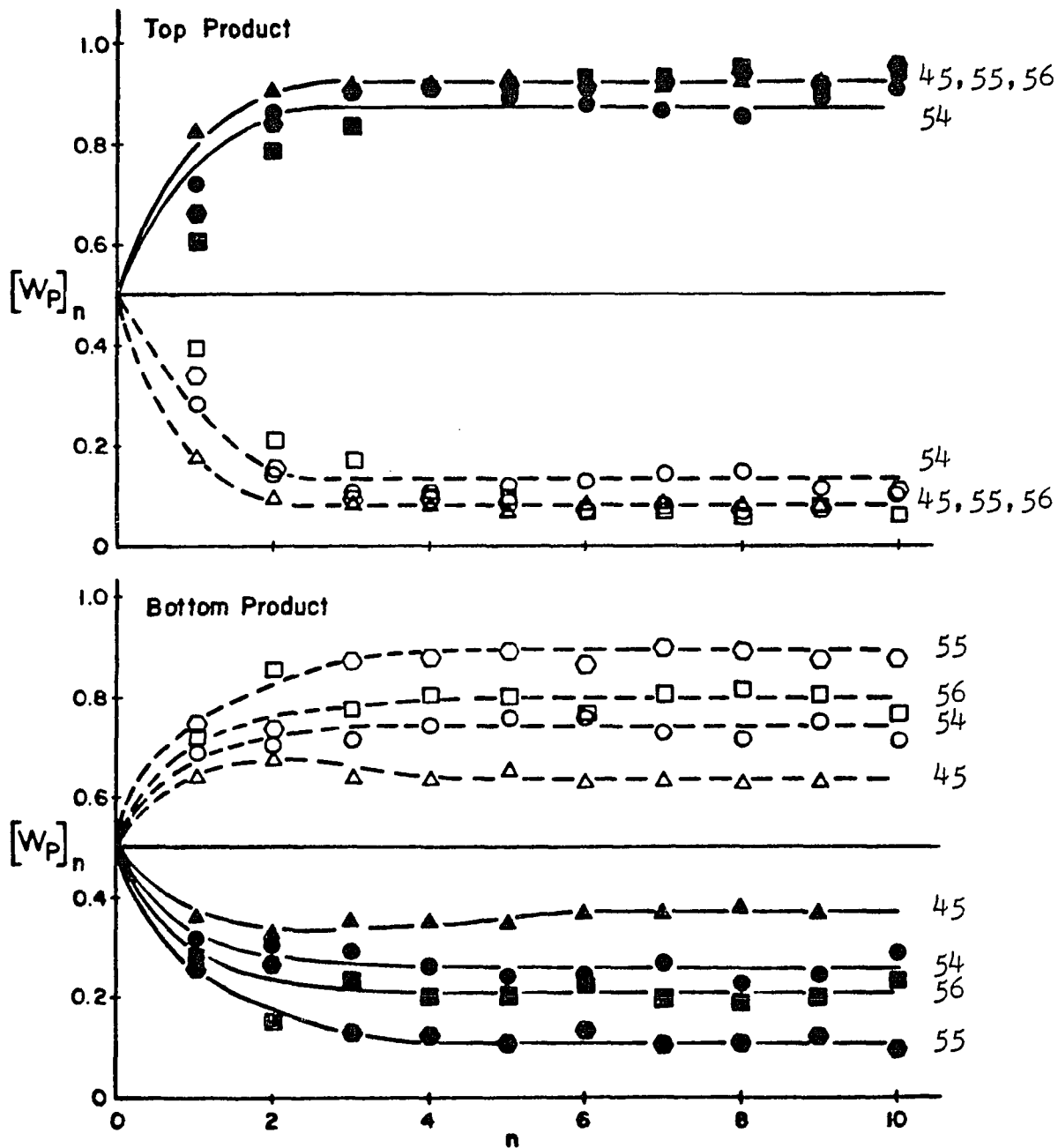


FIGURE 37. Experimental Results for the New Process

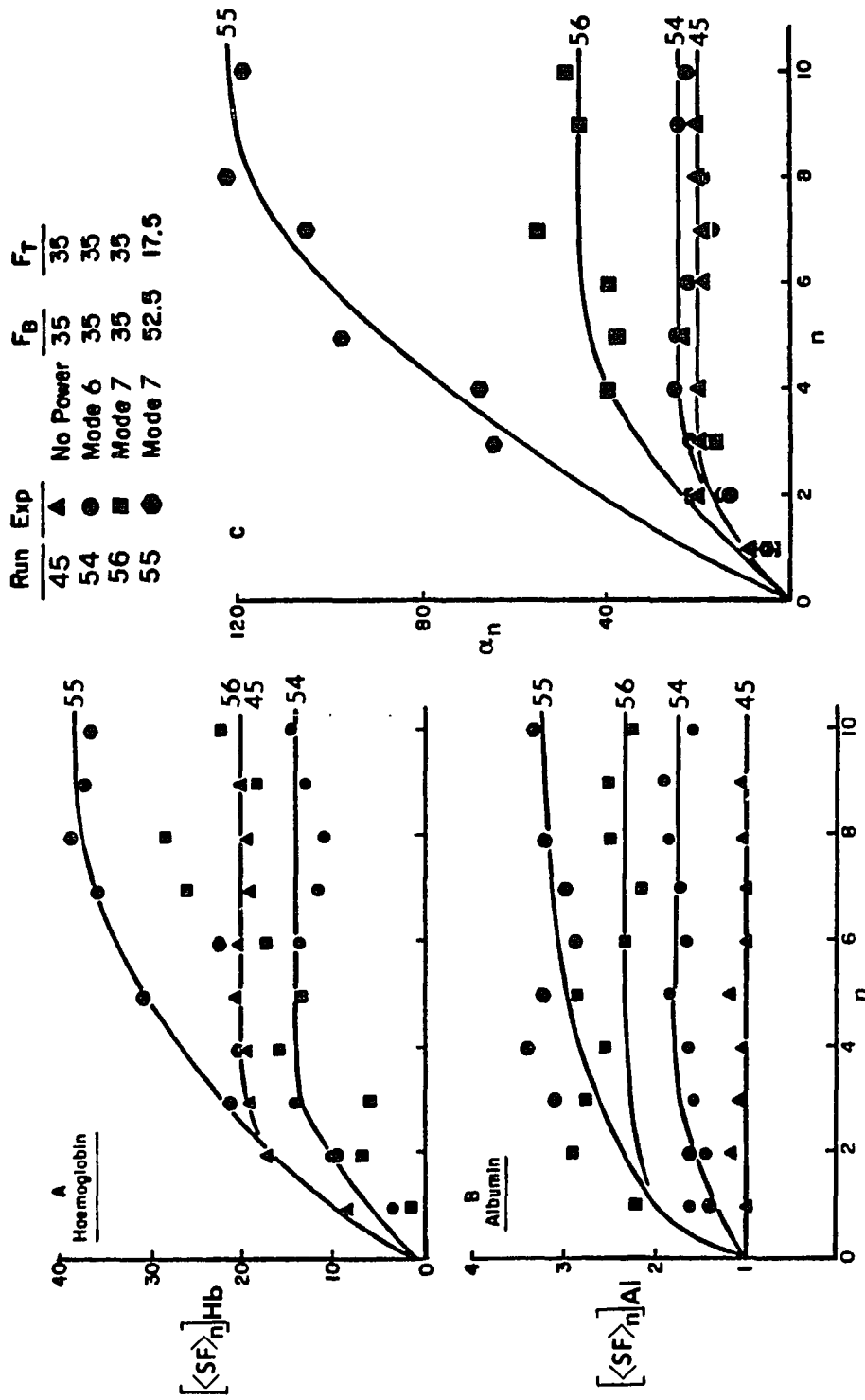


FIGURE 38. Experimental Separation Factors for the New Process

The new process is designed to improve the bottom product while maintaining the top product purity achieved via pH parametric pumping. The maximum hemoglobin weight fraction in the bottom stream for pH parapumping (Equations 2-26 and 2-27) is 66.7% if $F_B = F_T$ as in Runs 45, 54 and 56; and 80.0% if $F_B = 3F_T$ as in Run 55. Higher weight fractions of hemoglobin in the bottom product were obtained in all three runs with the new process as seen in Figure 37. With $F_B = F_T$, the hemoglobin weight fraction in the bottom product was increased from 63% in Run 45 to 74% in Run 54 (Mode 6) and 79% in Run 56 (Mode 7). Mode 7 is, therefore, a better mode of operation than Mode 6. The best case examined experimentally was Run 55 where the weight fraction of hemoglobin in the bottom product was increased to 89% by increasing the ratio of the bottom feed to top feed.

As shown in Figure 38C, a separation factor α_n as high as 120 was obtained via Mode 7 of the new process with $F_B = 3F_T$ (Run 55). All three runs considered in Figure 38 for the new process gave higher overall separations than the six-stage process (Run 45). The separations in the new process were also better than the batch separation achieved via electropolarization chromatography (Run 6, Figure 30), i.e., better than α equal to twenty.

As shown in Figure 38A, the separation factor for Protein A was approximately doubled in Run 55 as compared to Runs 45 and 56. Define the maximum concentration ratio ψ ,

$$\psi = \frac{F_B + F_T}{F_T} \quad (4-11)$$

Since the concentration ratio is 4.0 in Run 55 versus 2.0 in Runs 45 and 56, the observed increase for the hemoglobin separation in Run 55 may be expected to occur in any pH parapumping process. Chen et al., 1979a, experimentally examined the effect of concentration ratios of 1.5, 2.0, 2.5, and 3.0 on the separation in the four-stage pH parapump. Chen and co-workers proved that the separation increased as the concentration ratio increased up to some limiting value of F_B , after which a decrease in separation was observed.

The separation factor for Protein B is equal to one in a single-column pH parapumping process, but the albumin separation was significantly improved in the new splitting process as shown in Figure 38B. The increased albumin separation factors in Modes 6 and 7 are attributed to the addition of the electric field to the pH parapumping process, and will be discussed in more detail in the next chapter.

Chapter 5

MATHEMATICAL ANALYSIS OF THE ALBUMIN

SEPARATION IN THE NEW PROCESS

Two types of equations are needed in parapumping calculations: internal equations and external equations. The external equations are solute material balances on the streams flowing to and from the column and the reservoirs. The internal equations describe the events occurring within the column, i.e., the adsorption/desorption of Protein A (hemoglobin) and the electric field effects on both Proteins A and B. Since the increased separation in the new process relative to pH parametric pumping was due to improved albumin separation, a mathematical analysis of the Protein B separation was made in order to verify the trends observed for Runs 54-56 in Figure 38B.

In order to calculate the separation factor for Protein B from Equation 2-29, the top and bottom product concentrations must be calculated. The detailed calculations for $\langle y_{TP} \rangle_n$ and $\langle y_{BP} \rangle_n$ for Modes 6-10 are given below. The following assumptions were made:

- (1) Plug flow.
- (2) Negligible axial diffusion or dispersion.
- (3) Constant properties in the radial direction.
- (4) No interaction between solutes A and B, i.e., dilute solution.
- (5) The migration velocity $v_{E,A1}$ is assumed to be adequately represented by an average value determined from Runs 5 and 6 (Figure 29 and Table 7).

Given that $F_0 \leq V$, $Q_0 t_I \geq V$, and $Q_0 t_V \geq V$, four regions of operation may be considered as shown in Figure 39.

Region 1 - $F_B \leq V$ and $F_T \leq V$.

Region 2 - $F_B > V$ and $F_T \leq V$.

Region 3 - $F_B > V$ and $F_T > V$.

Region 4 - $F_B \leq V$ and $F_T > V$.

Experimental data was taken in all four regions for Mode 7 (Runs 55-59). For the other modes of operation, only Region 1 was investigated experimentally. The experimental work by Chen et al., 1979a, and the optimization studies at the end of this chapter both indicate that the point of optimum separability lies in Region 1. If three criteria are considered in the optimization, maximum separation, maximum production rate, and maximum recovery, the optimization studies indicate that the ideal feed ratio lies in Region 2.

The top and bottom product concentrations are calculated by following the albumin concentrations from stage-to-stage and repeating the calculations cycle-by-cycle until a repeating steady-state separation factor is obtained. A complete derivation will be given for Region 1, Mode 7. The changes in the external mass balances which are necessary for the other regions and/or modes of operation are easily handled by "IF" statements in a computer program. These changes will be specified following the complete derivation.

.....Mode 7 - Region 1.....

The initial conditions in Stage I of the n'th cycle are $[<y_{TR}>_n]_{A1}$, $[<y_{C,I}>_n]_{A1}$, and $[<y_{BR^*}>_n]_{A1}$, where

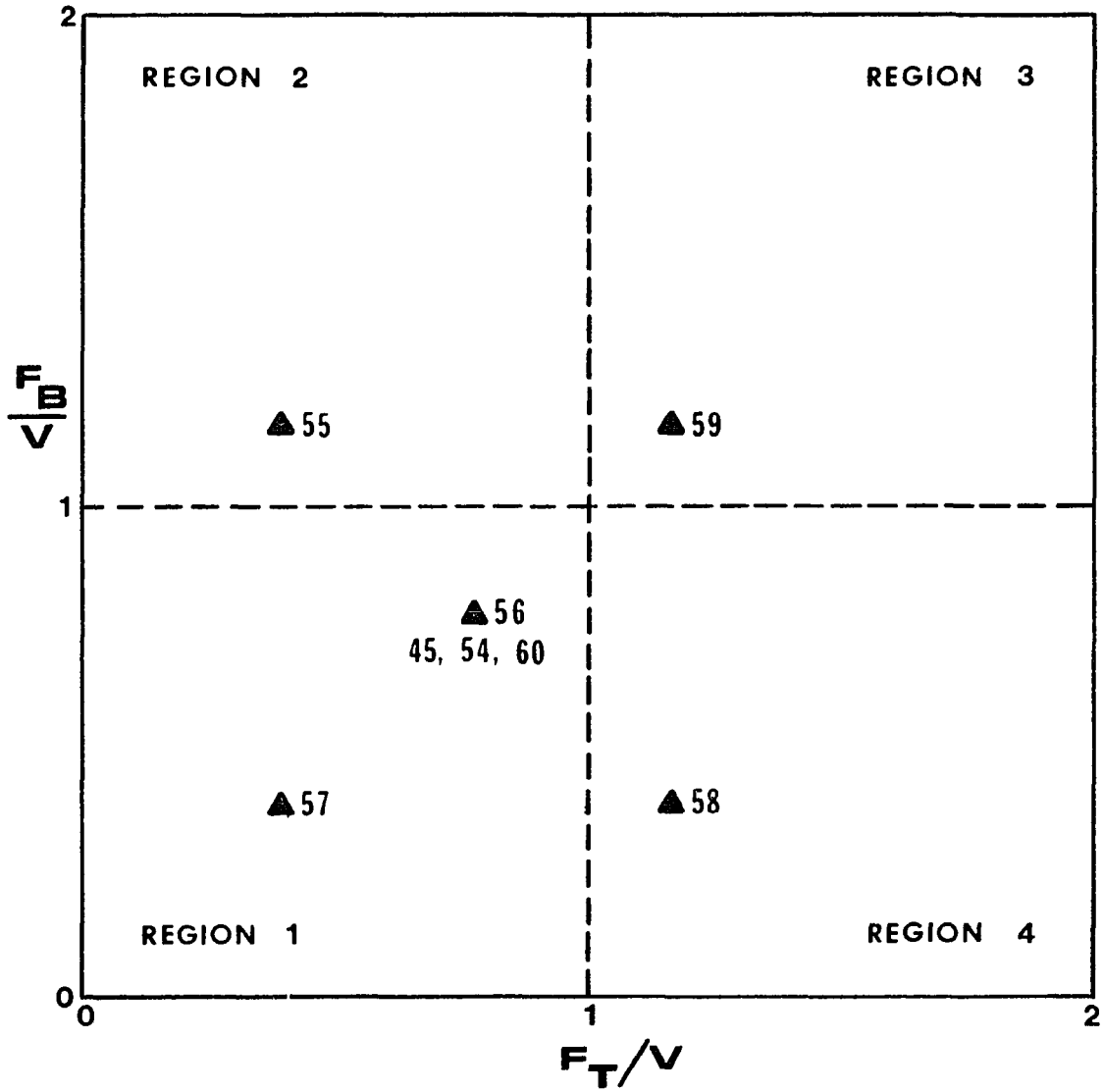


FIGURE 39. Regions of Operation based on Feed Volumes

$$[\langle y_{C,I} \rangle_n]_{A1} = [\langle y_{C,VI^*} \rangle_{n-1}]_{A1} \quad (5-1)$$

For cycles n to ∞ , with $n > 1$, these concentrations are calculated in the previous cycle. For the first cycle, i.e., $n = 1$,

$$\begin{aligned} [\langle y_{TR} \rangle_1]_{A1} &= [\langle y_{C,I} \rangle_1]_{A1} = [\langle y_{BR^*} \rangle_1]_{A1} = [\langle y_{BR} \rangle_1]_{A1} \\ &= [y_o]_{A1} \end{aligned} \quad (5-2)$$

In Stage I, y_{TR} remains the same. Stage II is a recycle stage and does not alter the albumin concentrations. The concentrations at the end of Stage I and the beginning of Stage III are,

$$[\langle y_{C,III} \rangle_n]_{A1} = [\langle y_{TR} \rangle_n]_{A1} \quad (5-3)$$

$$\begin{aligned} [\langle y_{BR} \rangle_n]_{A1} &= \frac{V_B [\langle y_{BR^*} \rangle_n]_{A1} + V [\langle y_{C,I} \rangle_n]_{A1}}{Q_o t_I + V_B} \\ &\quad + \frac{(Q_o t_I - V) [\langle y_{TR} \rangle_n]_{A1}}{Q_o t_I + V_B} \end{aligned} \quad (5-4)$$

In Stage III, y_{TR} and y_{BR} are unchanged. At the end of Stage III and the beginning of Stage IV, two concentration zones are present in the column due to the fact that $F_B \leq V$.

Top Zone : Volume = $(V - F_B)$

$$[\langle y_{C,IV1} \rangle_n]_{A1} = [\langle y_{TR} \rangle_n]_{A1} \quad (5-5)$$

Bottom Zone: Volume = F_B

$$[\langle y_{C,IV2} \rangle_n]_{A1} = [y_o]_{A1} \quad (5-6)$$

Also from Equation 5-3 and the overall mass balance,

$$[\langle y_{T,III} \rangle_n]_{A1} = [\langle y_{TR} \rangle_n]_{A1} \quad (5-7)$$

Where the total top product is a mixture from Stages III and IVA.

$$[\langle y_T \rangle_n]_{A1} = \frac{F_B [\langle y_{T,III} \rangle_n]_{A1} + F_0 [\langle y_{T,IVA} \rangle_n]_{A1}}{F_B + F_0} \quad (5-8)$$

In Stage IV, y_{BR} is constant. An electric field is placed across the column so that the fluid phase moves upward at ν_0 , while Protein B moves upward at $\nu_0 \pm \nu_{E,A1}$. The concentration of albumin in the fluid leaving the column is given by Equation 2-20, and the retardation coefficient for albumin is defined by Equation 3-2.

$$y_{out} = \frac{1}{R_{A1}} y_{column} \quad (2-20)$$

$$R_{A1} = \frac{\nu_0}{\nu_0 \pm \nu_{E,A1}} \quad (3-2)$$

By mass balance, if the concentration leaving the column is smaller, the volume containing the initial mass will be larger, i.e., it will take a longer time to exit from the column at ν_0 .

$$V_{out} = R_{A1} V_{initial} \quad (5-9)$$

The top product in Stage IVA is calculated from Equations 5-5, 5-6, 2-20, and 5-9 as follows:

If $F_0 \leq R_{A1} (V - F_B)$:

$$[\langle y_{T,IVA} \rangle_n]_{A1} = \frac{1}{R_{A1}} [\langle y_{TR} \rangle_n]_{A1} \quad (5-10)$$

If $F_0 > R_{A1} (V - F_B)$:

$$\begin{aligned}
\left[\langle y_{T,IVA} \rangle_n \right]_{A1} &= \frac{\left\{ R_{A1} (V - F_B) \right\} \left\{ \frac{1}{R_{A1}} \left[\langle y_{TR} \rangle_n \right]_{A1} \right\}}{F_0} \\
&\quad + \frac{\left\{ F_0 - R_{A1} (V - F_B) \right\} \left\{ \frac{1}{R_{A1}} \left[y_o \right]_{A1} \right\}}{F_0} \quad (5-11)
\end{aligned}$$

The total top product concentration is calculated from Equations 5-7, 5-8, 5-10, and 5-11.

If $F_0 \leq R_{A1} (V - F_B)$:

$$\left[\langle y_T \rangle_n \right]_{A1} = \frac{F_B + \frac{1}{R_{A1}} F_0}{F_B + F_0} \left[\langle y_{TR} \rangle_n \right]_{A1} \quad (5-12)$$

If $F_0 > R_{A1} (V - F_B)$:

$$\left[\langle y_T \rangle_n \right]_{A1} = \frac{V \left[\langle y_{TR} \rangle_n \right]_{A1} + \left\{ \frac{1}{R_{A1}} F_0 - (V - F_B) \right\} \left[y_o \right]_{A1}}{F_B + F_0} \quad (5-13)$$

During Stages IVA and IVB, the concentration of albumin in the fluid at the bottom of the column is calculated by Equations 2-19, 2-20, and 5-9.

$$\begin{aligned}
y_{\text{column}} &= R_{A1} \left[\langle y_{BR} \rangle_n \right]_{A1} \\
\text{@ volume} &= \frac{1}{R_{A1}} V_{\text{initial}} \quad (5-14)
\end{aligned}$$

This fluid leaves the top of the column in Stages IVB and IVC at a concentration which is equal to the inlet value.

$$y_{\text{out}} = \frac{1}{R_{A1}} \left\{ R_{A1} \left[\langle y_{BR} \rangle_n \right]_{A1} \right\} = \left[\langle y_{BR} \rangle_n \right]_{A1} \quad (5-15)$$

Using Equations 5-6, 2-20, 5-9, 5-14, and 5-15, the

concentrations at the end of Stage IV and the beginning of Stage V may be calculated.

$$\begin{aligned} \left[\langle y_{C,V} \rangle_n \right]_{A1} &= \frac{\frac{1}{R_{A1}} F_0 (0) + \left\{ (V - F_0) + F_0 \left(1 - \frac{1}{R_{A1}} \right) \right\}}{V} \\ &\quad \frac{\left\{ R_{A1} \left[\langle y_{BR} \rangle_n \right]_{A1} \right\}}{V} \end{aligned} \quad (5-16)$$

If $F_0 \leq R_{A1} (V - F_B)$:

$$\begin{aligned} \left[\langle y_{TR} \rangle_{n+1} \right]_{A1} &= \frac{(V + V_T - Q_0 t_I) \left[\langle y_{TR} \rangle_n \right]_{A1} + \left\{ R_{A1} (V - F_B) \right. \\ &\quad \left. - F_0 \right\} \left\{ \frac{1}{R_{A1}} \left[\langle y_{TR} \rangle_n \right]_{A1} \right\} + \left\{ R_{A1} F_B \right\}}{V + V_T} \\ &\quad \frac{\left\{ \frac{1}{R_{A1}} \left[y_0 \right]_{A1} \right\} + \left\{ Q_0 t_I - (R_{A1} V - F_0) \right\}}{V + V_T} \\ &\quad \frac{\left[\langle y_{BR} \rangle_n \right]_{A1}}{V + V_T} \end{aligned} \quad (5-17)$$

If $F_0 > R_{A1} (V - F_B)$:

$$\begin{aligned} \left[\langle y_{TR} \rangle_{n+1} \right]_{A1} &= \frac{(V + V_T - Q_0 t_I) \left[\langle y_{TR} \rangle_n \right]_{A1} + \left\{ R_{A1} F_B - \right. \\ &\quad \left. \left[F_0 - R_{A1} (V - F_B) \right] \right\} \left\{ \frac{1}{R_{A1}} \left[y_0 \right]_{A1} \right\} + \left\{ Q_0 t_I \right. \\ &\quad \left. - (R_{A1} V - F_0) \right\} \left[\langle y_{BR} \rangle_n \right]_{A1}}{V + V_T} \end{aligned} \quad (5-18)$$

In Stage V, y_{TR} and y_{BR} stay the same. The concentration in the column at the end of Stage V and the beginning of Stage VI is,

$$[\langle y_{C,VI} \rangle_n]_{A1} = [\langle y_{BR} \rangle_n]_{A1} \quad (5-19)$$

The bottom product is collected in a holding tank. The concentration is derived from Equation 5-16, where $V_B \geq Q_o t_V \geq P_B$. The following equation holds for $R_{A1} V \leq Q_p (t_{IVA} + t_{IVB})$, or for the operating conditions in Table 8 when $R_{A1} \leq 1.5$.

$$[\langle y_B \rangle_n]_{A1} = \frac{(R_{A1} V - F_o) [\langle y_{BR} \rangle_n]_{A1} + (Q_o t_V - V) [\langle y_{BR} \rangle_n]_{A1}}{Q_o t_V} \quad (5-20)$$

A volume of bottom product P_B is taken from the holding tank, and the remainder of the fluid is returned to the bottom reservoir. In Stage VI, y_{TR} is constant and at the end of Stage VI,

$$[\langle y_{BR}^* \rangle_{n+1}]_{A1} = \frac{(V_B - Q_o t_V) [\langle y_{BR} \rangle_n]_{A1} + (Q_o t_V - F_T)}{V_B} \\ \frac{[\langle y_B \rangle_n]_{A1} + F_T [\langle y_{BR} \rangle_n]_{A1}}{V_B} \quad (5-21)$$

Two concentration zones are present in the column at the end of Stage VI. From the overall mass balance and Equation 5-19, the average value is,

$$[\langle y_{C,VI}^* \rangle_n]_{A1} = \frac{F_T [y_o]_{A1} + (V - F_T) [\langle y_{BR} \rangle_n]_{A1}}{V} \quad (5-22)$$

This completes the n'th cycle of operation. Substituting Equations 5-1, 5-21, and 5-22 into Equation 5-4,

$$\begin{aligned}
 [\langle y_{BR} \rangle_{n+1}]_{A1} &= \frac{(V + V_B - Q_o t_V) [\langle y_{BR} \rangle_n]_{A1} + (Q_o t_V - F_T)}{Q_o t_I + V_B} \\
 &\quad \frac{[\langle y_B \rangle_n]_{A1} + F_T [y_o]_{A1} + (Q_o t_I - V)}{Q_o t_I + V_B} \\
 &\quad \frac{[\langle y_{TR} \rangle_{n+1}]_{A1}}{Q_o t_I + V_V} \tag{5-23}
 \end{aligned}$$

The albumin separation factors for Region 1 of Mode 7 are calculated from Equations 2-29, 5-2, 5-13 (or 5-12), 5-18 (or 5-17), 5-20, and 5-23.

.....Mode 7 - Region 2.....

If $F_B > V$ as in both Regions 2 and 3, the calculations for the top product concentration are slightly changed.

Equations 5-5 to 5-7 become,

$$[\langle y_{C,IV} \rangle_n]_{A1} = [y_o]_{A1} \tag{5-24}$$

$$[\langle y_{T,III} \rangle_n]_{A1} = \frac{V [\langle y_{TR} \rangle_n]_{A1} + (F_B - V) [y_o]_{A1}}{F_B} \tag{5-25}$$

Equations 5-10 and 5-11 become,

$$[\langle y_{T,IVA} \rangle_n]_{A1} = \frac{1}{R_{A1}} [y_o]_{A1} \tag{5-26}$$

Thus, $[\langle y_T \rangle_n]_{A1}$ can be calculated from Equations 5-8, 5-25, and 5-26. The resulting equation is identical to 5-13, so the final equations are identical to those for Region 1.

.....Mode 7 - Regions 3 & 4.....

If $F_T > V$ as in both Regions 3 and 4, the calculations for Stage VI are slightly changed. Equations 5-21 and 5-22 become,

$$\begin{aligned}
 [\langle y_{BR}^* \rangle_{n+1}]_{A1} &= \frac{(V_B - Q_o t_V) [\langle y_{BR} \rangle_n]_{A1} + (Q_o t_V - F_T)}{V_B} \\
 &\quad \frac{[\langle y_B \rangle_n]_{A1} + V [\langle y_{BR} \rangle_n]_{A1} + (F_T - V)}{V_B} \\
 &\quad \frac{[y_o]_{A1}}{V_B} \tag{5-27}
 \end{aligned}$$

$$[\langle y_{C,VI}^* \rangle_n]_{A1} = [y_o]_{A1} \tag{5-28}$$

Thus, $[\langle y_{BR} \rangle_{n+1}]_{A1}$ can be calculated from Equations 5-1, 5-4, 5-27, and 5-28. The resulting equation is identical to 5-23, so the final equations in this case are also identical to those for Region 1.

.....Mode 6.....

Mode 6 is identical to Mode 7 except for the operation of Stages V and VI. Equations 5-19 and 5-21 become,

$$\begin{aligned}
 [\langle y_{C,VI} \rangle_n]_{A1} &= [\langle y_{BR}^* \rangle_{n+1}]_{A1} \\
 &= \frac{(R_{A1} V - F_o) [\langle y_{BR} \rangle_n]_{A1} + V_B [\langle y_{BR} \rangle_n]_{A1}}{V + V_B} \tag{5-29}
 \end{aligned}$$

Equations 5-20, 5-22, and 5-23 are changed as follows,

If $F_T \leq V$:

$$[\langle y_B \rangle_n]_{A1} = [\langle y_{BR}^* \rangle_{n+1}]_{A1} \tag{5-30}$$

$$[\langle y_{C,VI}^* \rangle_n]_{A1} = \frac{F_T [y_o]_{A1} + (V - F_T) [\langle y_{BR}^* \rangle_{n+1}]_{A1}}{V} \tag{5-31}$$

$$\begin{aligned}
 [\langle y_{BR} \rangle_{n+1}]_{A1} &= \frac{(V + V_B - F_T) [\langle y_{BR}^* \rangle_{n+1}]_{A1} + F_T [y_o]_{A1}}{Q_o t_I + V_B} \\
 &\quad + \frac{(Q_o t_I - V) [\langle y_{TR} \rangle_{n+1}]_{A1}}{Q_o t_I + V_B} \quad (5-32)
 \end{aligned}$$

If $F_T > V$:

$$[\langle y_B \rangle_n]_{A1} = \frac{V [\langle y_{BR}^* \rangle_{n+1}]_{A1} + (F_T - V) [y_o]_{A1}}{F_T} \quad (5-33)$$

$$[\langle y_{C,VI}^* \rangle_n]_{A1} = [y_o]_{A1} \quad (5-34)$$

$$\begin{aligned}
 [\langle y_{BR} \rangle_{n+1}]_{A1} &= \frac{V_B [\langle y_{BR}^* \rangle_{n+1}]_{A1} + V [y_o]_{A1} + (Q_o t_I - V)}{Q_o t_I + V_B} \\
 &\quad \frac{[\langle y_{TR} \rangle_{n+1}]_{A1}}{Q_o t_I + V_B} \quad (5-35)
 \end{aligned}$$

The albumin separation factors for Regions 1 and 2 of Mode 6 are calculated from Equations 2-29, 5-2, 5-13, 5-18, 5-29, 5-30, and 5-32. The albumin separation factors for Regions 3 and 4 of Mode 6 are calculated from Equations 2-29, 5-2, 5-13, 5-18, 5-29, 5-33, and 5-35. Equations 5-12 and 5-17 are also needed for Regions 1 and 4.

.....Mode 8.....

Mode 8 is identical to Mode 7 except that the fluid flows in the opposite direction in Stage V. As shown in Figure 40, dead volume solution from the bottom reservoir enters the bottom of the column in Stage V, and the fluid in the column is pumped out of the top of the column and mixed in the holding tank. The equations for the albumin separation are identical for Modes 7 and 8. The various flow directions

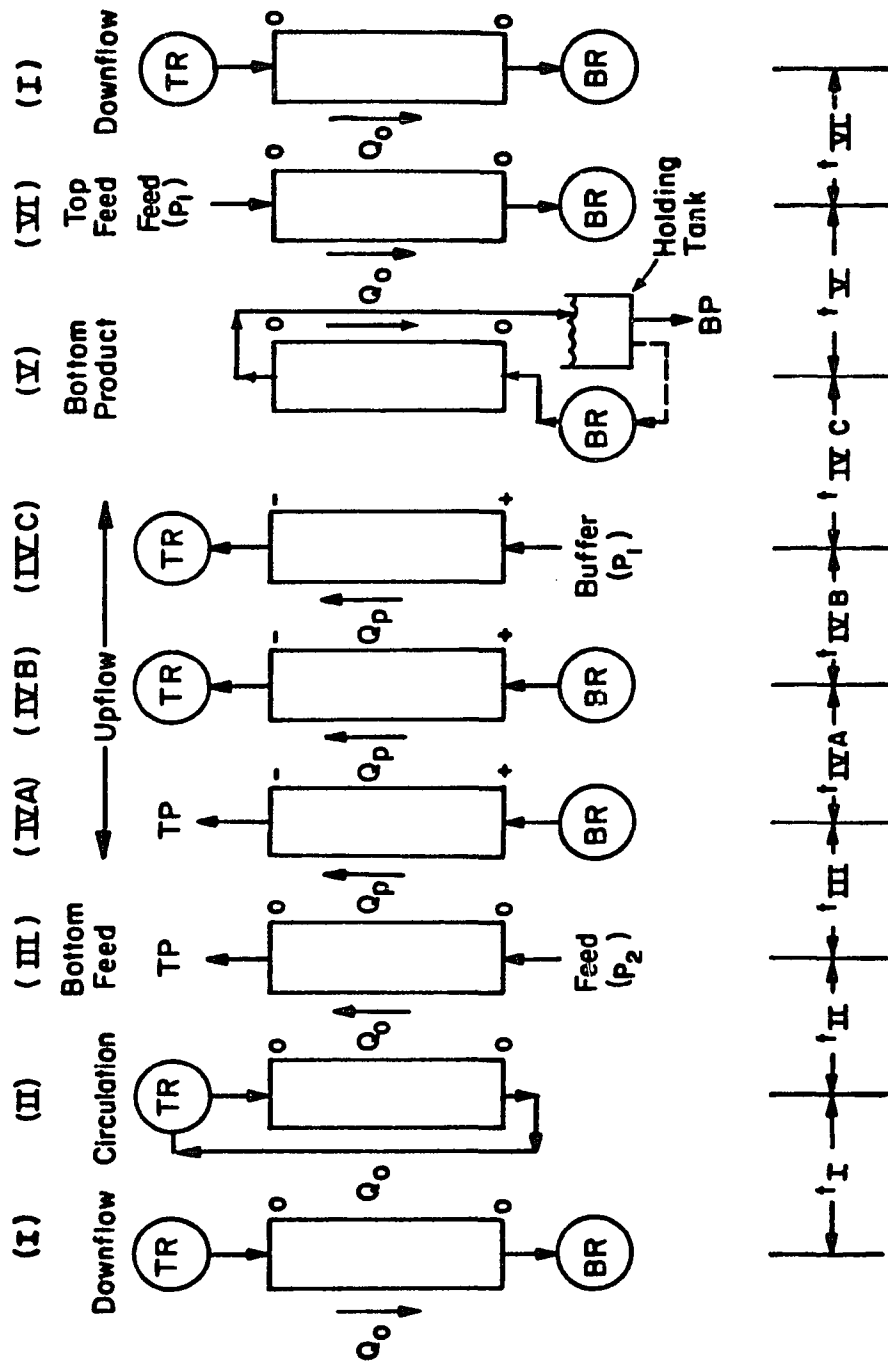


FIGURE 40. Process Diagram for the pH Parapump with Electric Field (Mode 8)

are incorporated into the parapumping computer program for the hemoglobin separation. Mode 8 has some experimental advantages over Mode 7 which will be discussed later in this chapter.

.....Mode 9.....

Mode 9 (Figure 41) is identical to Mode 7 except for the operation of Stage IV. The buffer solution with volume F_0 is mixed with the fluid in the bottom reservoir at the beginning of Stage IV rather than being added directly to the column in Stage IVC. The new concentration in the bottom reservoir is,

$$[\langle y_{BR}' \rangle_n]_{A1} = \frac{F_0 (0) + (Q_0 t_I + V_B) [\langle y_{BR} \rangle_n]_{A1}}{Q_0 t_I + V_B + F_0} \quad (5-36)$$

Equations 5-1 to 5-15 are unchanged. Equations 5-16, 5-19, and 5-20 become,

$$[\langle y_{C,V} \rangle_n]_{A1} = R_{A1} [\langle y_{BR}' \rangle_n]_{A1} \quad (5-37)$$

$$[\langle y_{C,VI} \rangle_n]_{A1} = [\langle y_{BR}' \rangle_n]_{A1} \quad (5-38)$$

$$[\langle y_B \rangle_n]_{A1} = \frac{(R_{A1} V + Q_0 t_V - V)}{Q_0 t_V} [\langle y_{BR}' \rangle_n]_{A1} \quad (5-39)$$

Equations 5-17, 5-18, 5-21, 5-22, and 5-23 are the same for Mode 9 except $[\langle y_{BR} \rangle_n]_{A1}$ is replaced by $[\langle y_{BR}' \rangle_n]_{A1}$. The albumin separation factors for Mode 9 are calculated from Equations 2-29, 5-2, 5-13 (or 5-12), 5-36, 5-39, plus the revised forms of Equations 5-18 (or 5-17), and 5-23.

.....Mode 10.....

Mode 10 is shown in Figure 42. The operation is similar

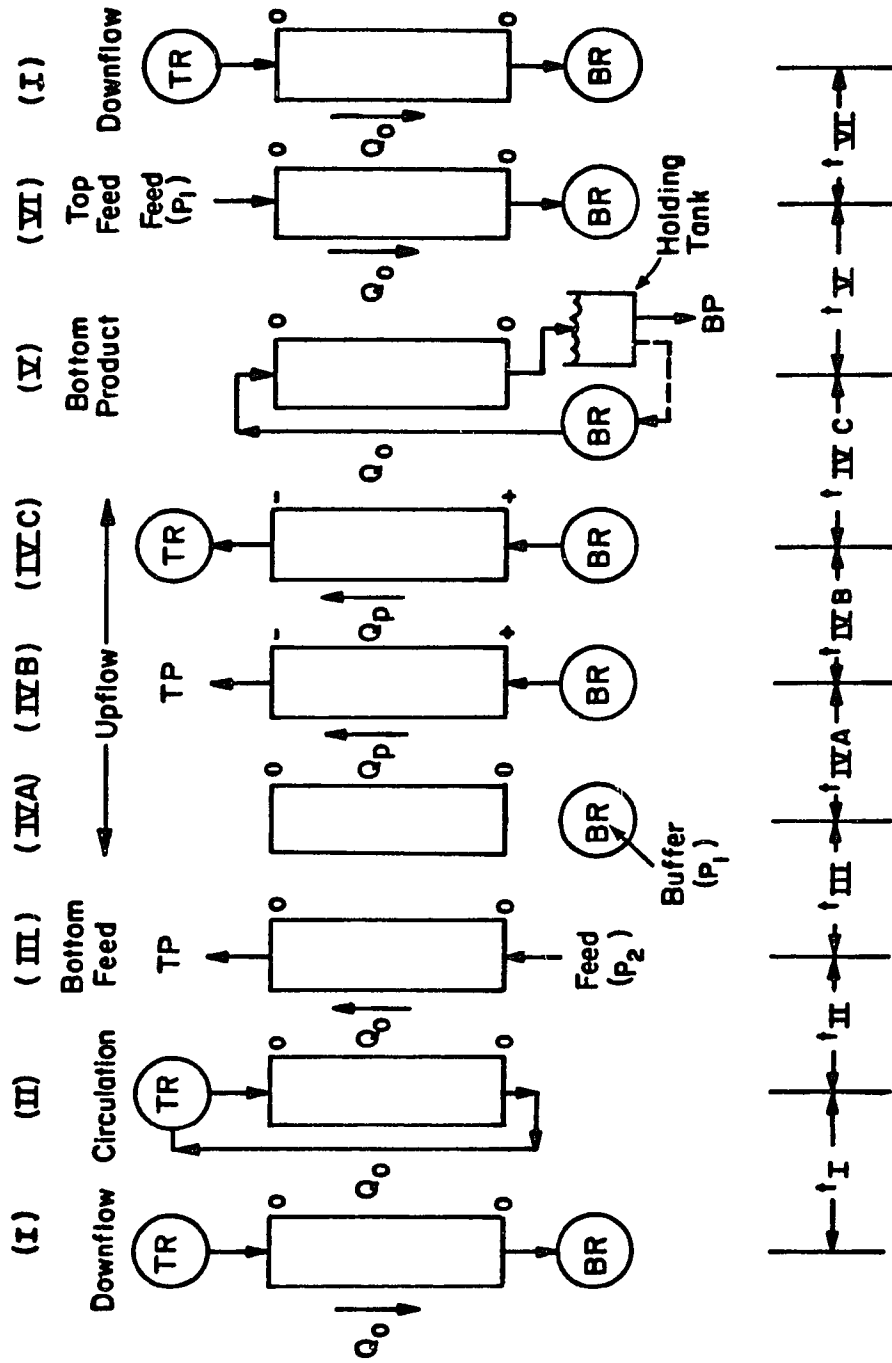


FIGURE 41. Process Diagram for the pH Parapump with Electric Field (Mode 9)

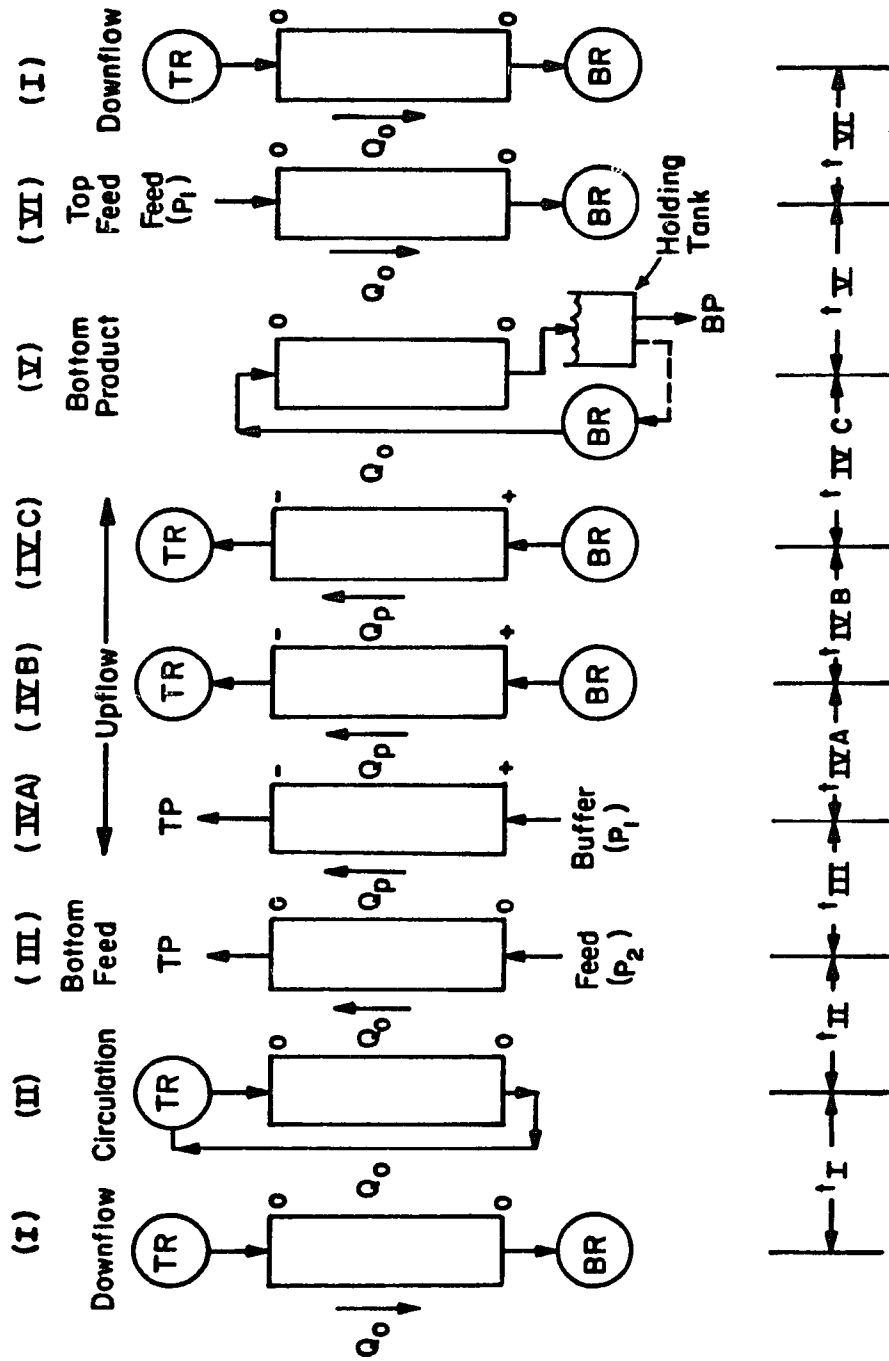


FIGURE 42. Process Diagram for the pH Parapump with Electric Field (Mode 10)

to Mode 7 except that the buffer is added in Stage IVA instead of Stage IVC. Equations 5-1 to 5-15, 5-19, and 5-21 to 5-23 are unchanged. Equations 5-16, 5-17, 5-18, and 5-20 become,

$$[\langle y_{C,V} \rangle_n]_{A1} = R_{A1} [\langle y_{BR} \rangle_n]_{A1} \quad (5-40)$$

If $F_0 \leq R_{A1} (V - F_B)$:

$$[\langle y_{TR} \rangle_{n+1}]_{A1} = \frac{(2V + V_T - Q_0 t_I - F_B - \frac{F_0}{R_{A1}}) [\langle y_{TR} \rangle_n]_{A1}}{V + V_T} + \frac{F_B [y_0]_{A1} + (Q_0 t_I - R_{A1} V) [\langle y_{BR} \rangle_n]_{A1}}{V + V_T} \quad (5-41)$$

If $F_0 > R_{A1} (V - F_B)$:

$$[\langle y_{TR} \rangle_{n+1}]_{A1} = \frac{(V + V_T - Q_0 t_I) [\langle y_{TR} \rangle_n]_{A1} + (V - \frac{F_0}{R_{A1}}) [y_0]_{A1} + (Q_0 t_I - R_{A1} V) [\langle y_{BR} \rangle_n]_{A1}}{V + V_T} \quad (5-42)$$

$$[\langle y_B \rangle_n]_{A1} = \frac{(R_{A1} V + Q_0 t_V - V) [\langle y_{BR} \rangle_n]_{A1}}{Q_0 t_V} \quad (5-43)$$

The albumin separation factors for Mode 10 are calculated from Equations 2-29, 5-2, 5-13 (or 5-12), 5-42 (or 5-41), 5-43, and 5-23. All of the equations for Modes 6 to 10 are included in Program #3 in Appendix C.

The equations above were used to calculate the experimental data for Runs 45, 54, 55, and 56 which were plotted in

Figure 38B. As seen in Figure 43, the agreement between the mathematical model and the experimental data is quite good. All three runs via the new process (Runs 54-56) show improved separation compared to the base case (Run 45 - no power). At comparable values of F_B and F_T (Runs 54 and 56), Mode 7 gives better albumin separation than Mode 6. The separation in Mode 7 is further increased by increasing the value of F_B relative to F_T (Runs 55 and 56).

An albumin migration velocity of $\nu_{E,A1} = -0.023$ cm/min was used in the calculations. This value was determined experimentally from Runs 5 and 6 (Figure 29 and Table 7). As shown in Figure 44, the albumin migration velocity must be included in the calculations in order to accurately predict the experimental data. A simple mass balance based on $\nu_{E,A1} = 0$ overpredicts the steady-state albumin separation in Runs 55A and 55C by 61% (5.43 vs. 3.37).

Figure 45 compares Mode 6 and Mode 7 operation. The main difference between Modes 6 and 7 is in the operation of Stages V and VI. As seen in Figure 45, the albumin separation is better in Mode 7, but Mode 6 separation will approach Mode 7 as the dead volume of the bottom reservoir in Mode 6 becomes small. This can be explained by comparing the equations for the bottom product in Modes 6 and 7. Rewriting Equations 5-20, 5-29, and 5-30,

For Mode 7:

$$[\langle y_B \rangle_\infty]_{A1} = \frac{(R_{A1} V - F_0) + (Q_0 t_V - V)}{V + (Q_0 t_V - V)} [\langle y_{BR} \rangle_\infty]_{A1} \quad (5-44)$$

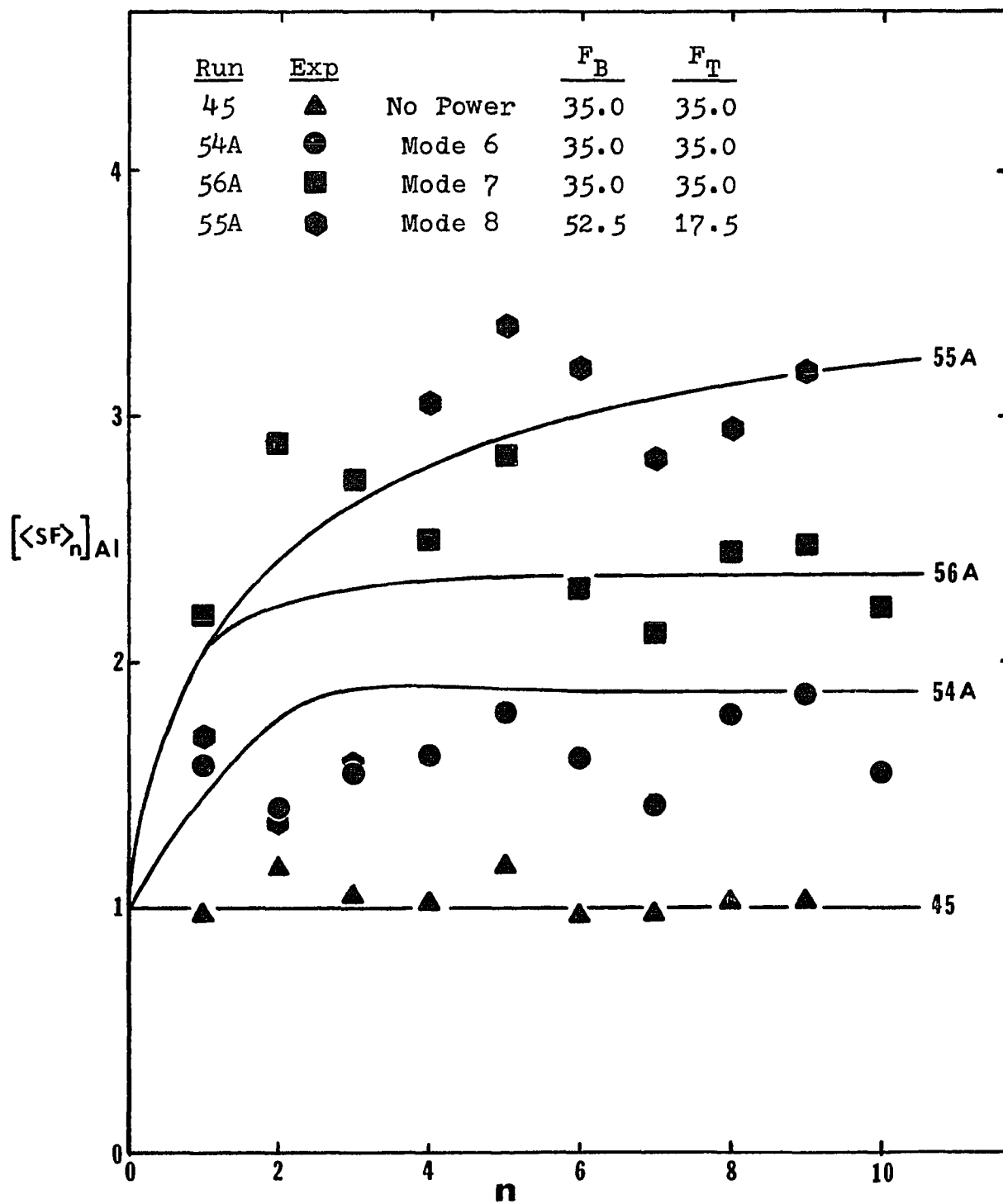


FIGURE 43. Calculated vs. Experimental Albumin Separation for the New Process

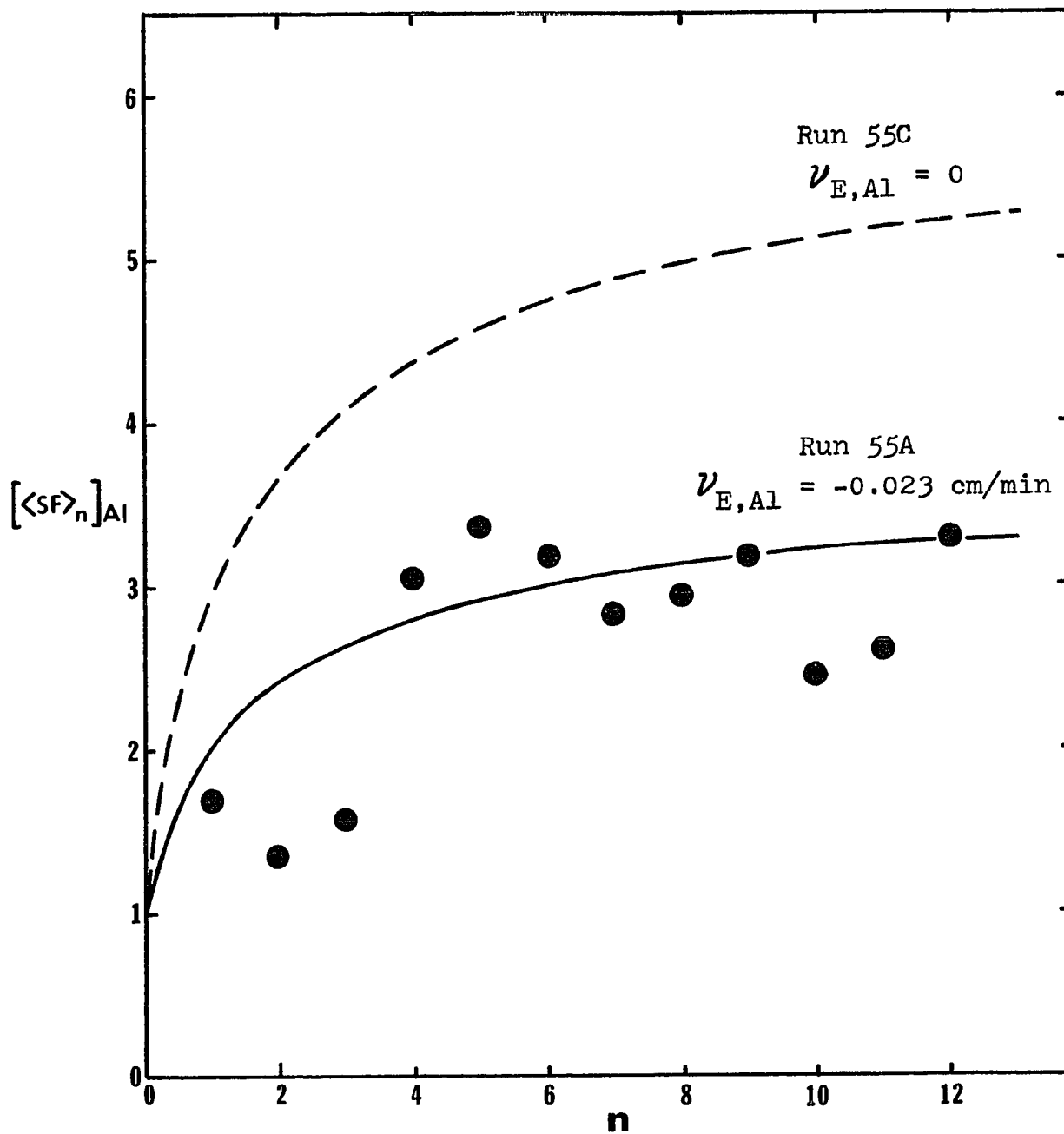


FIGURE 44. Effect of Albumin Migration Velocity on Calculated Results

$$F_B = F_T = 35, F_O = 40$$

Run	Mode	Exp	V_T	V_B
54C	6	●	82.5	60
56A	7	■	112.5	90
54F	6		22.5	60
54D	6		22.5	15
54E	6		112.5	15

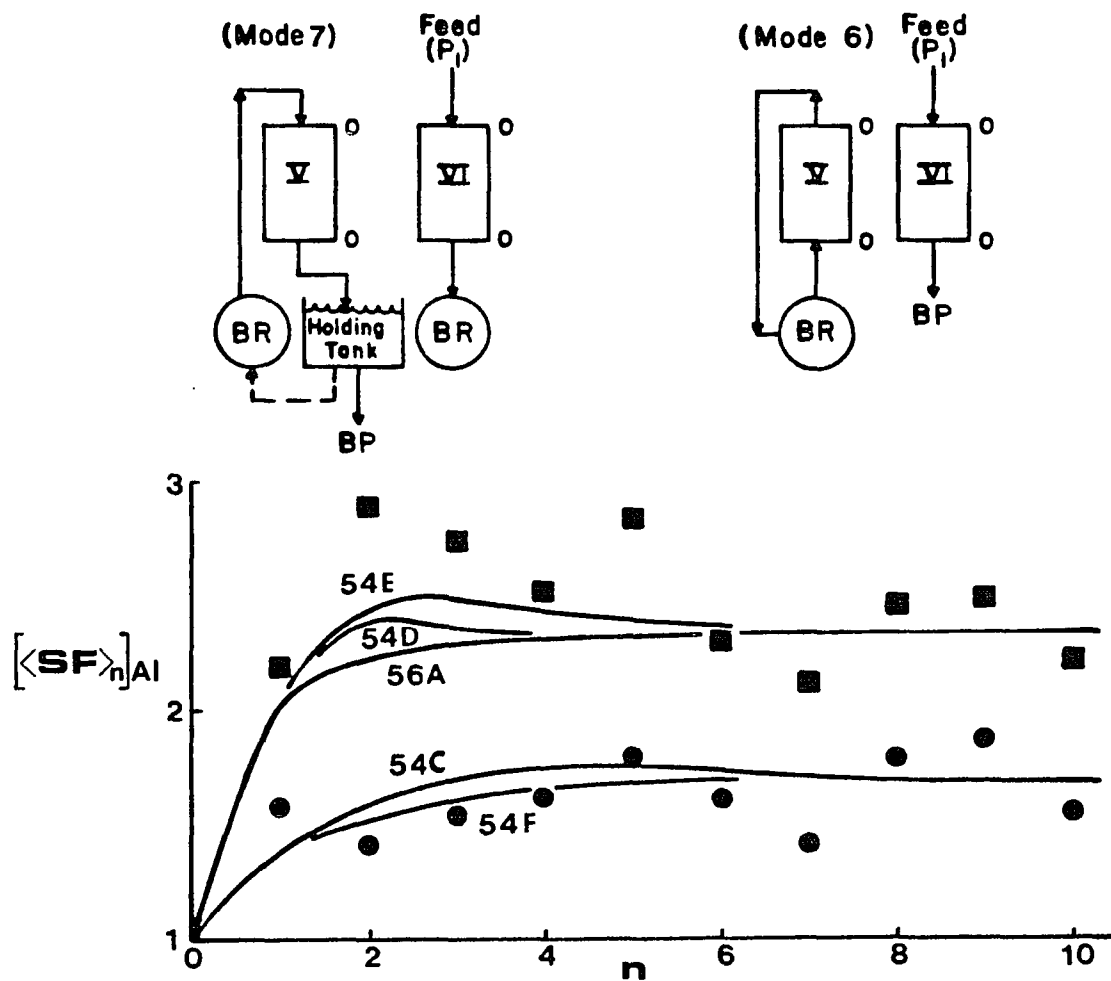


FIGURE 45. Calculated Effect of the Reservoir Dead Volumes on Albumin Separation (Mode 6)

For Mode 6:

$$[\langle y_B \rangle_{\infty}]_{A1} = \frac{(R_{A1}V - F_0) + V_B}{V + V_B} [\langle y_{BR} \rangle_{\infty}]_{A1} \quad (5-45)$$

For Mode 7 in Figure 45, $(Q_0 t_V - V) = 15$ cc. Comparing Equations 5-44 and 5-45 when $R_{A1} \geq 1$ and $V_B \geq Q_0 t_V - V$,

$$[\langle y_B \rangle_{\infty}]_{A1, \text{ Mode 7}} \leq [\langle y_B \rangle_{\infty}]_{A1, \text{ Mode 6}} \quad (5-46)$$

From Figure 45 and Equations 5-45, 5-12, and 5-13, note that the top reservoir dead volume V_T does not affect the separation in Mode 6. Also from Equations 5-44, 5-12, and 5-13 and from Runs 56A and 56C in Appendix C, neither the top nor the bottom reservoir dead volume affects the separation in Mode 7.

The concentration of albumin in both the bottom and the top products in the new process is less than the feed concentration, as determined from the mass balance at steady state.

$$[\langle y_T \rangle_{\infty}]_{A1} = \frac{(F_B + F_T)[y_0]_{A1} - F_T[\langle y_B \rangle_{\infty}]_{A1}}{F_B + F_0} \quad (5-47)$$

From Equations 5-46 and 5-47,

$$[\langle y_T \rangle_{\infty}]_{A1, \text{ Mode 7}} \geq [\langle y_T \rangle_{\infty}]_{A1, \text{ Mode 6}} \quad (5-48)$$

Therefore, from Equations 2-29, 5-46, and 5-48,

$$[\langle SF \rangle_{\infty}]_{A1, \text{ Mode 7}} \geq [\langle SF \rangle_{\infty}]_{A1, \text{ Mode 6}} \quad (5-49)$$

The albumin separation in Mode 7 will always be superior to the separation in Mode 6 when $(V_B)_{\text{Mode 6}} > (Q_0 t_V - V)_{\text{Mode 7}}$. The dead volume of the bottom reservoir V_B must be finite in order to accomplish the circulation step or Stage V. In order to maximize the albumin separation in the new process, the parapump must be operated with $[\langle y_T \rangle_{\infty}]_{A1}$ as close to $[y_0]$

and $[\langle y_B \rangle_\infty]_{A1}$ as close to zero as possible. This condition will also maximize the weight fraction of hemoglobin in the bottom product and, thus, the overall separation factor α . The experimental data and mathematical calculations are aimed at determining the best operating procedure and the experimental conditions which maximize this separation. The case where the quantity $(Q_0 t_V - V) = 0$ will be considered later in this chapter as Mode 8.

Figure 46 examines the effect of various feed volumes on the albumin separation in Mode 7. The following comparisons are based on Figure 46:

- (1) Runs 56 & 59 - When $F_B = F_T$, the albumin separation increases as the two feed volumes decrease.
- (2) Runs 55 & 59 - At constant bottom feed F_B , the separation increases as the value of F_T decreases.
- (3) Runs 56 & 62 - At constant top feed F_T , the separation increases as the value of F_B increases.
- (4) Runs 55 & 62 - If the ratio F_B/F_T is constant, the separation increases as the total feed $(F_B + F_T)$ decreases.
- (5) Runs 55, 56 & 61 - At a constant value of the total feed $(F_B + F_T)$, the separation increases as the ratio F_B/F_T increases.

The maximum albumin separation in Figure 46 was obtained when $F_B > V$ and $F_T \ll V$, which corresponds to Region 2 of operation. The five statements above suggest that some optimum values of F_B and F_T exist. Computer Runs 66-69 were made in order to search for the feed volumes which yield the maximum separation. The results of this optimization are analyzed in Figures 47-52.

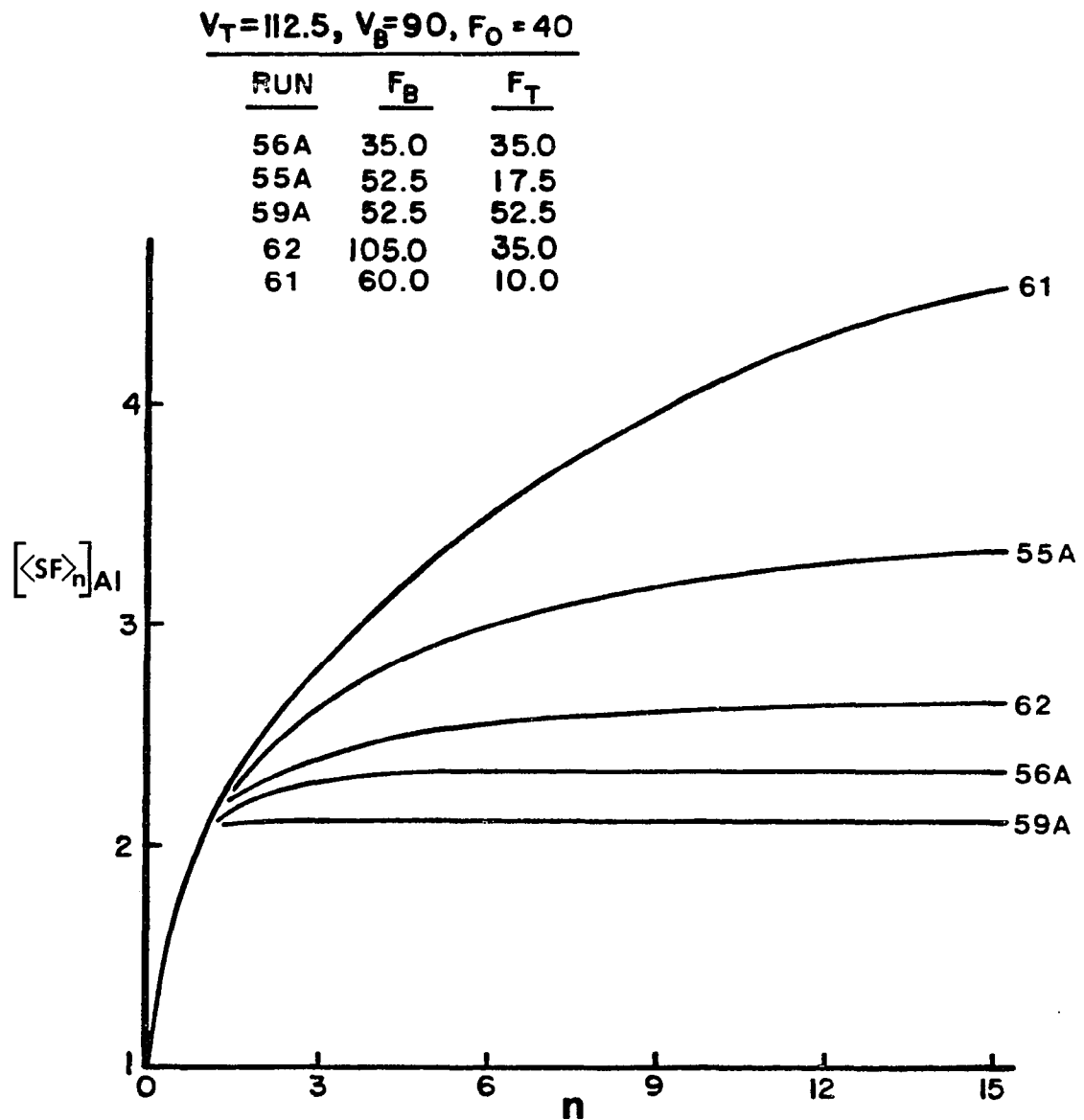


FIGURE 46. Calculated Effect of Feed Volumes on Albumin Separation in the New Process (Mode 7)

The separation factors SF_{Hb} , SF_{Al} , and α are defined by Equations 2-28, 2-29, and 2-30, respectively. The optimizations with respect to both hemoglobin and albumin are shown in Figures 47-52. The calculations for the hemoglobin separation will be explained in the next chapter. Since very little computer time is required to calculate the albumin separation, a large number of simulated experiments were run on the computer in order to generate the albumin curves in Figures 47 and 50. The major computer time requirement centers on the hemoglobin calculations, so only a limited number of points were calculated in generating the curves in the other four figures.

The albumin separation increases as ψ increases as seen in Figure 47, with the greatest effect observed in the region $F_T < 0.5V$ and $F_B > 0.5V$. The concentration ratio ψ is defined by Equation 4-11. Note that the ratio F_B/F_T discussed above equals $\psi-1$. Albumin which is fed to the top of the column in Stage VI (F_T) of the n 'th cycle ends up in the bottom reservoir at the end of Stage I of the $n+1$ 'th cycle. As F_T becomes large, $[\langle y_B \rangle_\infty]_{Al}$ approaches the feed value $[y_o]_{Al}$ which tends to decrease the albumin separation factor. On the other hand, albumin which is fed to the bottom of the column in Stage III (F_B) of the n 'th cycle ends up in either the top product of the n 'th cycle or the top reservoir of the $n+1$ 'th cycle. As F_B becomes large, $[\langle y_T \rangle_\infty]_{Al}$ approaches the feed value $[y_o]_{Al}$ which tends to increase the albumin separation factor. Since F_B and F_T are both finite and

both increase along the ψ curves, the albumin separation factor has the possibility of either increasing or decreasing as the feed volumes increase. As shown in Figures 47 and 50, the albumin separation factor goes through a maximum as F_T , F_B , or total feed ($F_T + F_B$) increases, and this maximum is more pronounced at greater values of ψ . If both F_B and F_T are very large, i.e., $F_B \gg F_0$ and $F_T \gg F_0$, the albumin separation factor decreases to 1.0.

Both the hemoglobin separation and the overall separation increase with ψ at low values of F_T , but at higher values of F_T these separations decrease as ψ increases (Figures 48 and 49). When $\psi > 2$ or $F_B/F_T > 1$, F_B is greater than F_T . Thus, if F_T is large F_B is very very large. Hemoglobin is adsorbed by the ion exchanger at low values of F_B , but if F_B becomes too large the hemoglobin from the feed breaks through the packed bed to the top product streams. This breakthrough decreases the hemoglobin separation as well as the overall separation.

The hemoglobin separation is expected to be a maximum for the batch process or when $F_B=0$ and $F_T=0$. The maximum hemoglobin separation in the new process, however, occurs at small but finite values of F_B and F_T . Since F_0 is finite in these calculations, an analogous batch case for the new process does not actually exist.

In practice, it is desirable to optimize both the overall separation and the production rate where,

$$\frac{\text{PRODUCTION RATE}}{\text{(mass/time)}} = \frac{\text{FEED RATE}}{\text{(mass/time)}} \times \text{RECOVERY} \quad (5-50)$$

Figures 50-52 examine these three variables -- separation, feed rate, and recovery. The recovery functions δ and ξ are defined as follows:

$$\text{ACCUMULATION} = \text{MASS IN} - \text{MASS OUT} \quad (5-51)$$

$$\begin{aligned} \delta_n &= \frac{\text{Mass Out in the } n\text{'th Cycle}}{\text{Mass In in the } n\text{'th Cycle}} \\ &= \frac{(F_B + F_O)[\langle y_T \rangle_n] + (F_T)[\langle y_B \rangle_n]}{(F_B + F_T)[y_O]} \end{aligned} \quad (5-52)$$

Note that during the initial transient period of a para-pumping experiment, the accumulation term may be positive or negative, but at steady-state accumulation equals zero or $\delta_\infty = 1.0$. Recovery for both the transient and the steady-state periods may be defined by the ξ function. However, the mathematical model must be reliable in order for the ξ function to be accurate.

$$[\langle \xi \rangle_n] = \frac{\sum_{n=1}^n \delta(\text{experimental})}{\sum_{n=1}^n \delta(\text{calculated})} \quad (5-53)$$

The maximum separation in Figures 50-52 occurs at low feed rate or $F_B + F_T < V$, but the protein recovery was very low for the one experimental run in that region. If the feed rate is too low, the residence time for the protein in the para-pumping system becomes large and the recovery drops. The protein recovery is excellent in the range $V < F_B + F_T < 2V$, and the separation is still relatively high. The separation drops off drastically for $F_B + F_T > 2V$. The experimental recovery dropped slightly at the high feed rate, but the

reason for this drop is not clear. The optimum feed volumes based on Figures 47-52 appears to be $V < F_B + F_T < 2V$ with $F_T < 0.5V$ and $F_B > 0.5V$. In this region, the overall separation may be increased from sixty to any desired level by increasing the ratio F_B/F_T to the appropriate level.

Figure 53 compares Mode 7, Mode 9, and Mode 10 operation. These modes were shown in detail in Figures 31, 41, and 42, respectively. The only difference between these modes is in the method of buffer addition to make-up the mass balance in Stage IV. The buffer is mixed with the fluid in the bottom reservoir in Mode 9. The buffer is added at the beginning of Stage IV in Mode 10 and at the end of Stage IV in Mode 7. As shown in Figure 53, the albumin separation is much better when the buffer is added at the end of Stage IV as in Mode 7.

Figure 54 compares Mode 7 and Mode 8 operation. From Equation 5-44, the albumin separation can be maximized in Mode 7 if $Q_0 t_V - V = 0$. Consider Mode 7 in more detail (Figure 31). At the end of Stage IV, the hemoglobin is concentrated at the top of the column in fluid with $P_2 < pH < P_1$. The fluid in the remainder of the column is at $pH = P_1$. The fluid in the column is pumped downward in Stage V of Mode 7. Since the pH wave velocity is slower than the bulk velocity, the displacement in Stage V must be greater than one void volume or $Q_0 t_V > V$ in order to push the hemoglobin concentration wave out of the bottom of the column. If the fluid is pumped upward in Stage V as indicated in Mode 8 (Figure 40),

the hemoglobin concentration wave has a shorter distance to travel and exits the column in $Q_o t_V = V$. As shown in Figure 54, Mode 8 operation is superior to Mode 7. Mode 8 was predicted mathematically and verified experimentally -- only one experimental run was made via this mode.

If the displacements in Mode 8 are optimized, the separation can be further improved as indicated in Figure 55. The data in Figure 32 suggested that $Q_o t_I$ could be reduced from 67.5 cc to 60.0 cc. Keeping everything else in Run 60, Mode 8 constant, the buffer volume F_o can be increased from 40.0 cc to 47.5 cc based on the relationship between $Q_o t_I$ and $Q_p t_{IV}$. This case is simulated in Run 63. The hemoglobin separation is unchanged from Run 60A to Run 63 (Appendix C), but the albumin separation and the overall separation are drastically improved. The overall separation is increased from 100 to 260 as shown in Figure 55. This is ten times better than the base case for $F_B = F_T = 35.0$ cc. The feed volumes lie in the optimum range determined from Figures 47-52, and as indicated above the separation can be further improved by increasing ψ .

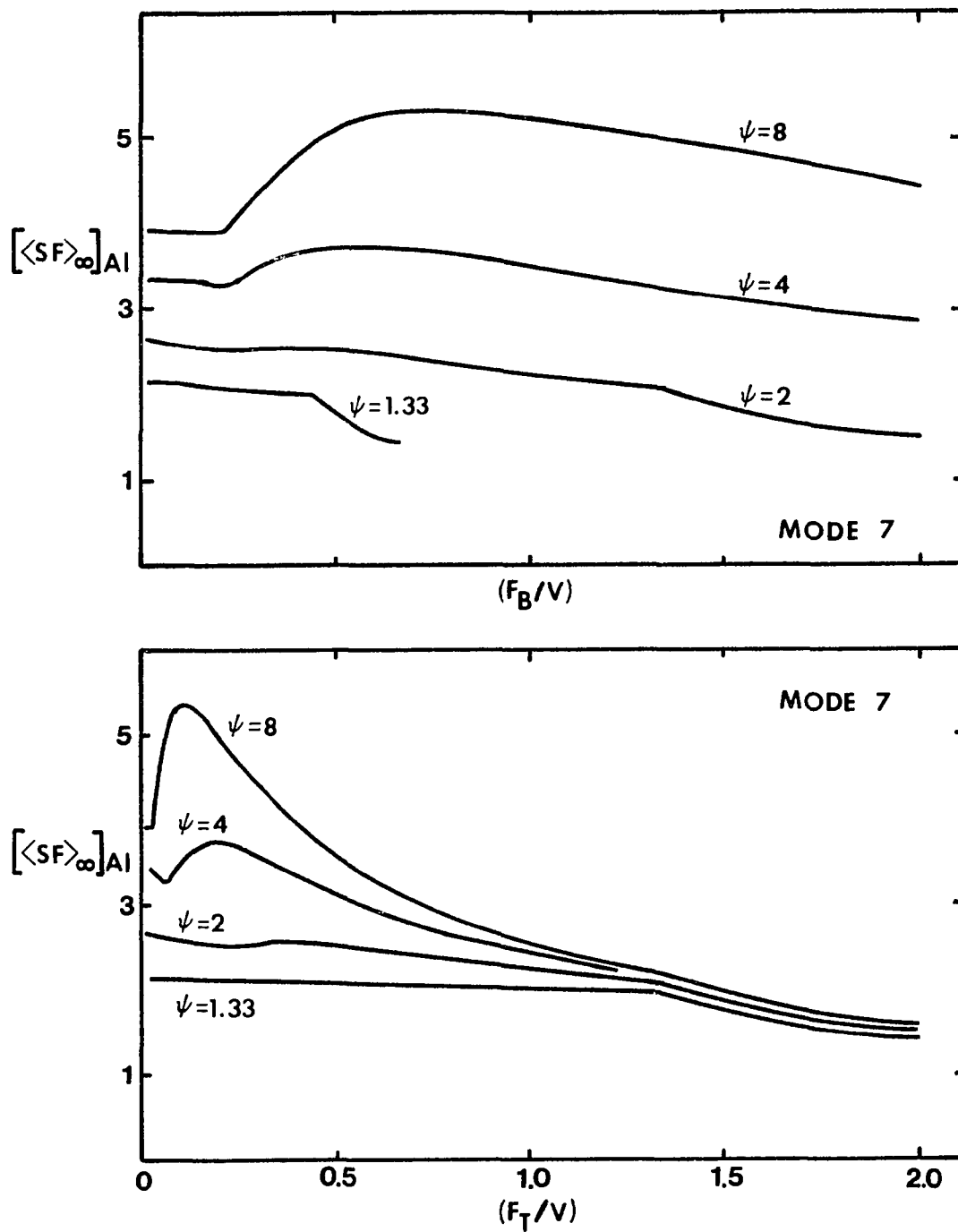


FIGURE 47. Albumin Separation as a Function of Top and Bottom Feed Rates (Runs 66B-69B)

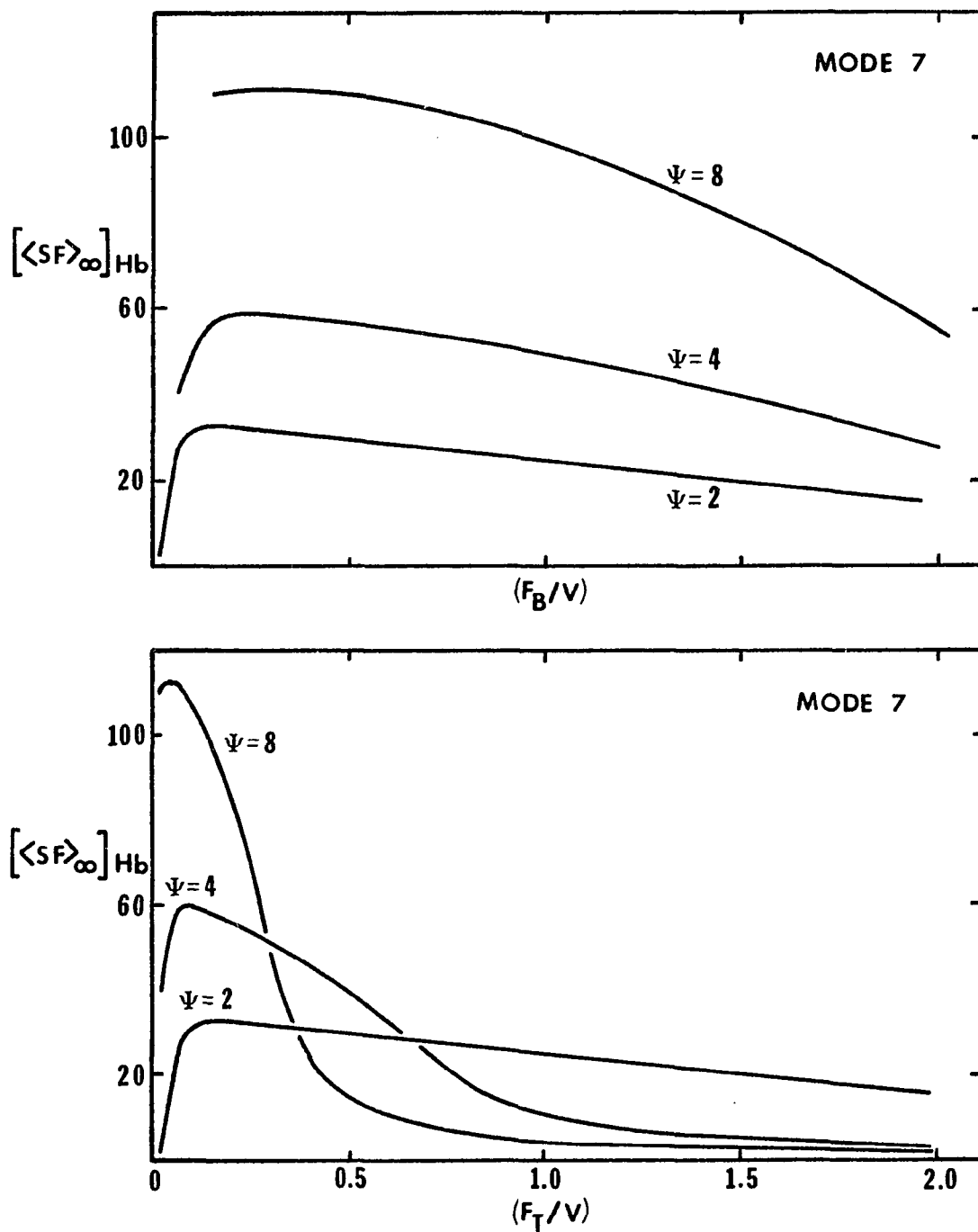


FIGURE 48. Hemoglobin Separation as a Function of Top and Bottom Feed Rates (Runs 66A-69A)

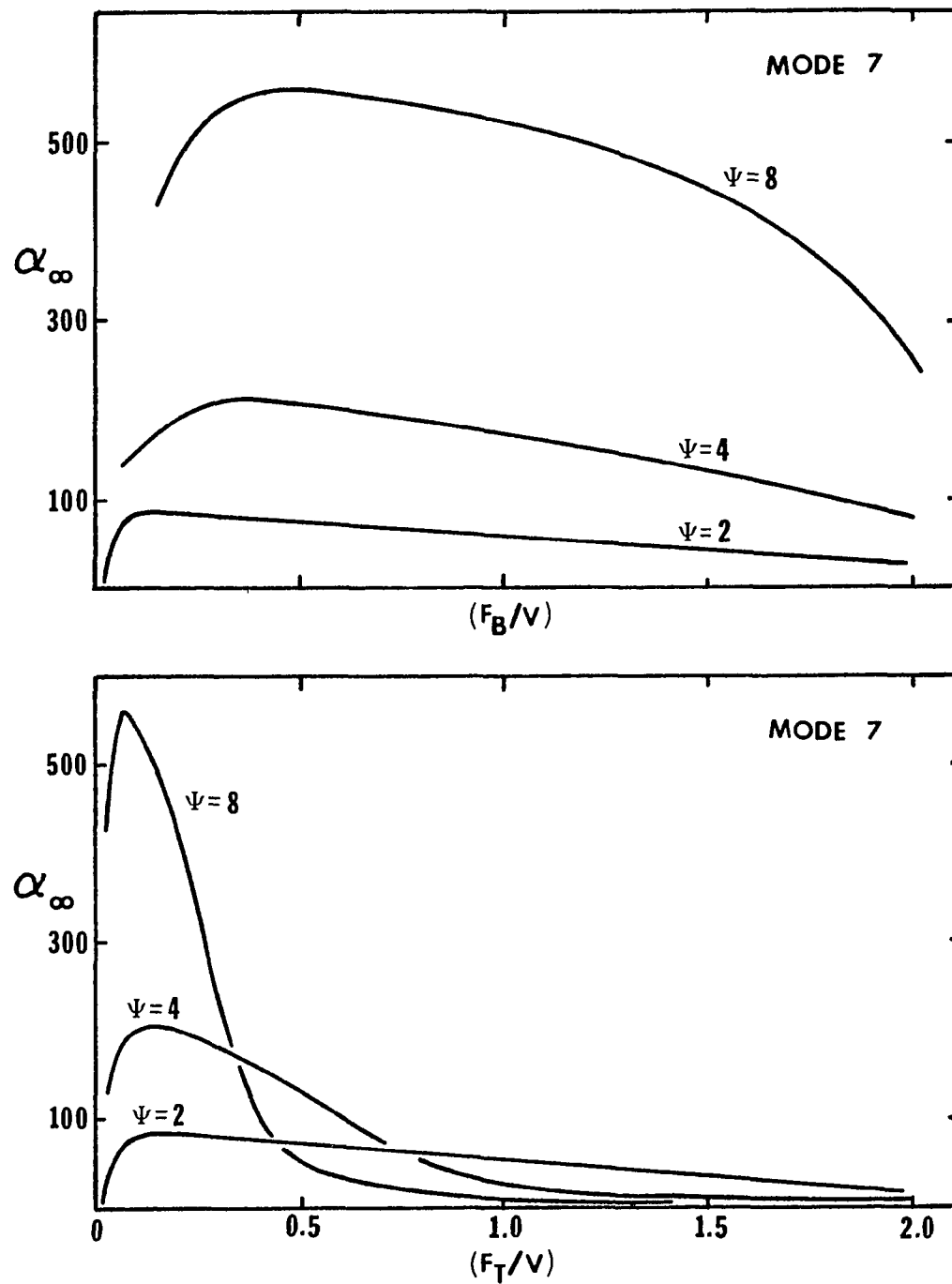


FIGURE 49. Overall Separation as a Function of Top and Bottom Feed Rates (Runs 66A-69A)

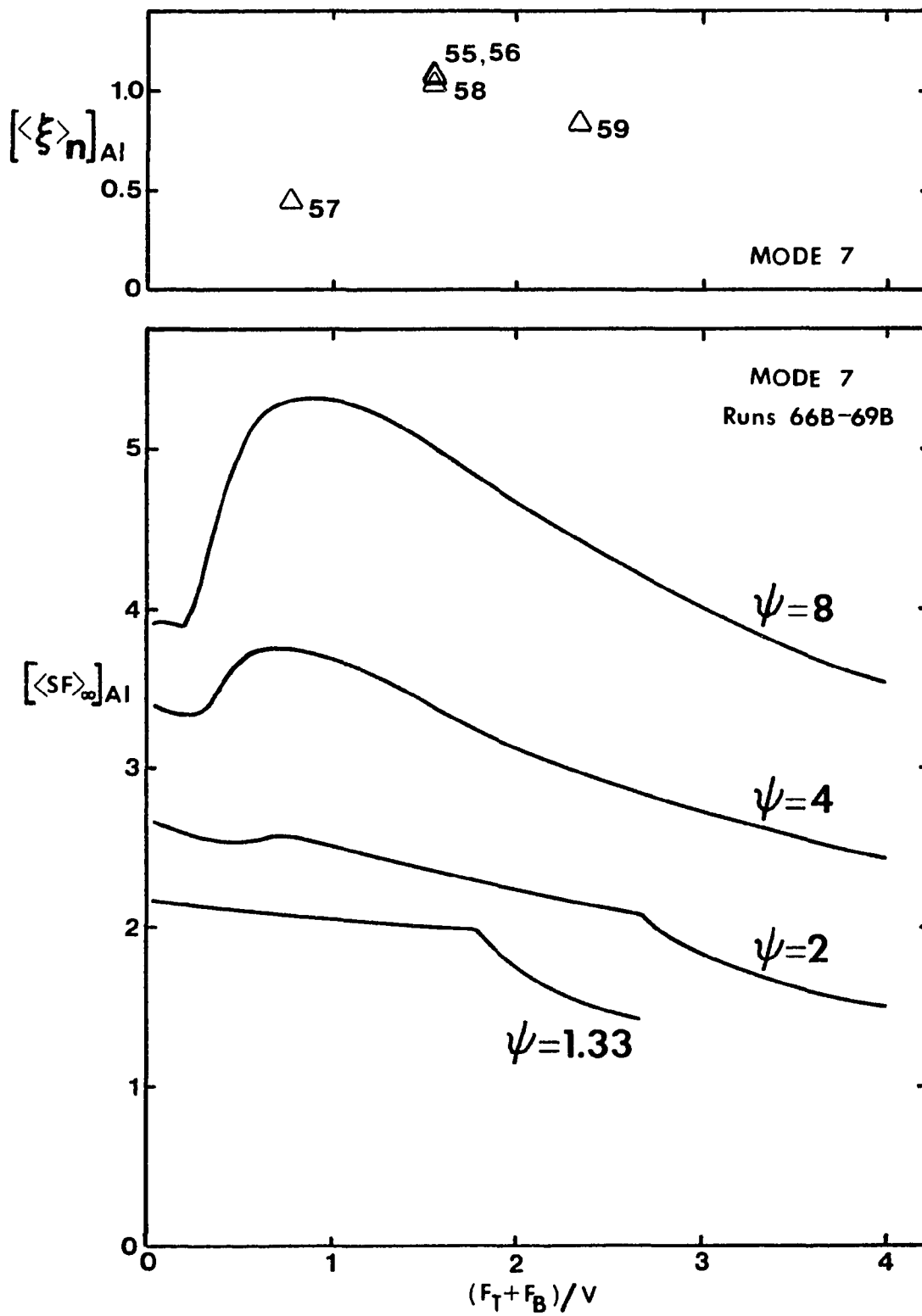


FIGURE 50. Optimization of Total Feed Rate Based on Albumin Recovery (top) and Albumin Separation Factor (bottom)

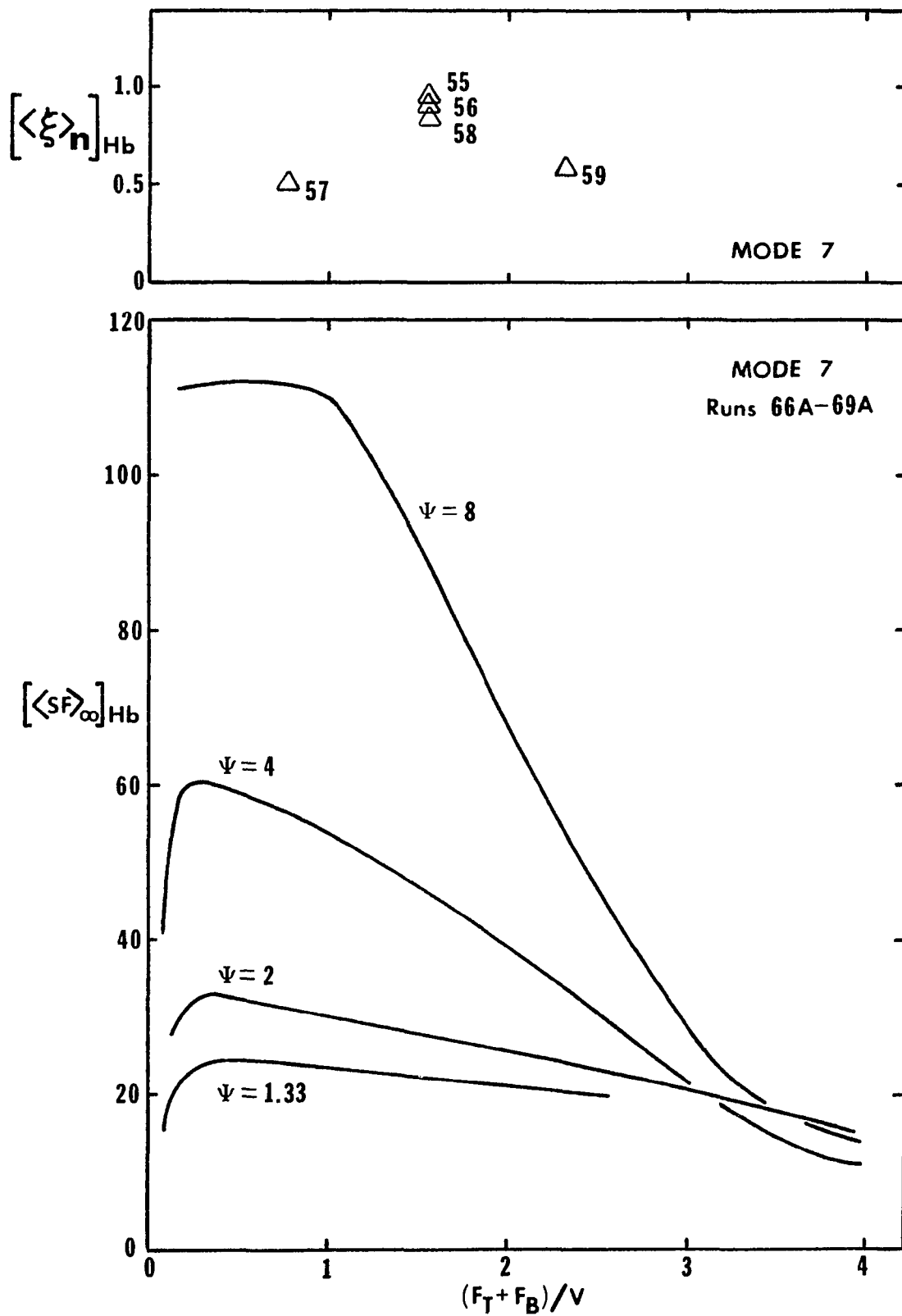


FIGURE 51. Optimization of Total Feed Rate Based on Hemoglobin Recovery (top) and Hemoglobin Separation Factor (bottom)

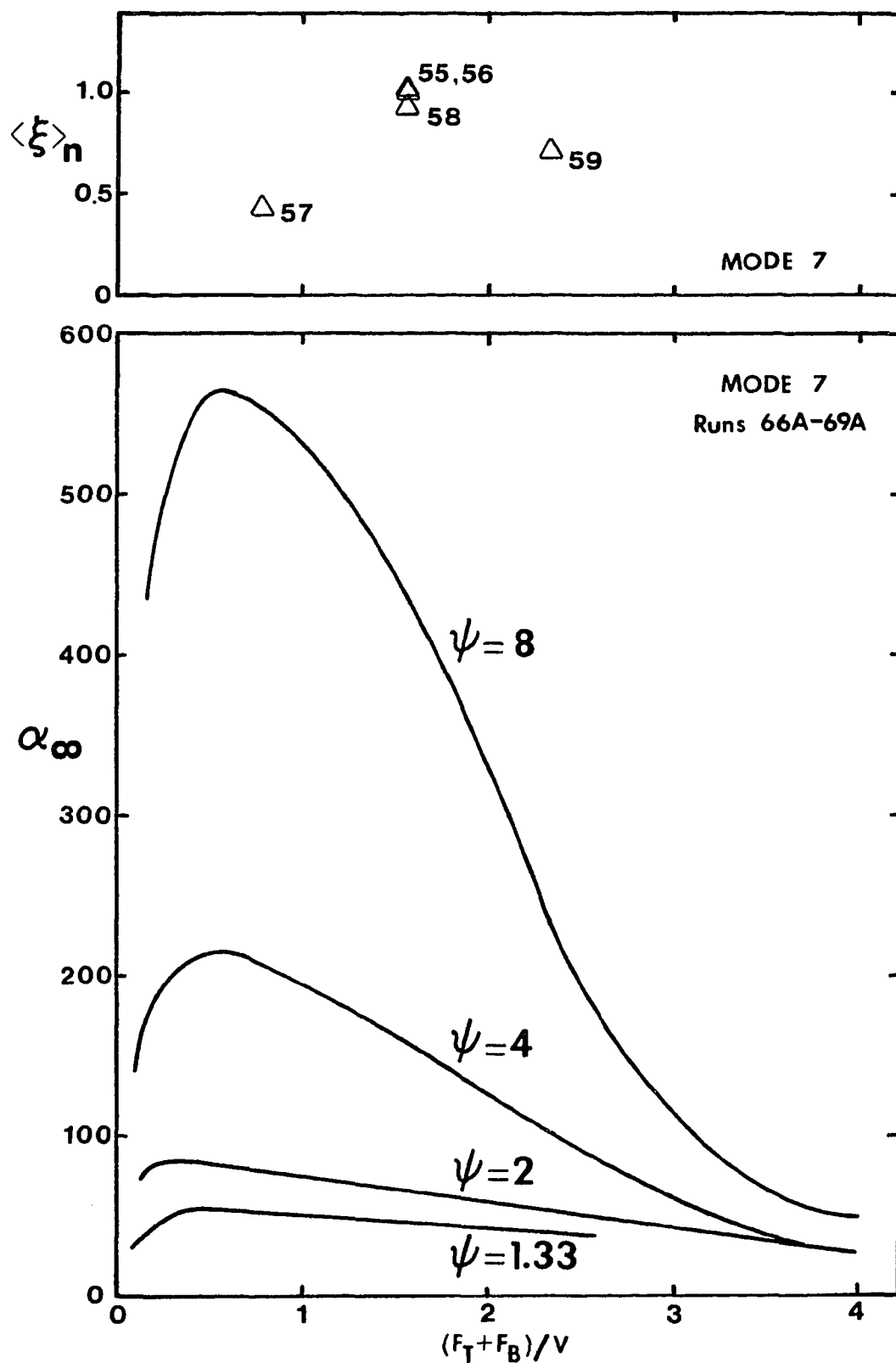


FIGURE 52. Optimization of Total Feed Rate Based on Protein Recovery (top) and Overall Separation Factor (bottom)

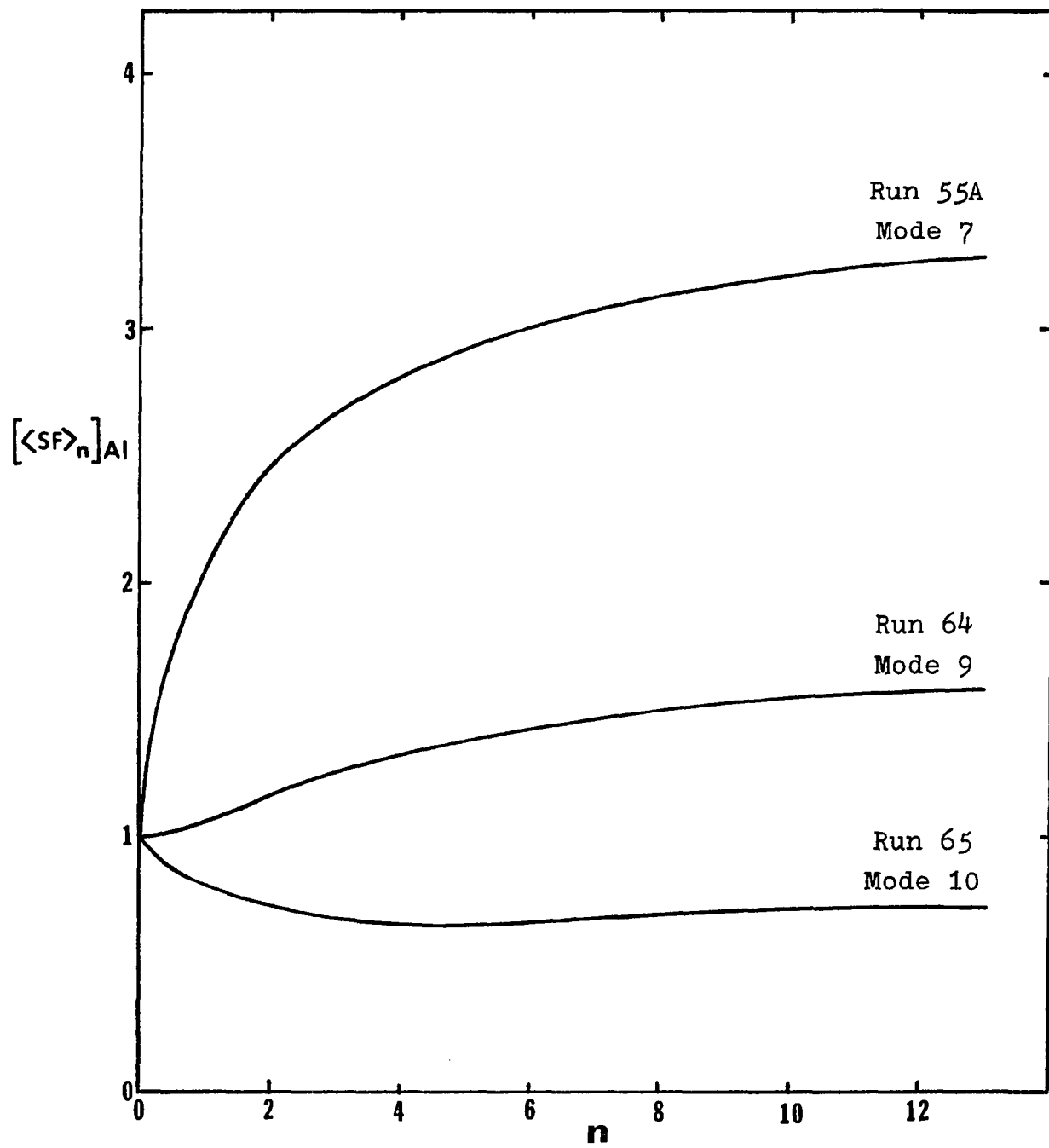


FIGURE 53. Optimization of Stage IV Operation

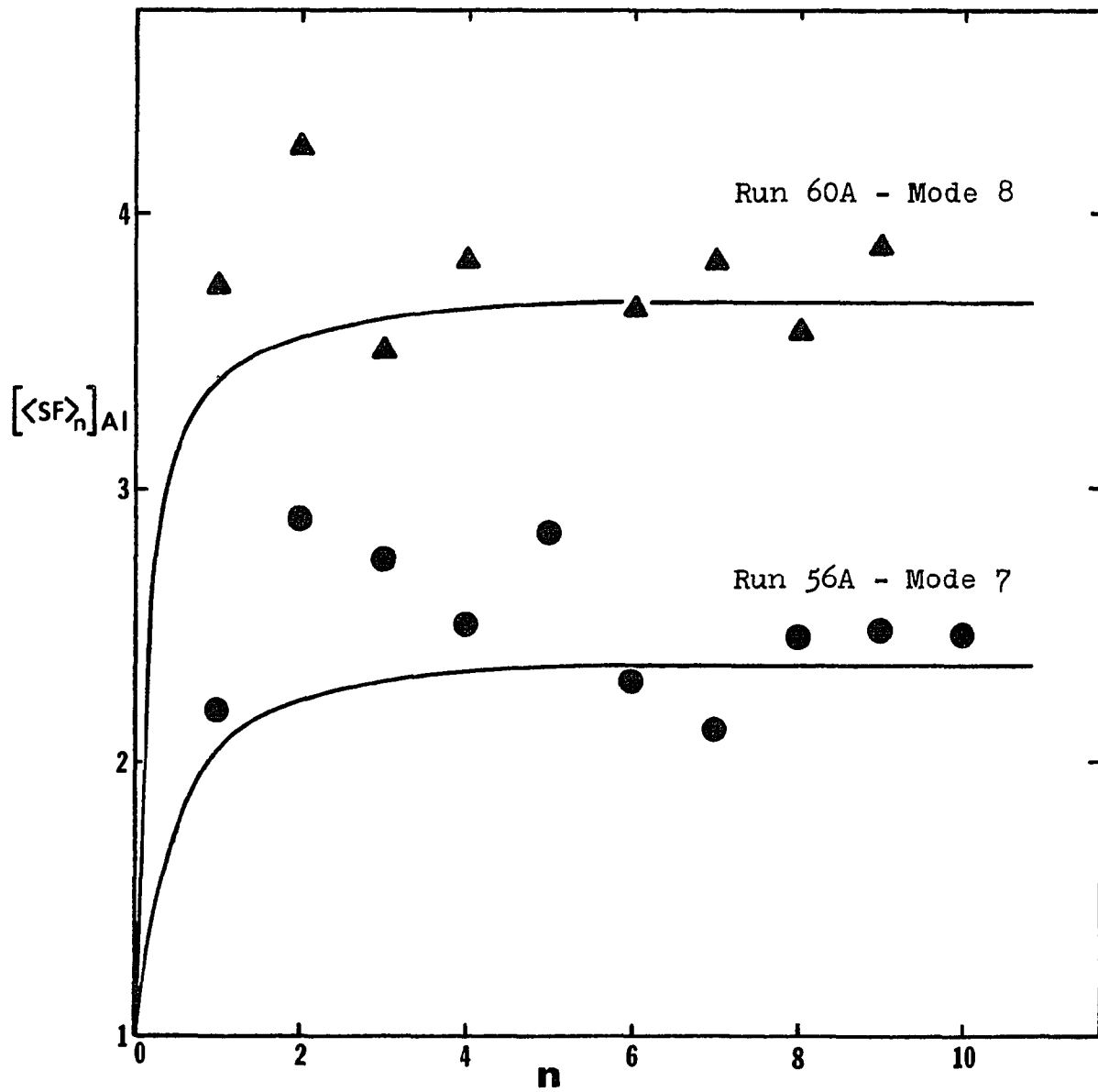


FIGURE 54. Optimization of Stage V Operation

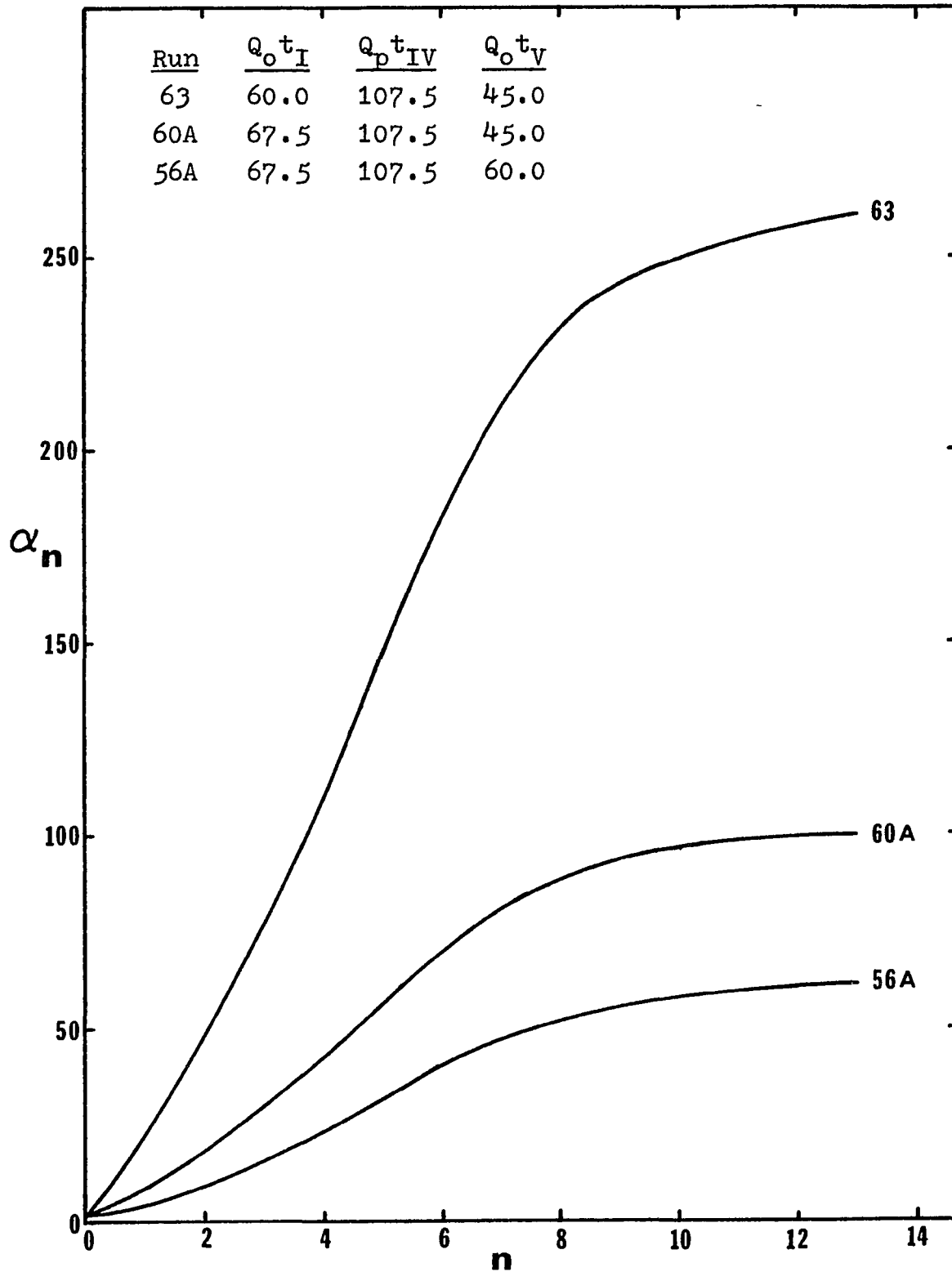


FIGURE 55. Optimum Displacements for Stages I, IV, and V

Chapter 6

MATHEMATICAL ANALYSIS OF THE ELECTRIC FIELD EFFECT ON THE HEMOGLOBIN SEPARATION

In order to calculate the hemoglobin separation factor from Equation 2-28, $[\langle y_{TP} \rangle_n]_{Hb}$ and $[\langle y_{BP} \rangle_n]_{Hb}$ must be determined. As discussed under the graphical method, the assumption is made that the only effect of the electric field on the overall separation is the electrophoretic migration of the proteins in the fluid phase. The external material balances and the internal equations for the electric field migration of hemoglobin may be handled in a manner similar to the albumin separation in the previous chapter. The internal equations for the adsorption/desorption of hemoglobin are developed in this chapter, and the parapumping runs via the new process (Runs 54-60) are calculated.

A single stage in the parametric pumping process involves unsteady-state mass transfer in a packed bed. Numerous solutions of the mass transport equations for this particular problem have been published, including Lapidus and Amundson, 1952; Mickley et al., 1957; Bird et al., 1960; Houghton, 1963; Chao and Hoelscher, 1966; Wilson, 1979; and Rasmuson, 1981. The mathematical model in this dissertation follows the finite mass transfer model by Chen et al., 1981a; Wu, 1981; and Yang, 1981. The 1981 pH parapumping model has been modified to include the electric field effect on the adsorbed protein as well as the pH wave lag which results from the low ionic strength buffer.

The graphical solution in Chapter 2 used Pigford's "local equilibrium" model (1969b) and Sweed and Wilhelm's STOP & GO algorithm (1969) with a single-transfer-step per half-cycle. The STOP & GO algorithm is used below to solve a model with finite mass transfer and multiple-cells-in-series. Since the only circulation stage in the new process is Stage II, and t_c (Equation 2-4) is finite in that stage; a single-cell model is not applicable to the experimental data. At the beginning of Stage I of the first cycle,

$$[\langle y_{TR} \rangle_1]_{Hb} = [\langle y_{C,I} \rangle_1]_{Hb} = [\langle y_{BR} \rangle_1]_{Hb} = [y_o]_{Hb} \quad (6-1)$$

A concentration gradient exists across the column in all other stages and all other cycles. This gradient may be stored in the computer memory by dividing the column in each stage of the process into a number of cells, and the memory may be carried forward as the initial condition for each successive stage and each successive cycle.

The appropriate equations for the hemoglobin adsorption are (Chen et al., 1981a),

$$\epsilon v_o \frac{\partial y}{\partial z} + \epsilon \frac{\partial y}{\partial t} = -(1 - \epsilon) \frac{\partial x}{\partial t} \quad (6-2)$$

$$\frac{\partial x}{\partial t} = \lambda (y - y^*) \quad (2-1)$$

$$y^* = \frac{x}{k_{pH}} \quad (2-2)$$

The following assumptions were made:

- (1) Plug flow.
- (2) Constant properties in the radial direction.
- (3) No interaction between solutes A and B, i.e., dilute solution.

- (4) Linear equilibrium relationship.
- (5) Surface adsorption with resistance to mass transfer through the film.
- (6) Negligible axial diffusion or dispersion.

Furthermore, it is assumed that:

- (7) The migration velocity $\nu_{E,Hb}$ may be adequately represented by an average value determined from Runs 5 and 6 (Figure 29 and Table 7).

The equilibrium constant k_{pH} in Equation 2-2 is dependent on the buffer composition and ionic strength as well as the pH. The linear form of the adsorption isotherm used by Mickley et al., 1957, gives a better fit to the experimental data for the 0.05M Tris-maleate/NaOH buffer. Equation 2-2 may be rewritten as follows, where the constant "b" may be positive, negative, or zero:

$$y^* = \frac{x}{k_{pH}} + b \quad (6-3)$$

The values of x and y^* may not be negative, and this constraint must be included in the computer program. For the Tris-maleate buffer, complete adsorption results in a non-zero value for hemoglobin in the fluid phase. From Runs 13, 15, and 16, a value of $b = 0.10$ is obtained.

Graham and Fook, 1982, recently published adsorption isotherms for bovine serum albumin on DEAE Protion, a cellulosic ion exchanger. Three isotherms were given, based on two pH levels and two different ionic strength buffers. All three isotherms are curved -- one has a positive value of "b" and the other two have negative values of "b". Similar isotherms may be expected for hemoglobin on CM Sepharose.

Butts et al., 1973, point out that the adsorption on ion exchange resins follows non-linear Langmuir type isotherms. The maximum capacity of the CM Sepharose ion exchanger is 10.0 g hemoglobin/ 100 ml gel (Pharmacia, 1980). Since the feed concentrations in the present study are only 0.01 weight % or 0.01 g hemoglobin/ 100 ml solution, it is reasonable to assume that the concentrations being studied are low enough to be in the linear region described by Equation 6-3.

The pore exclusion limit for CM Sepharose is "approximately 1×10^6 " (Pharmacia, 1980). The molecular weight of hemoglobin is 64500 (Table 2), which is certainly in this general range. It is, therefore, assumed that the adsorption is restricted to charged groups on the surface of the gel.

Chao and Hoelscher, 1966, studied axial dispersion in a packed bed both with and without adsorption. From experimental data, they reached the somewhat startling conclusion that the axial dispersion was significantly smaller under conditions of interphase mass transfer than under pure mixing conditions. Eddy diffusion is related to both molecular diffusion and Taylor dispersion. The molecular diffusivities in Table 2 indicate that this term is very very small. Convective dispersion is also assumed to be negligible.

The hemoglobin concentration waves exhibit a phenomena known as a self-sharpening wave front (Figure 6). Adsorbable proteins which move ahead of the wave front by axial

diffusion tend to be adsorbed onto the solid due to the fact that the leading fluid is at a different pH or concentration level. This phenomena was explained in detail for the pH wave in the present case in Chapter 1, and would be expected to minimize the measurable dispersion.

The finite difference method will be used to solve Equations 6-2, 2-1, and 6-3. Rolke and Wilhelm, 1969, proved mathematically that when an equation such as 6-2 is solved by this method, "a diffusive effect is introduced which is proportional to the differencing step size Δz or Δt ". This derivation is termed "diffusive differencing". In plain English, this means that either the number of cells-in-series or the eddy diffusivity may be determined from chromatographic data -- but not both. Wilson, 1979, presented a numerical solution of the transport equations for adsorption on activated carbon. His model included axial dispersion, pore diffusion, and non-linear isotherms. The choices are:

- (a) Use a very large number of cells-in-series and include a diffusion term in Equation 6-2.
- (b) Neglect axial diffusion and adjust the number of cells-in-series to some smaller number which fits the experimental data.

Wilson indicated that the second choice requires less computer time, and also avoids some mathematical instabilities in the numerical integration technique. The number of cells-in-series are adjusted to fit the data in the calculations below.

Replace the time and position derivatives in Equation 6-2 by the first backward-difference expressions.

$$\frac{\partial y}{\partial z} = \frac{y(i, j-1) - y(i-1, j-1)}{\Delta z} \quad (6-4)$$

$$\frac{\partial y}{\partial t} = \frac{y(i, j) - y(i, j-1)}{\Delta t} \quad (6-5)$$

$$\frac{\partial x}{\partial t} = \frac{x(i, j) - x(i, j-1)}{\Delta t} \quad (6-6)$$

The cell or the position along the axis of the column is denoted by "i" and the transfer step or time unit is denoted by "j". Substitute Equations 6-4 to 6-6 into Equation 6-2.

$$\begin{aligned} \epsilon \nu_0 \frac{y(i, j-1) - y(i-1, j-1)}{\Delta z} + \epsilon \frac{y(i, j) - y(i, j-1)}{\Delta t} \\ = -(1 - \epsilon) \frac{x(i, j) - x(i, j-1)}{\Delta t} \end{aligned} \quad (6-7)$$

Divide the column into "M" equal position increments or "M" cells-in-series. The time and position increments are related as follows:

$$\Delta z = \nu_0 \Delta t \quad (6-8)$$

Substitute Equation 6-8 into Equation 6-7 and multiply by Δt .

$$\begin{aligned} \epsilon [y(i, j-1) - y(i-1, j-1)] + \epsilon [y(i, j) - y(i, j-1)] \\ = -(1 - \epsilon) [x(i, j) - x(i, j-1)] \end{aligned}$$

$$\begin{aligned} \text{Or: } \epsilon y(i, j) + (1 - \epsilon) x(i, j) \\ = \epsilon y(i-1, j-1) + (1 - \epsilon) x(i, j-1) \end{aligned} \quad (6-9)$$

Alternately Equation 6-9 may be obtained by dividing the column into "M" cells and writing a mass balance on cell "i" from time "j-1" to time "j". Figure 56 shows this balance schematically via the STOP & GO algorithm. The

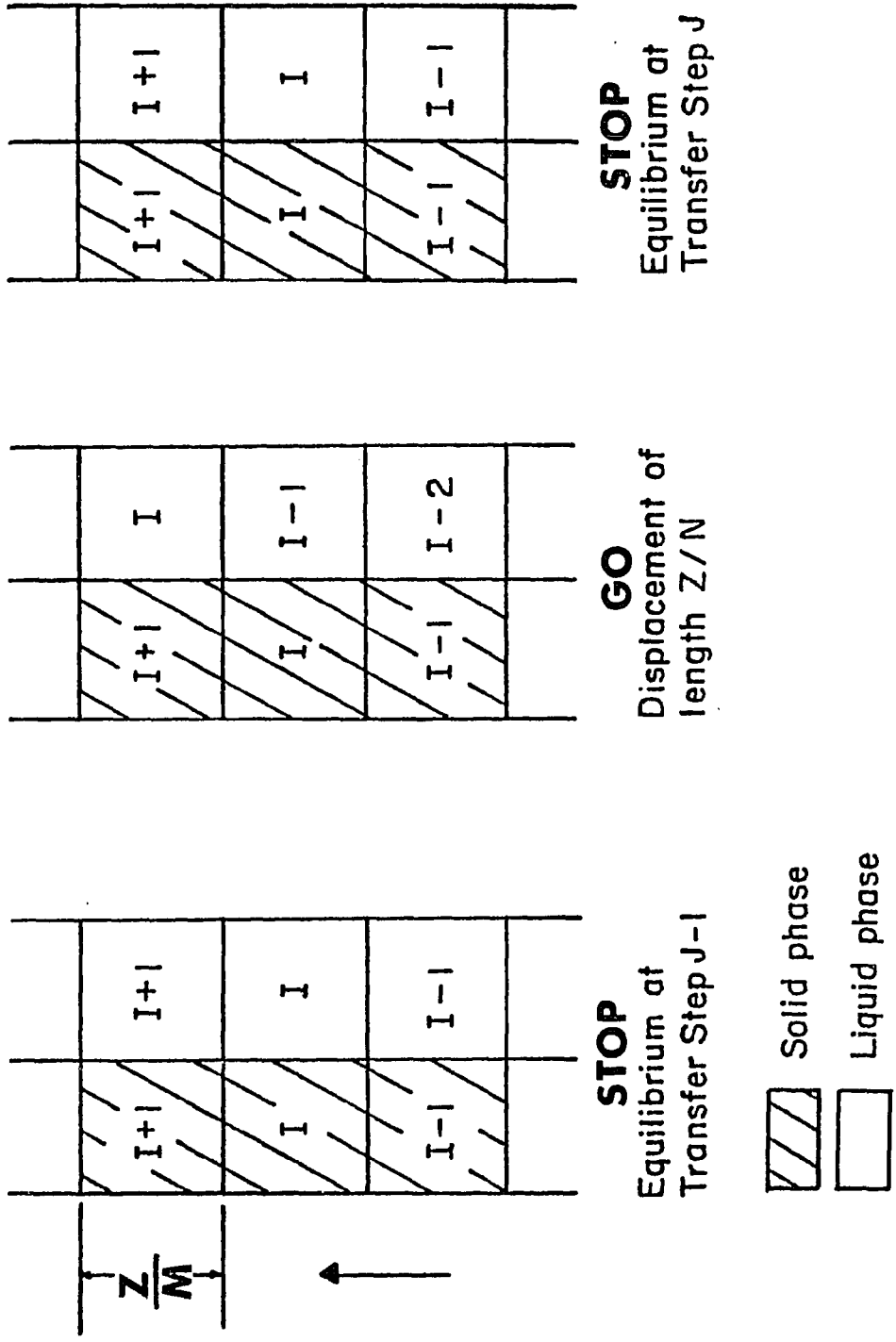


FIGURE 56. Multi-Cells-in-Series Model with STOP & GO Algorithm

Ref: Chen et al., Sep. Sci., 15, 1379 (1980)

initial conditions are shown in transfer step "j-1". The fluid is moved from cell "i-1" to cell "i" in the GO step or displacement without mass transfer. During the STOP step or mass transfer without fluid displacement, the solid phase changes from composition $x(i, j-1)$ to $x(i, j)$. The fluid phase composition in transfer step "j" may be calculated from the mass balance or from Equation 6-9.

$$y(i, j) = y(i-1, j-1) + \left(\frac{1-\epsilon}{\epsilon}\right) [x(i, j-1) - x(i, j)] \quad (6-10)$$

The total volume of the cell $\Delta(V + \bar{V})$ cancels out in these equations. The individual volumes are,

$$\text{Fluid Phase: } \Delta V = \frac{V}{M} = \epsilon \Delta(V + \bar{V}) \quad (6-11)$$

$$\text{Solid Phase: } \Delta \bar{V} = (1 - \epsilon) \Delta(V + \bar{V}) \quad (6-12)$$

In order to calculate $y(i, j)$, a value for $x(i, j)$ must also be calculated. In Equation 6-10, the compositions from transfer step "j-1" are known. Therefore, $y(i-1, j-1)$ and $x(i, j-1)$ may be treated as constants. Rewrite Equations 2-1 and 6-3 for transfer step "j".

$$\frac{\partial x(i, j)}{\partial t} = \lambda [y(i, j) - y^*(i, j)] \quad (6-13)$$

$$y^*(i, j) = \frac{x(i, j)}{k} + b \quad (6-14)$$

Substitute Equations 6-10 and 6-14 into Equation 6-13.

$$\begin{aligned} \frac{\partial x(i, j)}{\partial t} = & \lambda \left[y(i-1, j-1) + \left(\frac{1-\epsilon}{\epsilon}\right) x(i, j-1) \right. \\ & \left. - \left(\frac{1-\epsilon}{\epsilon}\right) x(i, j) - \frac{1}{k} x(i, j) - b \right] \end{aligned}$$

$$\text{Or: } \frac{d x(i, j)}{d t} = \lambda [c_1 - c_2 x(i, j)] \quad (6-15)$$

$$\text{Where, } c_1 = y(i-1, j-1) + \left(\frac{1-\epsilon}{\epsilon}\right) x(i, j-1) - b$$

$$c_2 = \frac{1-\epsilon}{\epsilon} + \frac{1}{k}$$

Equation 6-15 can be integrated analytically and solved for $x(i, j)$. If a non-linear equilibrium relationship is used instead of Equation 6-14, a numerical integration will be required at this point.

Group variables and integrate Equation 6-15 from transfer step "j-1" at time "t" to transfer step "j" at time "t + Δt".

$$\int_{x(i, j-1)}^{x(i, j)} \frac{d x}{(c_1 - c_2 x)} = \lambda \int_t^{t + \Delta t} d t \quad (6-16)$$

$$\left[-\frac{1}{c_2} \ln(c_1 - c_2 x) \right]_{x(i, j-1)}^{x(i, j)} = \lambda \left[t \right]_t^{t + \Delta t}$$

$$\ln \left[\frac{(c_1 - c_2 x(i, j))}{(c_1 - c_2 x(i, j-1))} \right] = -c_2 \lambda \Delta t$$

$$\frac{c_1 - c_2 x(i, j)}{c_1 - c_2 x(i, j-1)} = \text{EXP} (-c_2 \lambda \Delta t)$$

Solve for $x(i, j)$.

$$x(i, j) = \frac{c_1}{c_2} [1 - \text{EXP} (-c_2 \lambda \Delta t)] + x(i, j-1) \text{EXP} (-c_2 \lambda \Delta t) \quad (6-17)$$

From Equation 6-8,

$$\Delta t = Z / M \nu_0, \text{ where } Z = 15 \text{ cm} \quad (6-18)$$

Finally, the working equation for the finite mass transfer model is,

$$\begin{aligned}
 x(i,j) = & \frac{y(i-1,j-1) + \left(\frac{1-\epsilon}{\epsilon}\right) x(i,j-1) - b}{\frac{1-\epsilon}{\epsilon} + \frac{1}{k}} \\
 & \left[1 - \text{EXP} \left(-\frac{\lambda Z}{M \nu_0} \left\{ \frac{1-\epsilon}{\epsilon} + \frac{1}{k} \right\} \right) \right] \\
 & + x(i,j-1) \left[\text{EXP} \left(-\frac{\lambda Z}{M \nu_0} \left\{ \frac{1-\epsilon}{\epsilon} + \frac{1}{k} \right\} \right) \right] \quad (6-19)
 \end{aligned}$$

Everything on the right-hand-side of Equation 6-19 is known except M , k_{pH} at $\text{pH} = P_1$, k_{pH} at $\text{pH} = P_2$, λ at Q_0 , and λ at Q_p . These variables will be determined by computer trial-and-error to find the best-fit parameters using the pulse or breakthrough experiments without power for the 0.05M Tris-maleate/NaOH buffer. The best-fit parameters will then be used to calculate the pulse experiments with power and also the parametric pumping runs via the new process in order to simultaneously check the model and verify the experimental data. If one run fits -- it may be sheer luck!! If a number of sets of experimental data can be accurately calculated in this manner, however, the calculated parameters and the mathematical model must be reasonably valid.

The best way to determine k_{pH} is from experimental equilibrium adsorption isotherms. This is easily done for the no power case by well-documented methods. However, the possibility must be considered that the adsorptive properties of the charged ion exchange resin may be altered in the presence of an externally imposed electric field. No

precedent exists for determining these k_{pH} values under the influence of an electric field. The pulse experiments in Chapter 3 were run under identical conditions, except that in each set of runs the power was on in one case and off in the other case. These experiments afford a method for determining k_{pH} both with and without the electric field. In previous calculations by Chen and co-workers, the k_{pH} values were computer-fit directly to the pH parapumping experiments.

In order to estimate a value for the mass transfer coefficient λ , Equation 11 from Chen et al., 1981a, must be written in an appropriate form for scale-up.

$$\lambda = 2.036 \times 10^{-8} Q^{-0.693} \quad (6-20)$$

with λ (sec^{-1}) and Q (m^3/sec)

Since the dimensions of the experimental apparatus in this study are larger than in previous work, consider the equation for the dimensionless mass-transfer factor J_D from Geankopolis, 1978. For mass transfer of liquids in packed beds at Reynolds numbers of 0.016 to 55 and Schmidt numbers of 165 to 70,000, the equation is,

$$J_D = \frac{1.09}{\epsilon} N_{Re}^{-0.667} \quad (6-21)$$

Estimate the Reynolds and Schmidt numbers by using the density and viscosity of water at 278^oK and the other parameters as follows:

$$D_p = 40-160 \mu\text{m} \approx 1 \times 10^{-4} \text{ m}$$

$$D_{Hb,Al} = 7.6 \times 10^{-11} \text{ m}^2/\text{sec} \quad (\text{Table 2})$$

$$N_{Sc} = \frac{\mu}{\rho D_{AB}} \approx 20,000$$

At $Q_o = 2.5$ cc/min or $\nu = 1.39 \times 10^{-4}$ m/sec,

$$N_{Re} = \frac{D_p \nu_o \rho}{\mu} \approx 0.0091$$

At $Q_p = 0.5$ cc/min or $\nu = 2.78 \times 10^{-5}$ m/sec,

$$N_{Re} \approx 0.0018$$

Since the mass transfer is proportional to the Reynolds number in the equation above, Equation 6-20 should be rewritten in terms of linear velocity ν for scale-up. The numbers in Equation 6-20 are based on a column with a diameter of 0.016 m and a void volume of 0.75. Using these values to calculate the effective cross-sectional area,

$$\lambda = 9.061 \times 10^{-6} \nu^{-0.693} \quad (6-22)$$

with λ (sec⁻¹) and ν (m/sec)

Since tris-maleate buffer is used in the present work instead of phosphate buffer and the ionic strength of the buffer is lower, Equation 6-22 only gives the order of magnitude of λ .

$$\lambda \approx 0.3 \text{ min}^{-1} \quad \text{at } Q_o = 2.5 \text{ cc/min}$$

$$\lambda \approx 0.8 \text{ min}^{-1} \quad \text{at } Q_p = 0.5 \text{ cc/min}$$

Furthermore, if the exponential function in Equation 6-19 is small, λ cannot be determined from computer fit of that particular experimental data.

If the exponential function is small compared to "1", Equation 6-19 simplifies to the local equilibrium model. The local equilibrium model may be used as a first estimate in the calculations.

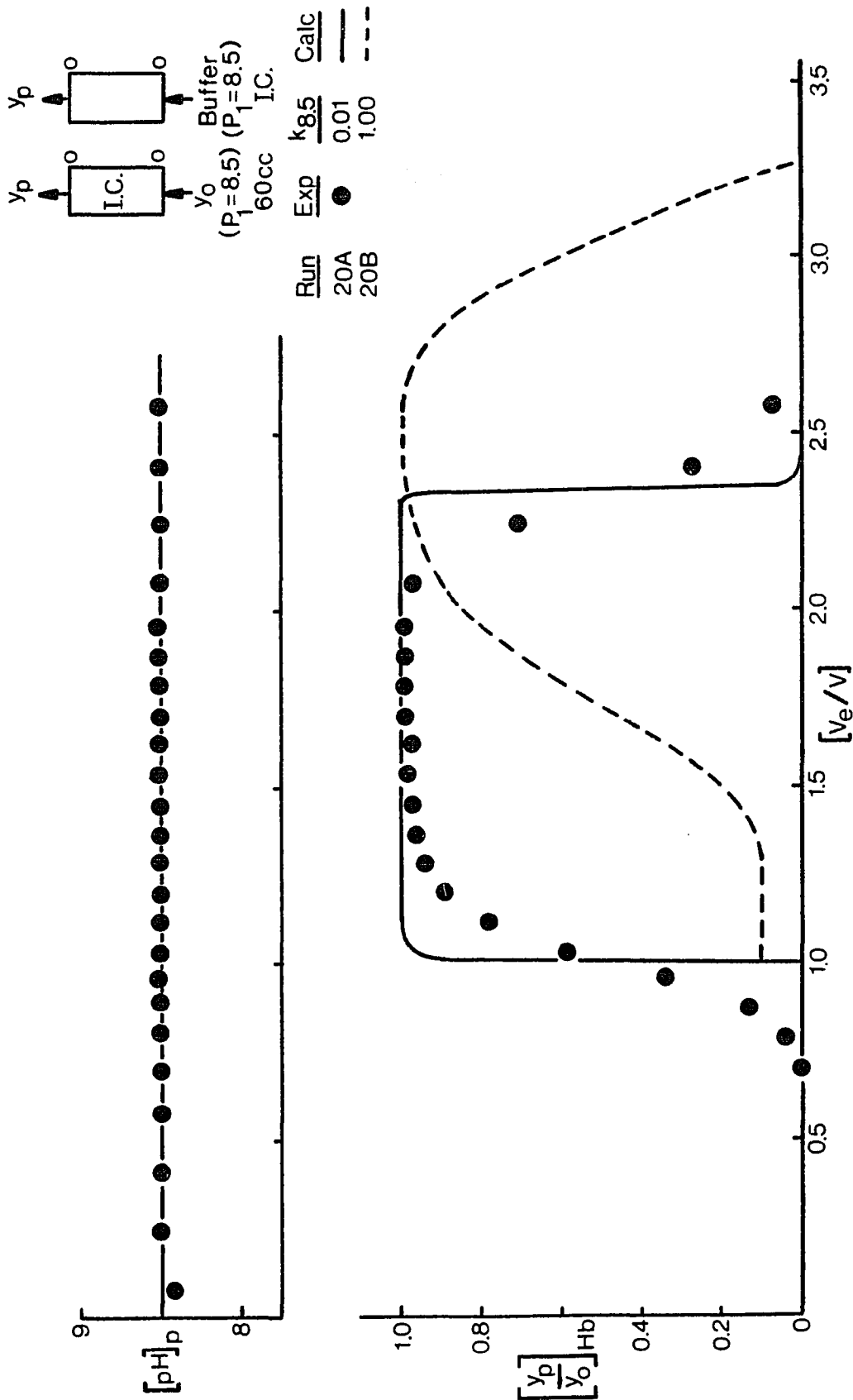


FIGURE 57. Determination of Equilibrium Constant at High pH

$$x(i,j) = \frac{y(i-1,j-1) + \left(\frac{1-\epsilon}{\epsilon}\right) x(i,j-1) - b}{\left(\frac{1-\epsilon}{\epsilon}\right) + \frac{1}{k}} \quad (6-23)$$

In Equation 6-23, the unknowns are M , k_{pH} at $\text{pH} = P_1$, and k_{pH} at $\text{pH} = P_2$.

The k_{pH} at $P_1 = 8.5$ may be determined from Run 20A in Figure 57. The adsorption is very small at high pH or at $P_1 > I_A$, because hemoglobin is negatively charged under these conditions. A higher value for k_{pH} predicts that the concentration peak exits at a later time as shown in Run 20B. All of the following figures are based on data using a feed mixture of 0.01 weight percent hemoglobin plus 0.01 weight percent albumin. Single component data cannot be used, because the hemoglobin adsorption is less in the presence of albumin as discussed in Chapter 2 (Figure 17).

The pH Wave Equation

The remaining pulse experiments and the parapumping experiments all involve a change in pH. The equilibrium adsorption constant k_{pH} will be assumed to vary linearly with pH.

$$k(i,j) = k_{\text{pH}=P_1} + \frac{k_{\text{pH}=P_2} - k_{\text{pH}=P_1}}{P_2 - P_1} (\text{pH}(i,j) - P_1) \quad (6-24)$$

In order to calculate $x(i,j)$ from Equation 6-19, a value for $k(i,j)$ from Equation 6-24 must be calculated. A value for $k(i,j)$ cannot be calculated unless $\text{pH}(i,j)$ is known. The model of Wankat, 1974b; Busbice and Wankat, 1975; and Dore

and Wankat, 1976, will be used to calculate $\text{pH}(i,j)$.

The phenomena involved in the pH change in the pH parapump is identical to the situation which occurs when a hot fluid is introduced into a bed packed with cold solids. The incoming fluid will decrease in temperature and the solid will simultaneously increase in temperature, with the relative temperature changes depending on the relative heat capacities. After one void volume of fluid has exited from the column, the outlet temperature will still be less than the inlet temperature. If there are no heat losses from the system, the fluid and solid in the column and the outlet fluid will eventually reach the inlet temperature. In the pH parapump, the relative pH changes of the fluid and solid phases depend on the relative hydrogen ion capacities. Instead of making a heat balance in order to calculate the velocity of the temperature wave, it is necessary to make a hydrogen ion balance in order to calculate the velocity of the pH wave.

The dimensionless pH wave velocity "B" may be determined experimentally.

$$B = \frac{\nu_{\text{pH}}}{\nu_0} , \quad \text{where} \quad \nu_{\text{pH}} \leq \nu_0 \quad (6-25)$$

The velocity of a pH wave which is changing from $\text{pH} = P_1$ to $\text{pH} = P_2$ is measured at the point where $\text{pH} = \frac{1}{2}(P_1 + P_2)$. The magnitude of "B" varies with buffer composition, pH, and ionic strength as seen in Table 9. The pH wave velocity is also different in identical experiments with and without power.

TABLE 9
Dimensionless pH Wave Velocities

<u>Run</u>	<u>Buffer</u>	<u>Q</u>	<u>pH</u>		<u>B</u>
			<u>OLD</u>	<u>NEW</u>	
5	0.05M Tris-maleate/NaOH	0.5	6.0	8.5	0.436
13	"	"	8.5	6.0	0.493
17	0.2M Tris-maleate/NaOH	2.5	5.8	8.5	0.512
4	0.05M Phosphate	2.7	6.0	8.0	0.502
4	"	"	8.0	6.0	0.610
9	0.1M Phosphate	2.5	6.0	8.0	0.610
9	"	"	8.0	6.0	0.638
11	"	1.0	6.0	8.0	0.647
11	"	"	8.0	6.0	0.671

It is hypothetically possible to analytically determine the hydrogen ion balance in the pH parapump, and to calculate "B" from this hydrogen balance. The calculations for the buffer or fluid phase are included in any general chemistry textbook, and the mechanism for the hydrogen ion interaction with the ion exchanger is probably similar. Note that:

$$\text{pH} = -\log[\text{H}^+]$$

The hydrogen ion capacity of the fluid phase is associated with the type of buffer and the buffer concentration. The buffer has the capacity to accept or donate hydrogen ions from the acid HA. An organic acid is only partially dissociated into its component ions in aqueous solution.



The ionization constant of the acid K_a is defined as,

$$K_a = \frac{[\text{A}^-][\text{H}^+]}{[\text{HA}]}$$

Titration curves of organic acids are "S-shaped" curves, where the relatively straight horizontal portion of the curve between $\text{pH} = \text{p}K_a - 1$ and $\text{pH} = \text{p}K_a + 1$ is the buffering region.

The STOP & GO model in Figure 56 may be used to write a hydrogen ion balance. Assume that the pH of the fluid in a given cell "i" equals the pH of the solid in that same cell at the end of each transfer step "j".

$$\begin{aligned} \Delta\text{H}^+ (\text{fluid}) + \Delta\text{H}^+ (\text{solid}) &= 0 \\ \Delta V M_{\text{HA}} c_{\text{H}^+, \text{F}} (\text{pH}(i, j) - \text{pH}(i-1, j-1)) \\ &= - \Delta\bar{V} \rho_S c_{\text{H}^+, \text{S}} (\text{pH}(i, j) - \text{pH}(i, j-1)) \quad (6-26) \end{aligned}$$

M_{HA} = molarity of acid (moles HA/liter)

$c_{H^+,F}$ = hydrogen ion capacity of fluid
(moles H⁺/mole HA • pH unit)

ρ_S = moles of hydrogen ion sites/liter of gel

$c_{H^+,S}$ = hydrogen ion capacity of solid
(moles H⁺/mole sites • pH unit)

Substitute Equations 6-11 and 6-12 into Equation 6-26 and solve for the pH at the end of transfer step "j".

$$pH(i,j) = B pH(i-1,j-1) + (1-B) pH(i,j-1) \quad (6-27)$$

Where,

$$B = \frac{M_{HA} c_{H^+,F}}{M_{HA} c_{H^+,F} + \left(\frac{1-\epsilon}{\epsilon}\right) \rho_S c_{H^+,S}} \quad (6-28)$$

In order to calculate the dimensionless pH velocity from Equation 6-28, the hydrogen ion capacities of the fluid and solid phases must be determined experimentally. The hydrogen ion capacity of the Tris-maleate buffer in the region from pH = 6.0 to pH = 8.5 is fairly constant as calculated from the data in Figure 58. If Φ is the slope of the hydroxyl ion titration curve, then $-\Phi$ is the slope of the hydrogen ion titration curve and

$$c_{H^+,F} = -\frac{1}{\Phi} = -2.214 \frac{\text{moles H}^+}{\text{mole HA} \cdot \text{pH unit}}$$

The titration curve for CM Sepharose in 1M KCl solution is nearly vertical in the pH = 6.0 to pH = 8.5 region (Pharmacia, 1980). This implies that the pH capacity of the solid is zero in this region, but since $B < 1$ in Table 9 $c_{H^+,S} \neq 0$. An attempt to calculate $c_{H^+,S}$ from the data in Table 9 and from Equation 6-28 shows that $c_{H^+,S}$ is not zero and not constant.

$$c_{H^+,S} = f(\text{pH, I.S.})$$

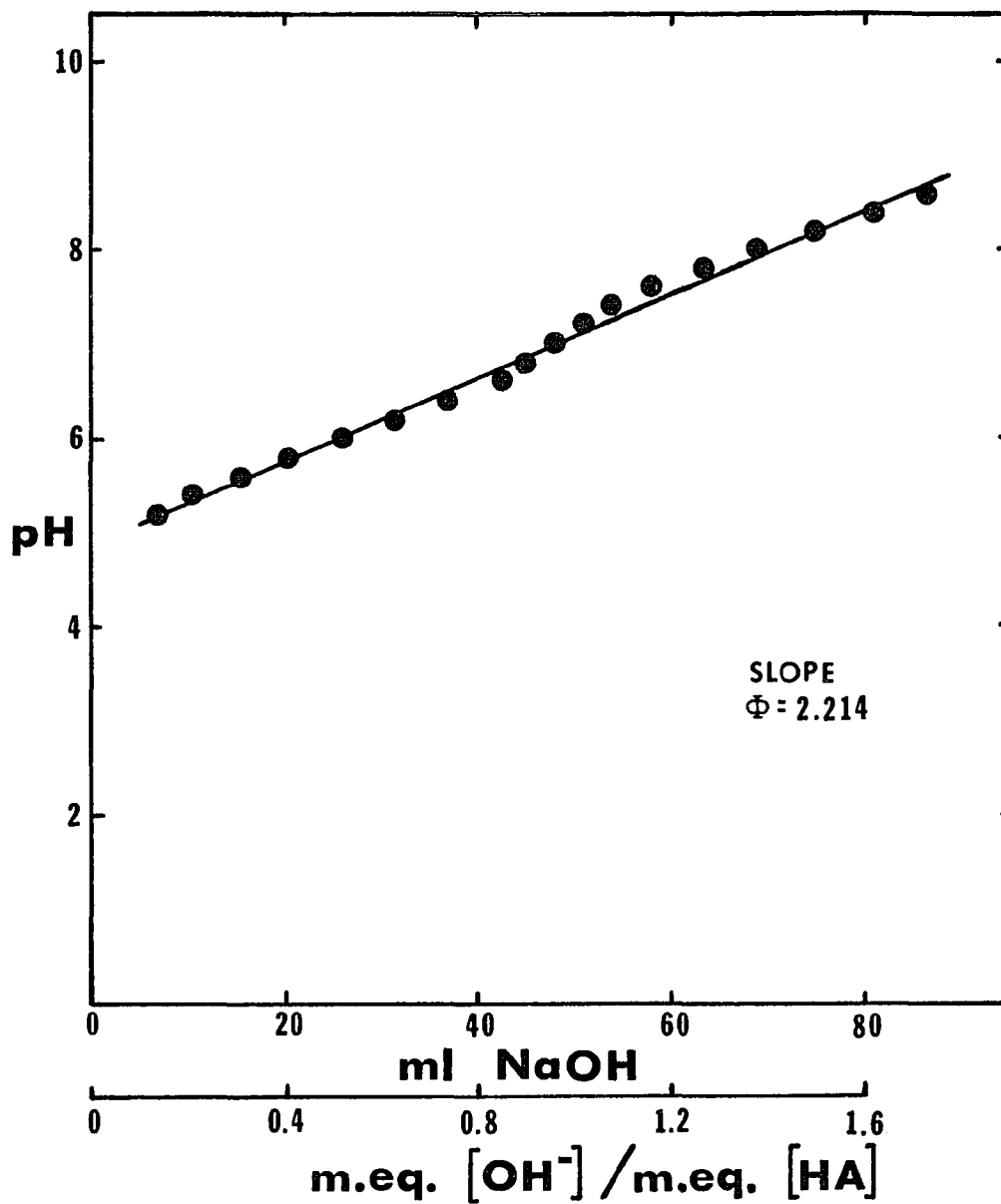


FIGURE 58. Titration Curve for Tris-maleate Buffer

Ref: Colowick & Kaplan, 1955

I.C.: 50 ml of 0.2M Tris-maleate

Titrate with 0.2M NaOH

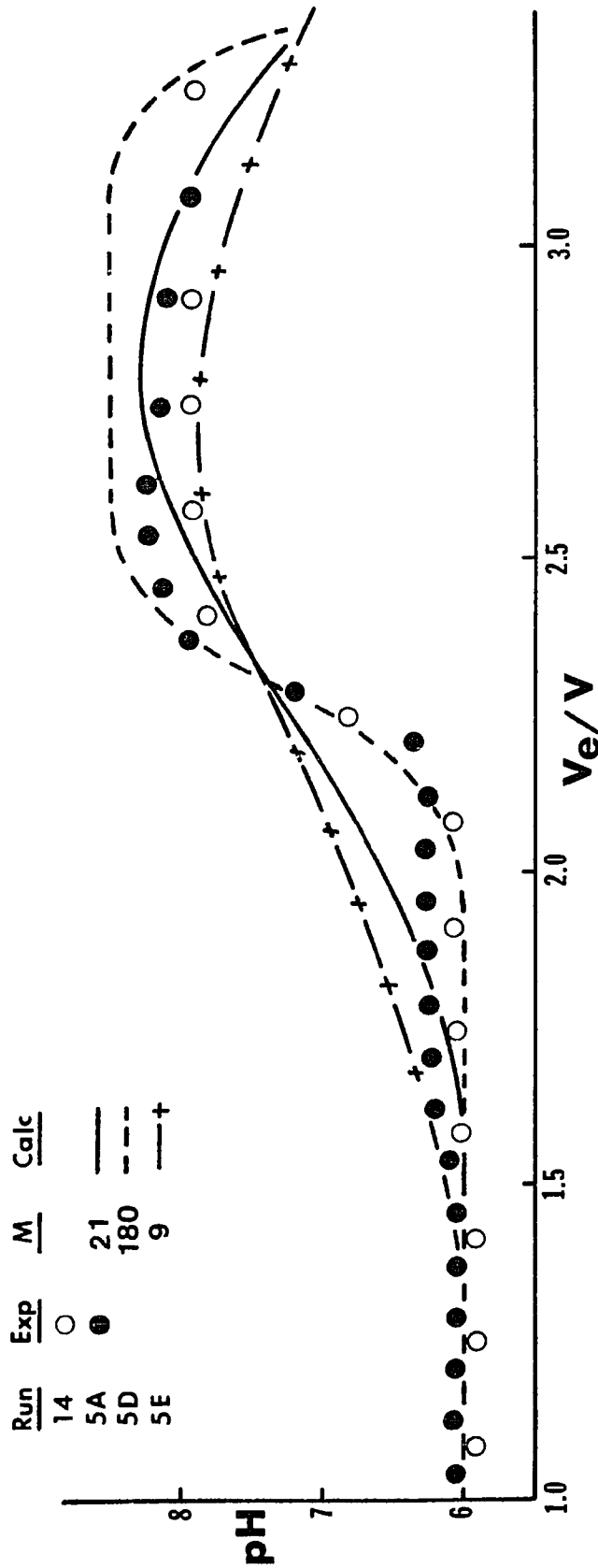
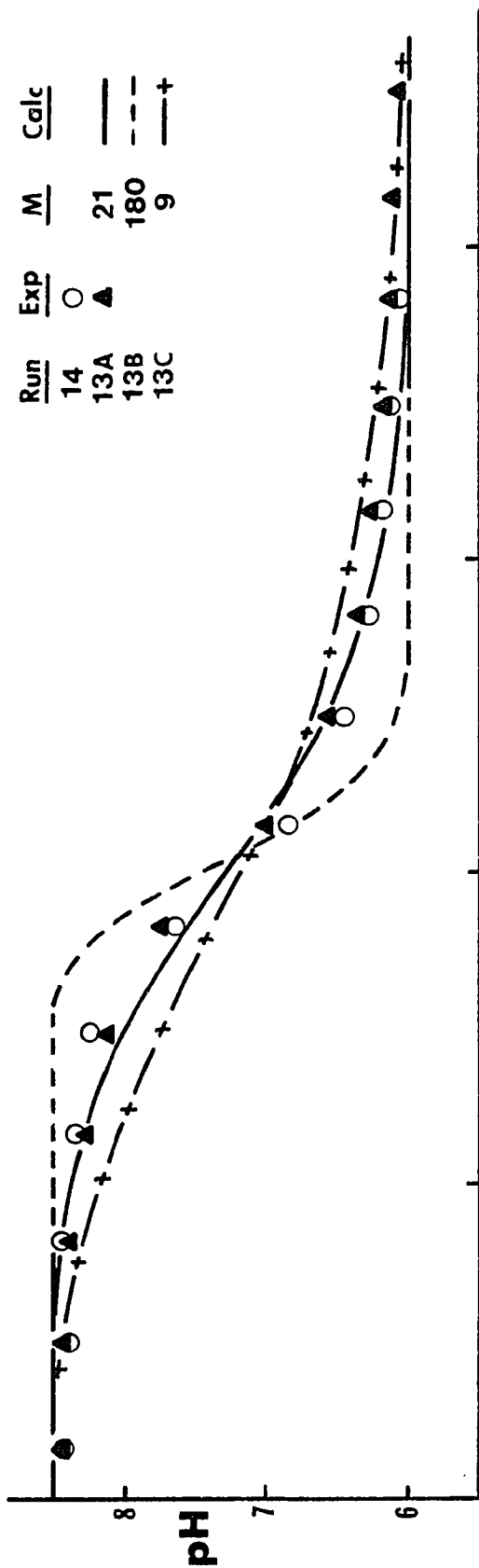


FIGURE 59. pH Waves without Electric Field

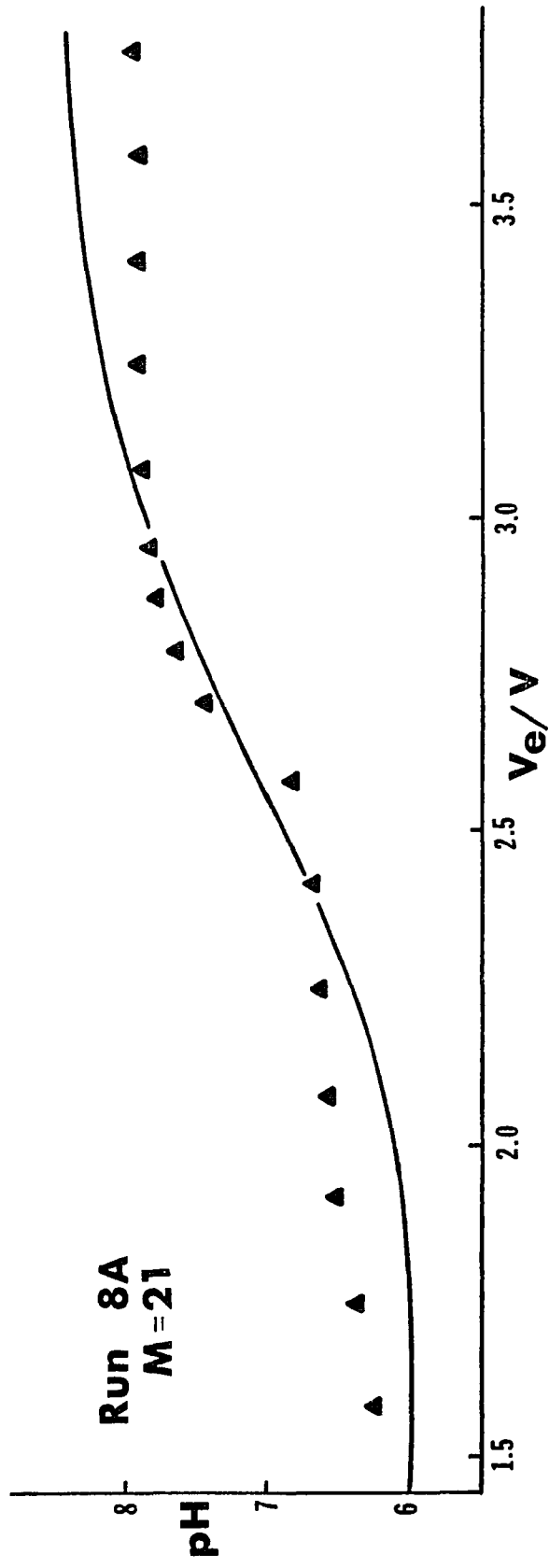
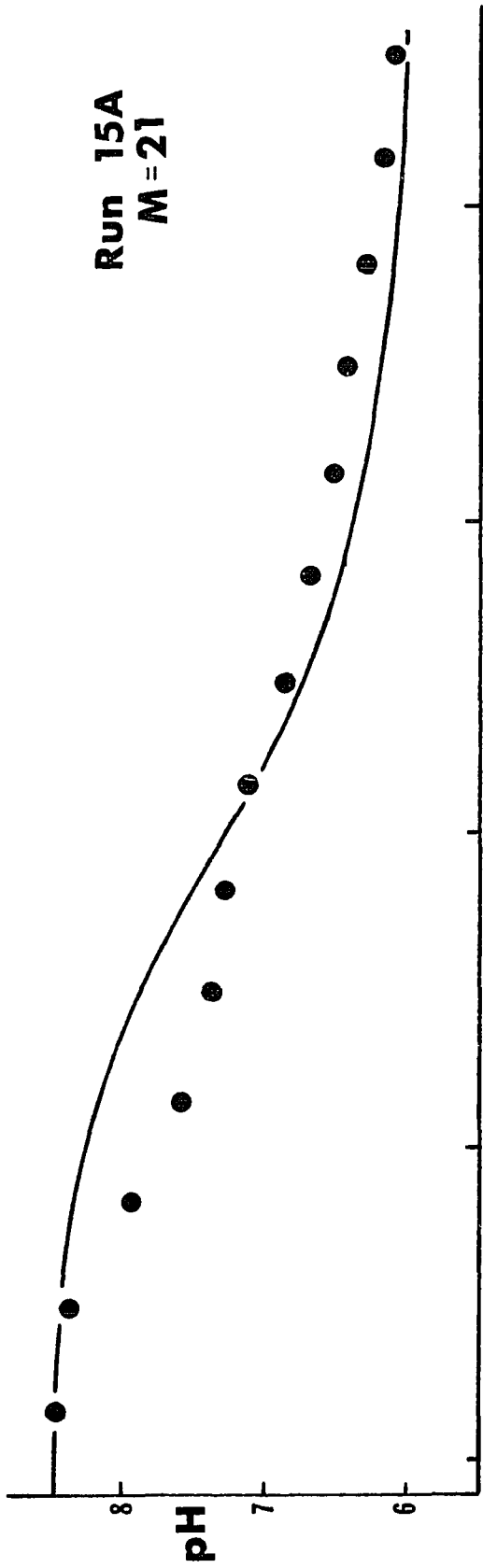
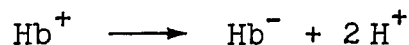


FIGURE 60. pH Waves with Electric Field

The dimensionless pH velocity "B" is calculated directly from the pH wave data or Equation 6-25 as suggested by Wankat, 1974b. In order to fit the pH data from Equation 6-27, the number of cells-in-series "M" is also needed. A larger number of cells calculates less mixing or sharper pH changes as shown in Figure 59. The data in Figure 59 was run twice -- once with feed solution and once with pure buffer. The data is the same in both cases, so the hemoglobin adsorption or desorption does not significantly affect the hydrogen ion balance. Note that the desorption of Hb^+ releases hydrogen ions.



The quantity of hydrogen ions in this reaction is evidently negligible compared to the total buffering capacity of the system. A value of $M = 21$ was chosen based on the figures to be discussed below. As shown in Figures 59 and 60, this value gives a reasonable fit to the pH data both with and without power.

Calculation of the Concentration Waves

The concentration wave exits the column at a later time when the power is applied with the cathode at the entrance of the column as discussed in conjunction with Figure 29. In Figure 57, it was shown that a larger value for k_{pH} also delays the concentration wave. This coincidence suggests that the imposed electric field increases the equilibrium constant k_{pH} for the ion exchanger. The pulse experiment without power in Figure 61 supports the alternate explanation,

i.e., that the concentration wave is married to the pH wave and both are delayed simultaneously. The pH wave for a low ionic strength buffer is delayed relative to the bulk velocity in any case, but the concentration wave is only delayed significantly when P_2 fluid moves across the packed bed in front of P_1 fluid. This phenomenon was explained in Chapter 1.

$$\nu_{\text{Hb},P_1} > \nu_{\text{pH}} > \nu_{\text{Hb},P_2} \quad (1-1)$$

The relationship between the pH and concentration waves was not recognized until after the data was analyzed -- so it is a major piece of serendipity that the pH waves were even measured! Fortunately, the pH of a sample is needed to correct the spectrophotometer reading at $403 \mu\text{m}$ (Appendix A).

The k_{pH} at $\text{pH} = 8.5$ was calculated in Run 20 (Figure 57). If the pH wave were not the controlling factor, the concentration wave in Run 5 would be identical to the wave in Run 20, as shown by the dotted line in Figure 61. Both experiments have identical feeds. The concentration wave in Run 20 is a square wave of height 1.0 beginning at one void volume. A pH change is instituted in Run 5 by initially filling the packed bed with low pH buffer, then introducing high pH feed. The concentration wave in Run 5 exits at greater than two bed volumes as a peak of 3.4 times the feed concentration. This peak immediately follows the point of pH change in Figure 61. The peak is calculated by using the equations derived in this chapter in the following sequence:

- (1) Determine "B" from the pH wave data and Equation 6-25.
- (2) Set the initial conditions and feed conditions.
- (3) Calculate the pH in cell "i" from Equation 6-27.
- (4) Calculate k_{pH} in cell "i" from Equation 6-24.
- (5) Calculate the concentrations in cell "i" from Equations 6-19 and 6-10.

The experimental parameters for the new process are collected in Table 10. The computer-fit values and the constants from raw data are all included. The effect of the remaining parameters on the concentration waves are shown in Figures 62-64.

The equilibrium constant at low pH is calculated in Figure 62. Again, a larger value for k_{pH} delays the concentration wave.

As shown in Figure 63, a large value for "M" gives an extremely sharp peak. The value for "M" is reduced to a number of cells-in-series which is low enough to simulate the curve broadening due to diffusion. A mass balance constraint must be placed on the number of cells used for the calculations. The value for "M" must be selected so that the total number of transfer steps $j = \Gamma$ is a whole number.

$$\Gamma = \frac{\text{Volume Pulse}}{\Delta V} = \frac{\text{Volume Pulse}}{V} M$$

= WHOLE NUMBER (6-29)

The volume of the pulse was 60 cc, so $M = 3, 6, 9, 12, \dots$ are possible values. Good results were obtained with $M = 21$.

All of the pulse experiments were run at $Q_p = 0.5$ cc/min. A range of values for λ at the low flow rate are examined in Figure 64. The concentration peak occurs in Run 5 because the hemoglobin is adsorbed at P_2 , then desorbed at P_1 . If the mass transfer coefficient λ is zero, no adsorption takes place and a square wave of height 1.0 exits after one void volume (Run 5J). Any value of λ above 1.3 min^{-1} fits the data. This means that the flow rate is low enough that Pigford's local equilibrium model (Equation 6-23) is applicable. The exponential function from Equation 6-19 is negligible at both pH conditions for the low flow rate. The value for λ at high flow rate will be considered in the section on the parapumping calculations.

The parameters from Table 10 were used to calculate the pulse experiments with power in Figures 65 and 66. The model fits very well! The electric field changes the dimensionless pH wave velocity "B", and moves both the hemoglobin and the albumin in the fluid phase, but has no effect on the adsorptive properties of the ion exchanger.

The hemoglobin migration velocity is added in the GO step of the STOP & GO model (Figure 56). A new value for y_{Hb} is calculated at the end of the GO step. At the end of transfer step "j-1", $y_{\text{Hb}} = y(i-1, j-1)$; but after displacement with the electric field and without mass transfer, $y_{\text{Hb}} = y^*(i-1, j-1)$. If the cells in each transfer step are calculated from the inlet $i = 1$ to the outlet $i = M$, three equations are needed to calculate the concentration of

hemoglobin in the fluid phase at the end of the displacement step. At the outlet from Equation 2-20,

$$y^*_{\text{out}} = \frac{1}{R_{\text{Hb}}} y(M, j-1) \quad (6-30)$$

By mass balance at the inlet,

$$y^*(0, j-1) = y_0 + \left(1 - \frac{1}{R_{\text{Hb}}}\right) y(1, j-1) \quad (6-31)$$

For the intermediate cells,

$$y^*(i-1, j-1) = \frac{1}{R_{\text{Hb}}} y(i-1, j-1) + \left(1 - \frac{1}{R_{\text{Hb}}}\right) y(i, j-1) \quad (6-32)$$

In the equations above, the retardation coefficient R_{Hb} for hemoglobin is defined by Equation 3-1.

Most of the parameters necessary to calculate the hemoglobin peak in the presence of the electric field are taken from Runs 5 and 6. The data in Figure 65 is from Run 8 so none of the data is back-calculated. This independent calculation is required to support the validity of the model.

Figure 66 shows the case where no peak occurs due to the fact that the leading fluid is at a higher pH than the trailing fluid. The feed is adsorbed as the P_2 fluid enters the column, and the effluent concentration drops after one void volume of fluid has been pumped out of the column. The pH wave model also predicts this data, but a model which neglects the pH wave or with $B = 1.0$ will work just as well in this case.

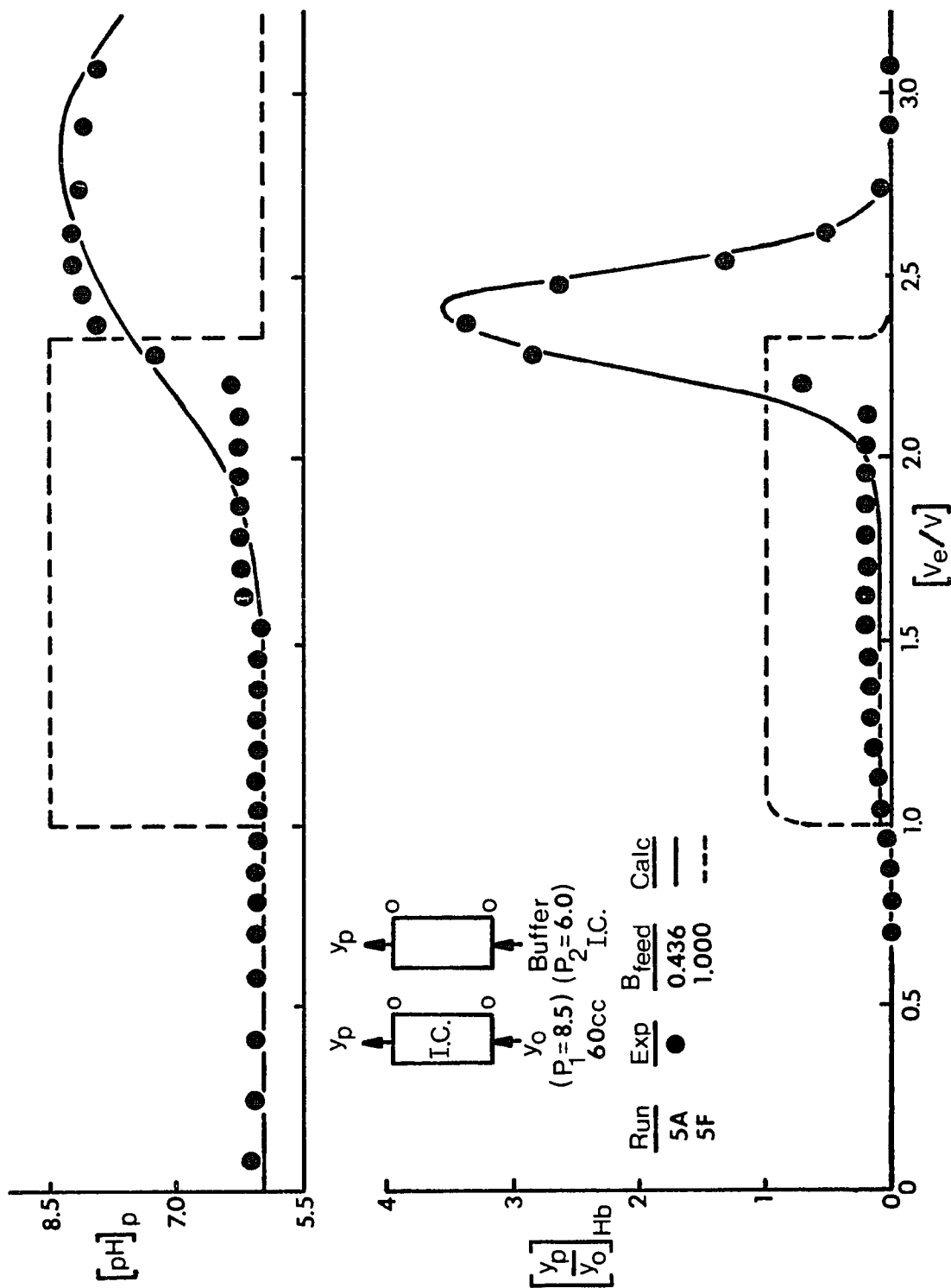


FIGURE 61. Relationship Between pH and Concentration Waves for the Adsorbed Protein

TABLE 10

Experimental Parameters for the New Process

<u>Parameter</u>	<u>Source</u>
No Power, B (8.5 \rightarrow 6.0) = 0.493	Runs 13 & 14
" B (6.0 \rightarrow 8.5) = 0.436	Runs 5 & 14
Power, B (8.5 \rightarrow 6.0) = 0.391	Run 15
" B (6.0 \rightarrow 8.5) = 0.371	Run 8
b = 0.10	Runs 13, 15 & 16
$k_{pH} = 0.01$ @ $P_1 = 8.5$	Run 20
$k_{pH} = 3.8$ @ $P_2 = 6.0$	Run 5
M = 21 cells-in-series	Run 5
V = 45 cc	Measurement
$[y_o]_{Al} = 1.0$	Definition
$[y_o]_{Hb} = 1.0$	Definition
$\epsilon = 0.565$	Measurement
λ at $Q_p \geq 1.3 \text{ min}^{-1}$	Run 5
$\nu_o = 0.167 \text{ cm/min}$	Variable
$\nu_{E,Al} = -0.023 \text{ cm/min}$	Runs 5 & 6
$\nu_{E,Hb} = -0.048 \text{ cm/min}$	Runs 5 & 6

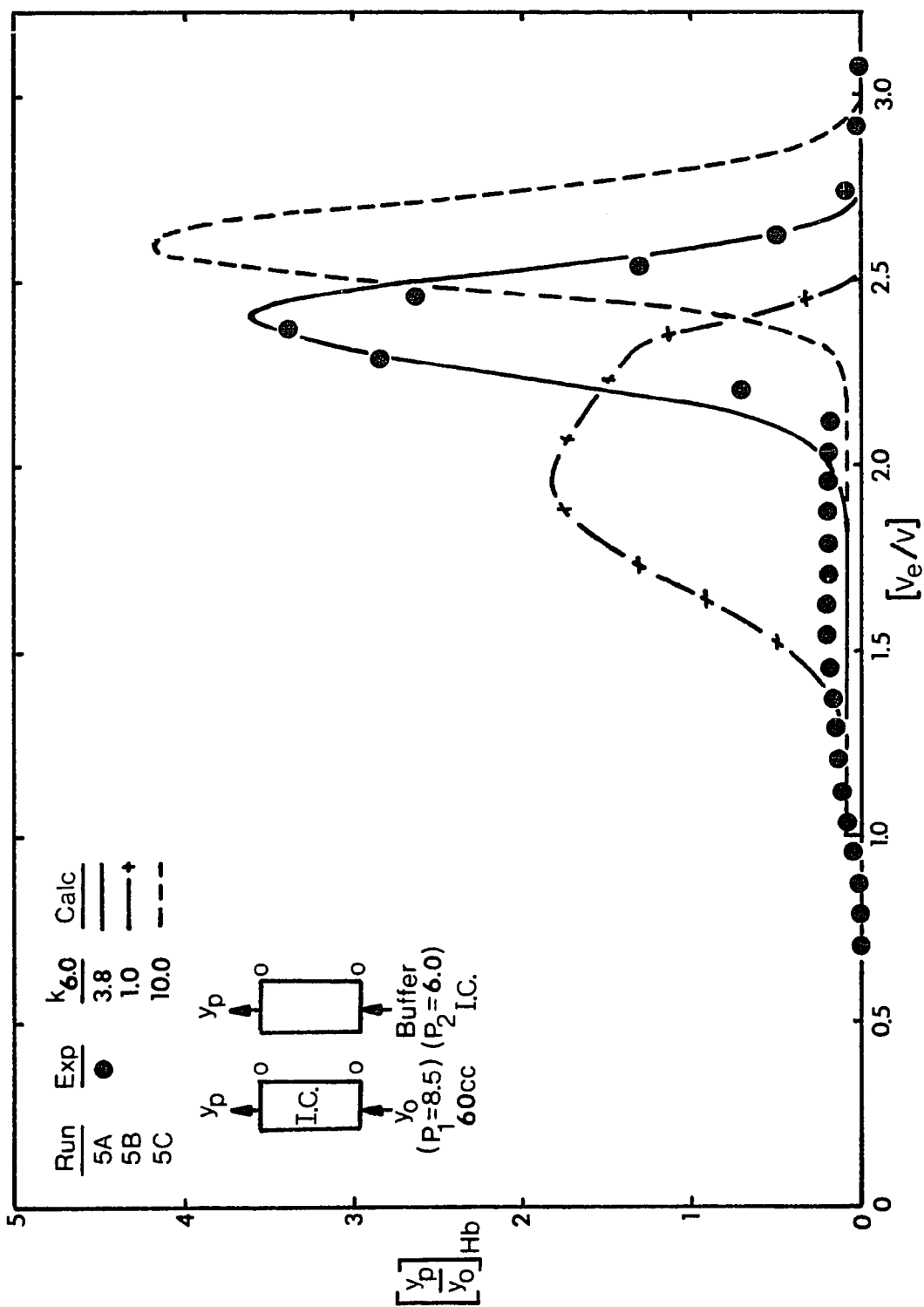


FIGURE 62. Determination of Equilibrium Constant at Low pH

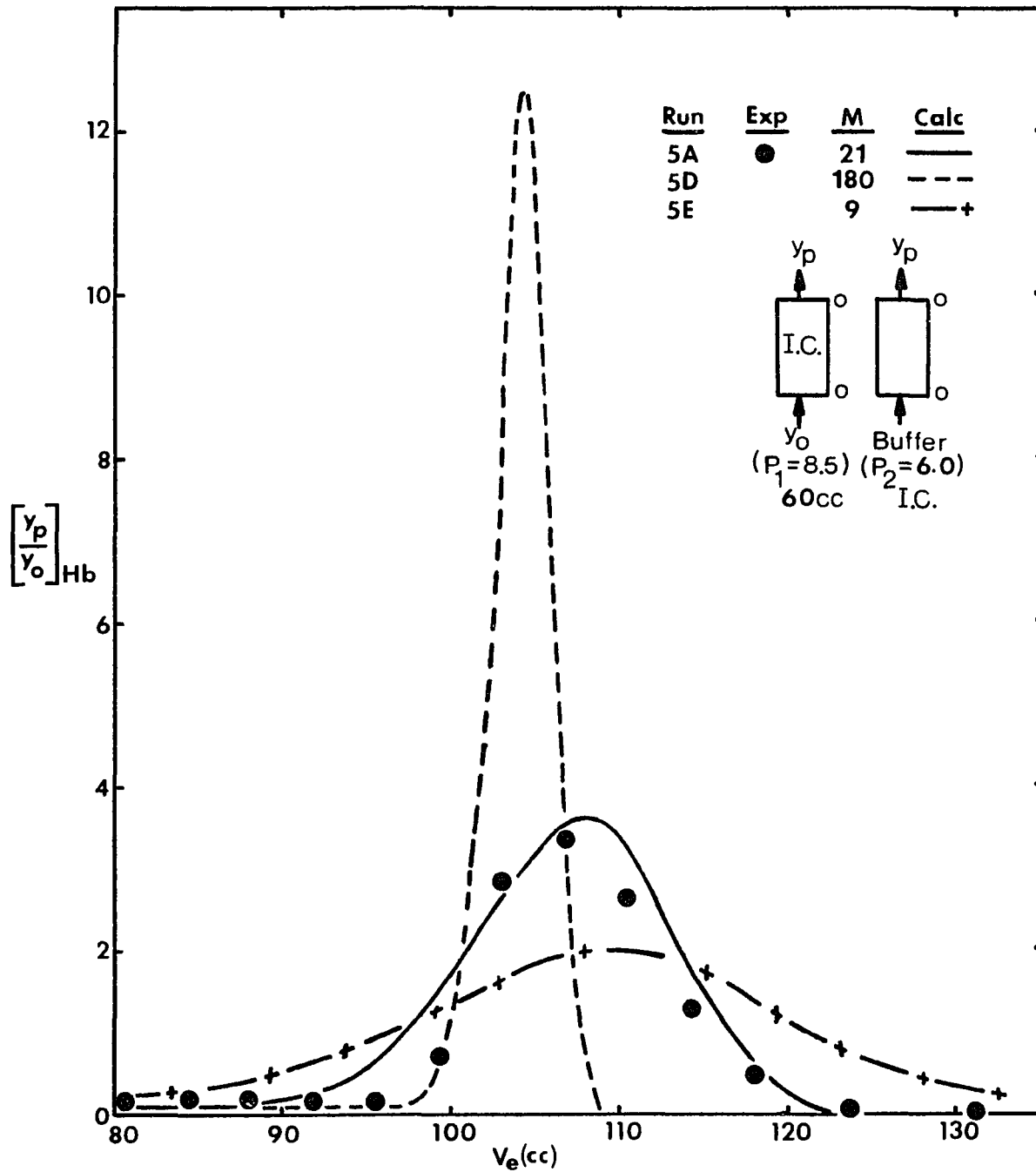


FIGURE 63. Shape of Concentration Wave as a Function of Number of Cells used for the Multi-Cells-in-Series Model

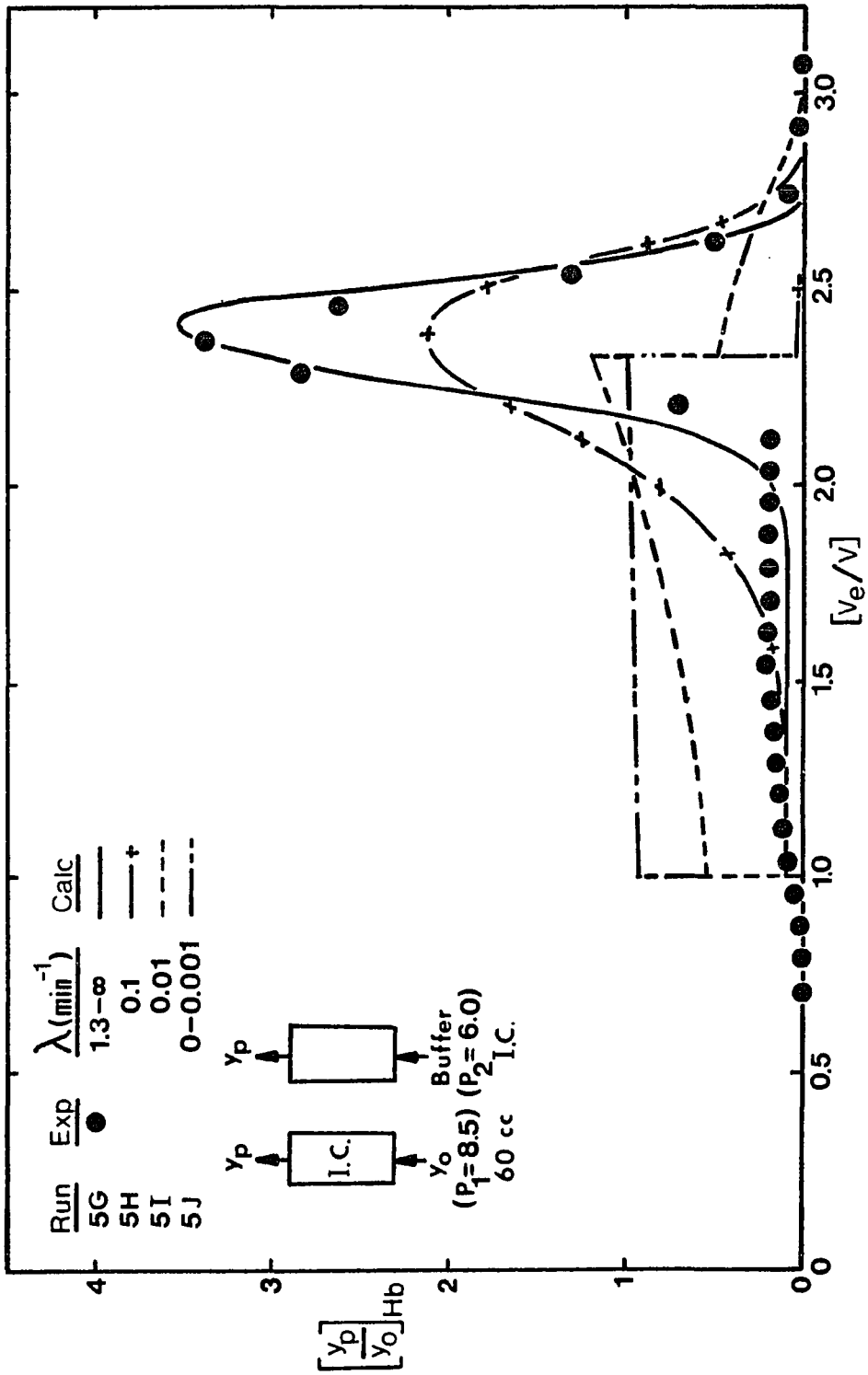


FIGURE 64. Effect of Mass Transfer Coefficient on Shape of Concentration Wave

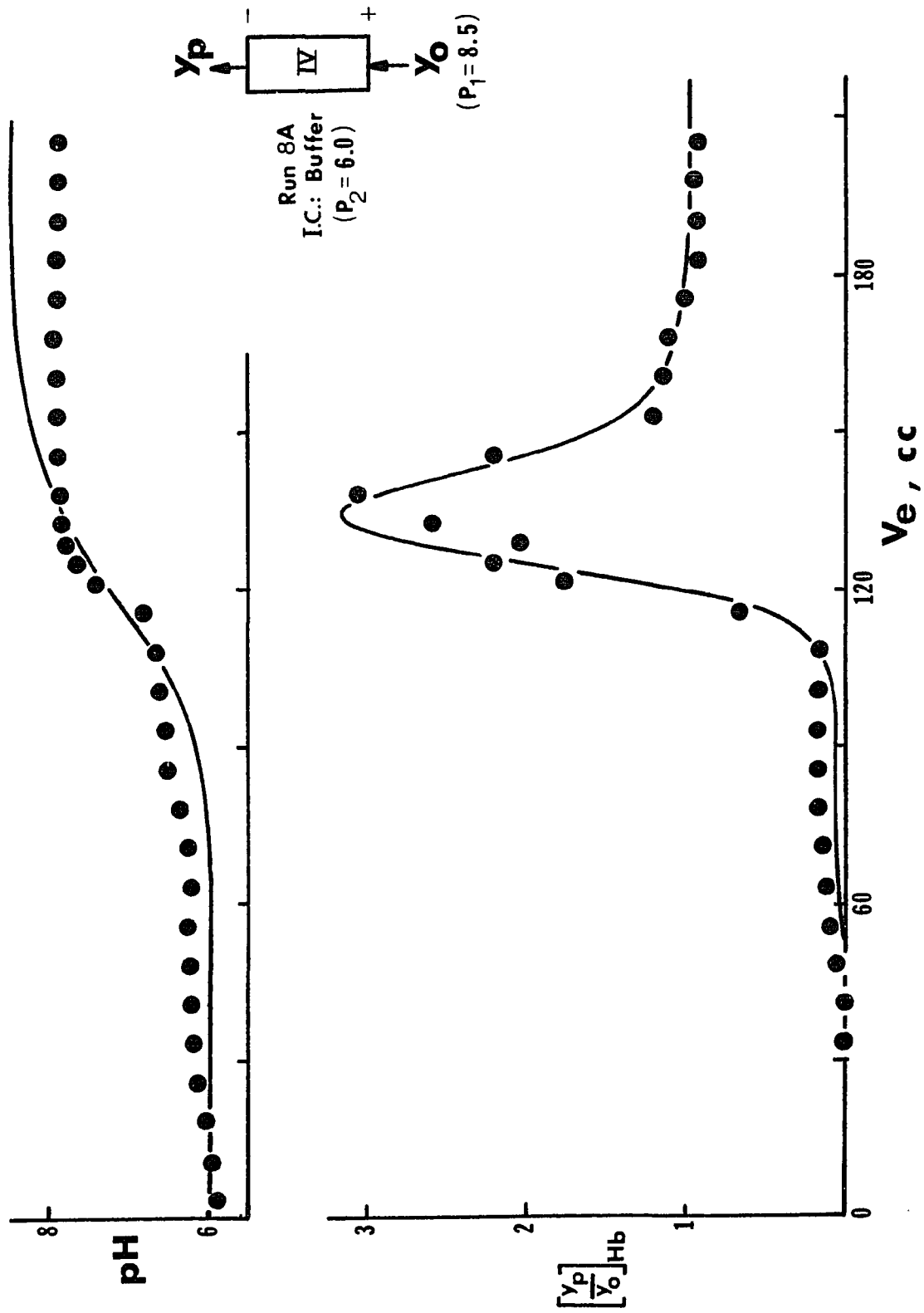


FIGURE 65. Calculated Desorption with Electric Field

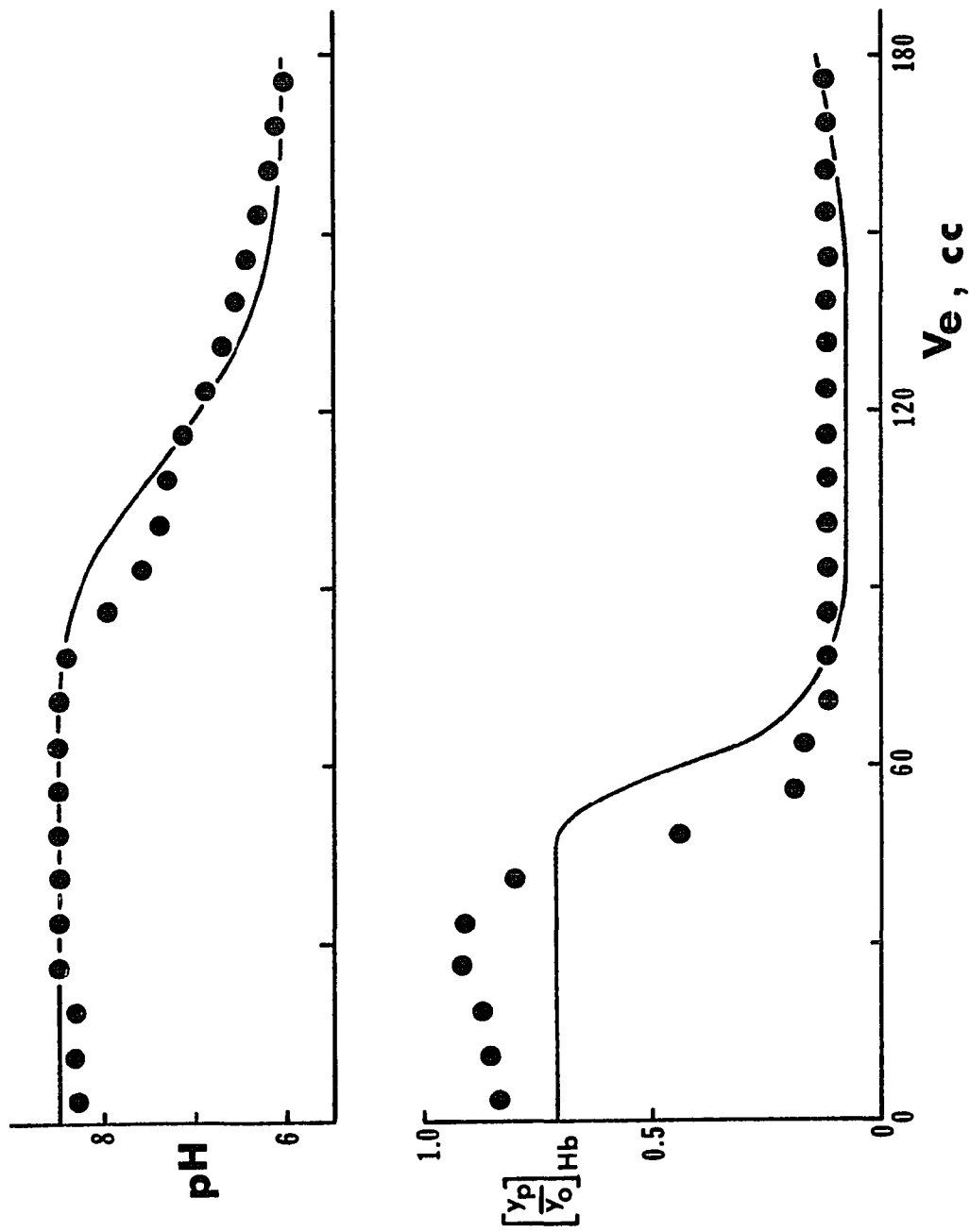
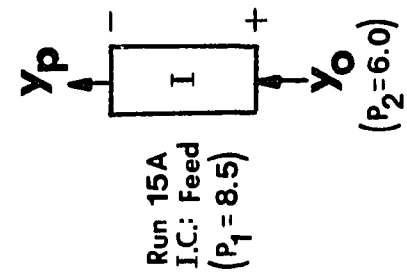
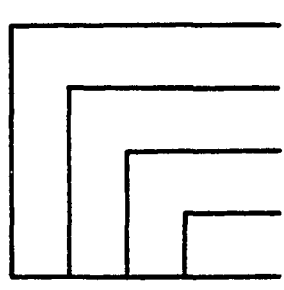


FIGURE 66. Calculated Adsorption with Electric Field

Hemoglobin Separation in the New Process

The mathematical model developed thus far, will now be used to calculate the parametric pumping data for the new process (Modes 6-8). The complete computer program with the appropriate external mass balances is given in Appendix C (Program #2). Albumin and hemoglobin are assumed not to interact and are calculated separately. The hemoglobin segment of the program is set-up with four nested "DO loops".



```

DO 540 n = 1,N (cycles)
DO 540 l = 1,8 (stages)
DO 540 j = 1,Γ (transfer steps)
DO 540 i = 1,M (cells-in-series)
540 CONTINUE

```

The preceding calculations are repeated for M cells, Γ transfer steps, 8 stages (Stage IV becomes three stages), and N cycles with appropriate changes in inlet and outlet conditions for each stage. The experimental parameters in Table 10 are used except as noted below.

Run 55 will be calculated first because this run has the best experimental separation (Figures 67-70). Based on the constraint in Equation 6-29 for a whole number of transfer steps, $M = 18, 36, 54, \dots$ should be used instead of $M = 21$. Run 55A with $M = 36$ cells-in-series gave a better fit to the data than Run 55B with $M = 18$. Runs 55A and 55B are both given in Appendix C. The individual concentrations in Figure 67 as well as the weight percentages and the albumin and hemoglobin separations in Figure 68 are well-predicted

by the model.

Different values of the mass-transfer coefficient λ were examined as shown in Table 11. The value for λ at low flow rate $Q_p = 0.5$ cc/min is larger than 1.0 as previously calculated. The separation drops off sharply as the magnitude of λ at Q_p decreases from 1.0 to 0.1. Any value of λ from zero to infinity gives the same separation at high flow rate $Q_o = 2.5$ cc/min. Since the adsorption steps (Stages I to III) take place at Q_o and adsorption does in fact occur, the mass-transfer coefficient at the high flow rate cannot possibly be zero. This simply means that the exponential function in Equation 6-19 is small enough under the experimental conditions, that the value of λ at Q_o cannot be determined in this manner.

The top and bottom products in each cycle are measured as an average value for the entire volume taken. The concentration profiles for these products are calculated as a function of cycle number in Figures 69 and 70. The advantage of parametric pumping over one-way processes is the improvement in these products from cycle-to-cycle up to some limiting separation. As shown in Figure 69, the effluent to the top product in Cycle 1 resembles a portion of a typical breakthrough curve and approaches 1.0. By Cycle 10 the top product concentration is reduced to $b = 0.10$ or less. The bottom product increases in concentration from cycle-to-cycle as shown in Figure 70. The concentration profiles shown in Figure 70 are the effluent streams from the bottom of the

column to the holding tank at the end of Stage V. This fluid is mixed in the holding tank prior to taking the bottom product, so that the bottom product stream is at a constant concentration value. Both product streams continue to improve with the top product decreasing in hemoglobin concentration and increasing in albumin concentration, and the bottom product increasing in hemoglobin concentration and decreasing in albumin concentration until steady-state is reached.

The other runs via the new process are calculated in Figures 71-76. Run 56 in Figure 71 is the case with $F_B = F_T = 35.0$ cc which can be compared directly to the base case previously discussed in Figure 21. In the calculations in Chapter 2, the graphical solution for the enrichment case predicted improved hemoglobin separation due to the addition of the electric field. The hemoglobin separation is twenty-five in Run 56 at the tenth cycle as compared to twenty in the base case. The effect of this improvement on the overall separation is small, however, compared to the improvement achieved in the albumin separation.

The recoveries in the new process are listed in Table 12. Since the initial cycles are not at steady-state, the δ functions are not expected to be 100%. The ξ function is a better measurement for recovery, and should be 100%. The model fits the data very well in Runs 55 and 56 where the ξ function is close to 100%, and reasonably well in Runs 54, 58, and 60 where the ξ function is around 90%. The

data is not well-predicted when the recovery is low as in Runs 57 and 59, because the model assumes 100% recovery. The decreased recovery in these two runs is not well-understood. Conditions such as high wattage which are known to decrease recovery have been avoided in the process, since the optimum operating conditions require maximum recovery. It has also been recommended that the low feed rate in Run 57 be avoided, since it is desirable to maximize production rate. Using a low feed rate in the four-stage pH parapump similarly reduces both separation and recovery as shown in Figure 77.

The external buffer system was cooled to 288°K in all the runs discussed thus far. As shown in Figure 78, the separation and recovery are lower if the experimental set-up does not include buffer cooling. The decreased protein recovery has little effect on the top product, but the hemoglobin concentration in the bottom product decreases as the recovery decreases. For some reason, part of the adsorbed protein sticks permanently to the gel when the recovery is low. In order to add an a priori correction for recovery to the model, the physical mechanism for this limited desorption needs to be understood. The mathematical model as written works very well as long as the recovery is high.

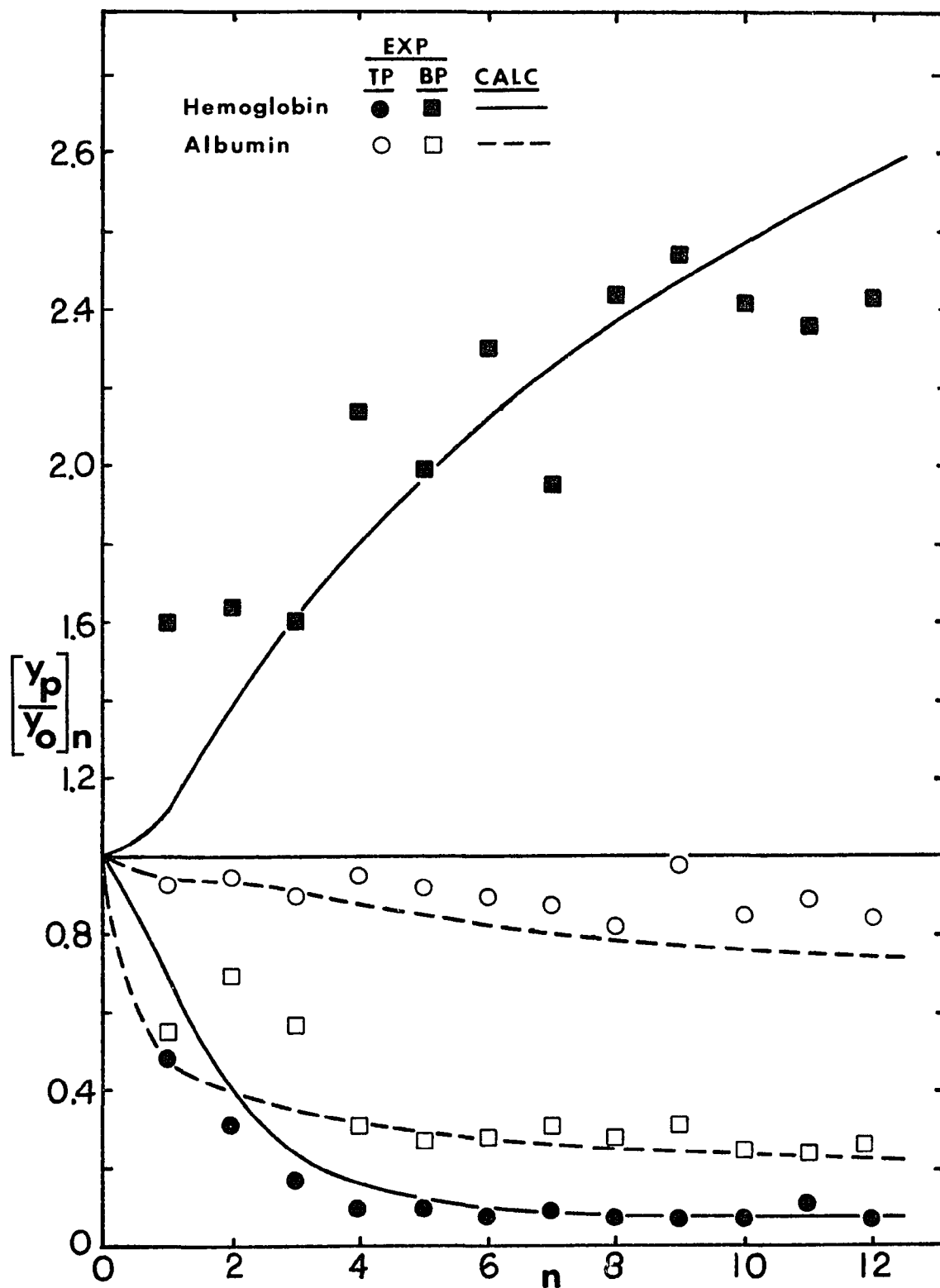


FIGURE 67. Calculated vs. Experimental Concentrations
 (Mode 7 - Run 55A: $F_B = 52.5$ cc & $F_T = 17.5$ cc)

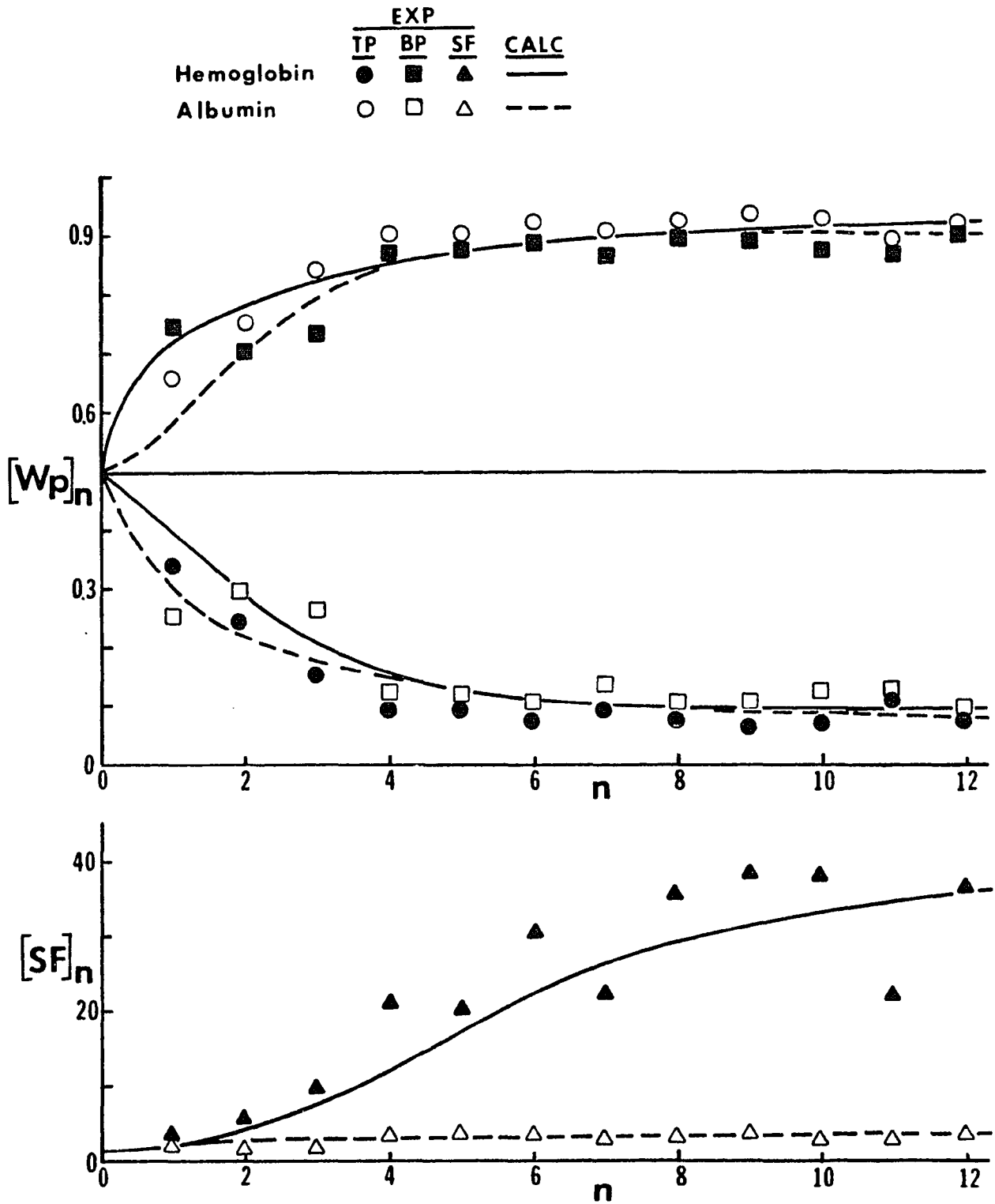


FIGURE 68. Calculated vs. Experimental Results
 (Mode 7 - Run 55A: $F_B = 52.5$ cc & $F_T = 17.5$ cc)

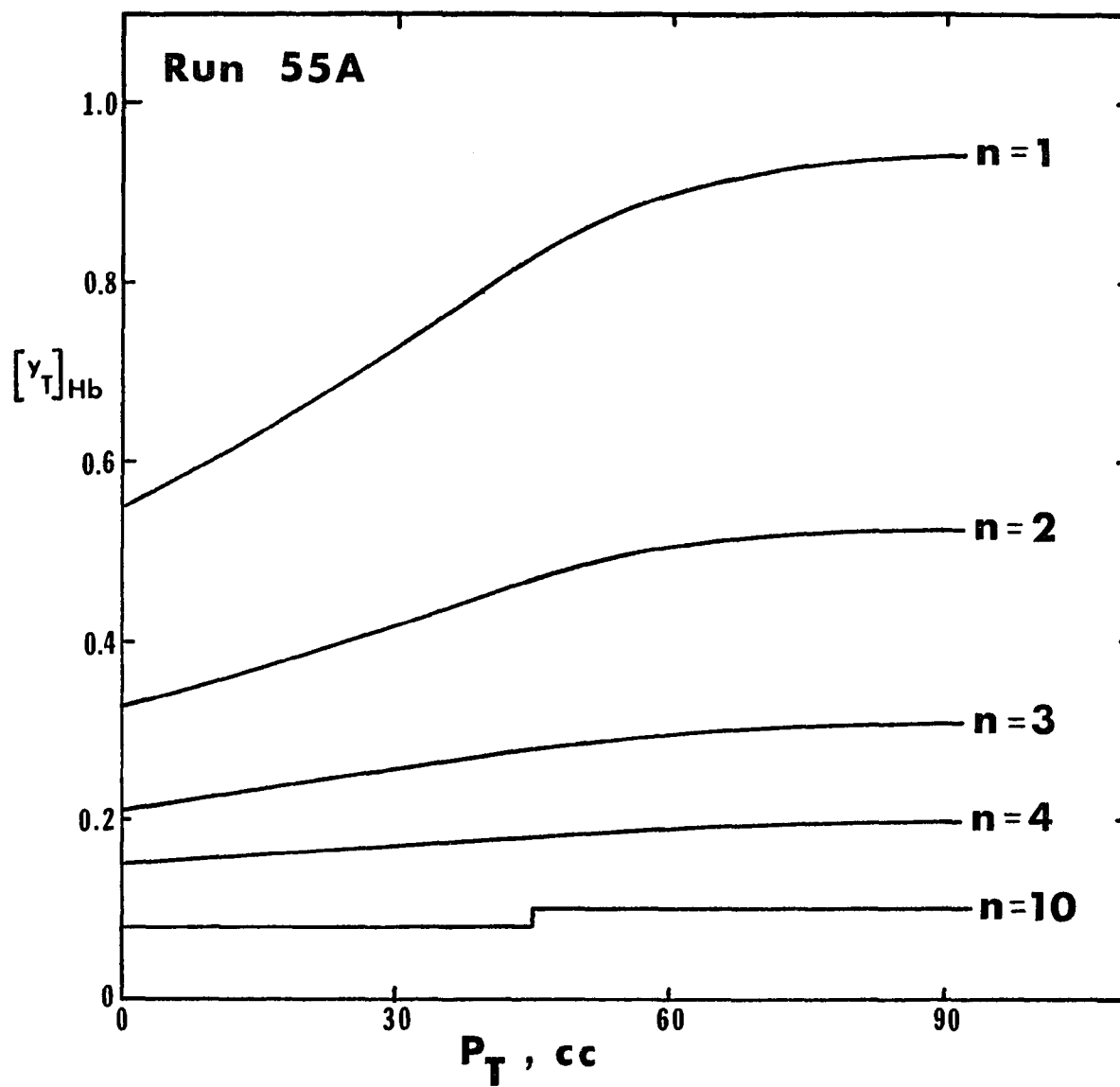


FIGURE 69. Decrease in Top Product Hemoglobin Concentration with Repeating Cycles

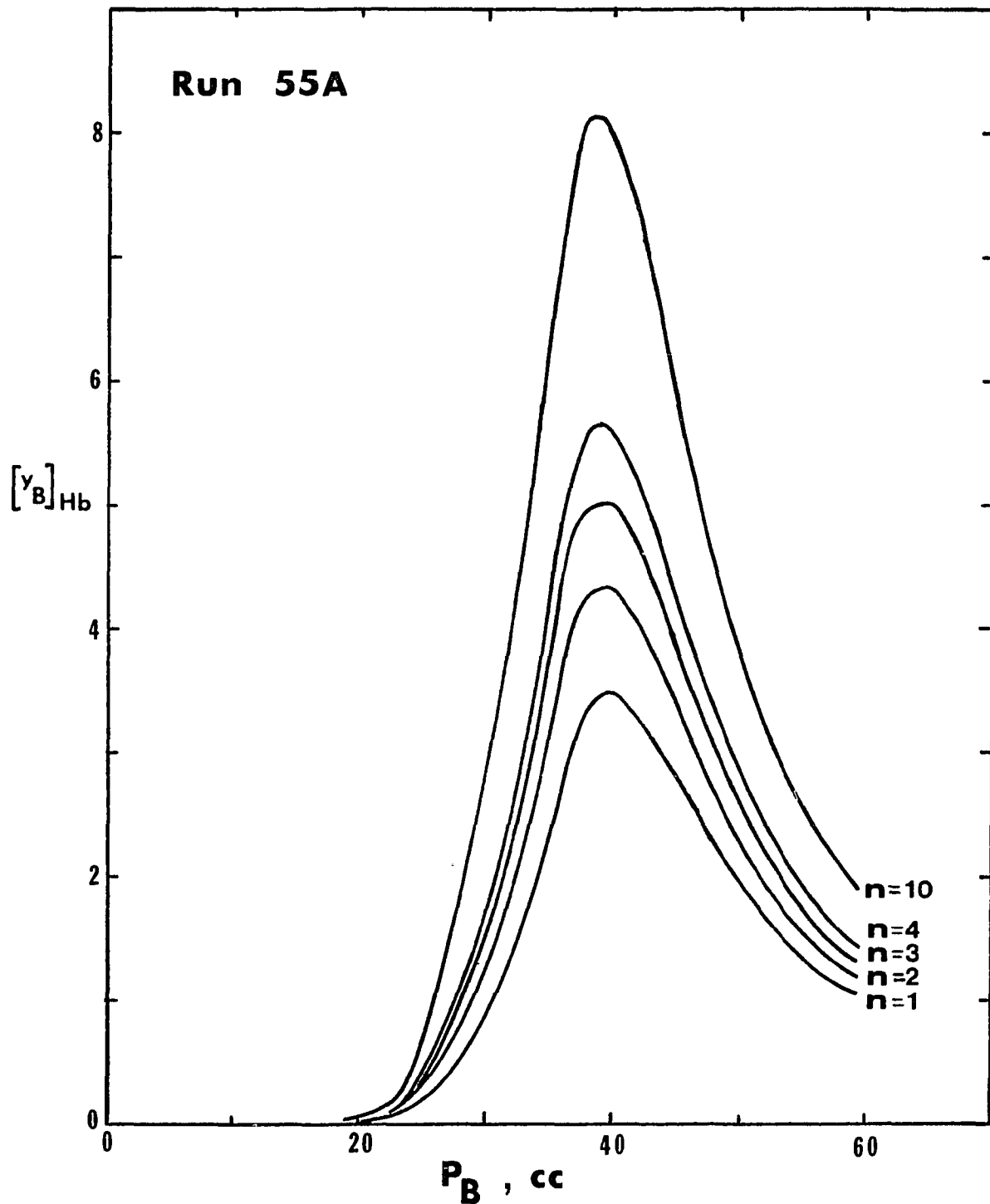


FIGURE 70. Increase in Bottom Product Hemoglobin Concentration with Repeating Cycles

TABLE 11
Effect of λ on the Overall Separation

<u>λ at Q_0</u>	<u>λ at Q_p</u>	<u>α_{12}</u>	<u>α_{25}</u>
∞	∞	118	148
2.5	2.5	118	148
"	2.0	118	148
"	1.5	118	148
"	1.0	116	146
"	0.9	115	145
"	0.8	114	143
"	0.7	112	141
"	0.6	110	137
"	0.5	105	132
"	0.4	98	122
"	0.3	85	104
"	0.2	63	75
"	0.1	33	37
2.0	2.5	118	148
1.5	"	118	148
1.0	"	118	148
0.1	"	118	148
0.01	"	117	148
0.001	"	117	148
0.0001	"	117	148
0.00001	"	117	148
0.000001	"	117	148

Note: Calculations based on Run 55A.

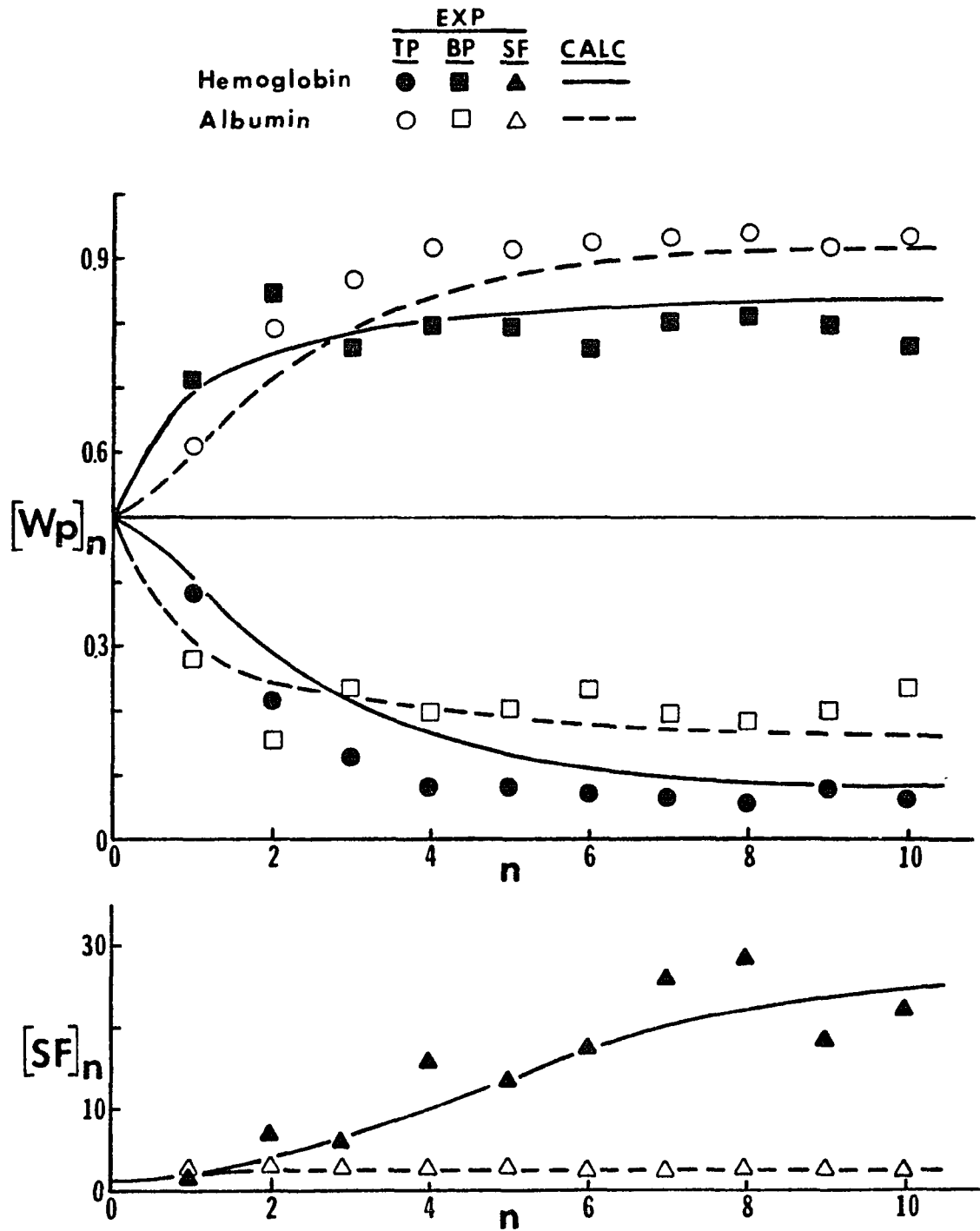


FIGURE 71. Calculated vs. Experimental Results

(Mode 7 - Run 56A: $F_B = 35.0$ cc & $F_T = 35.0$ cc)

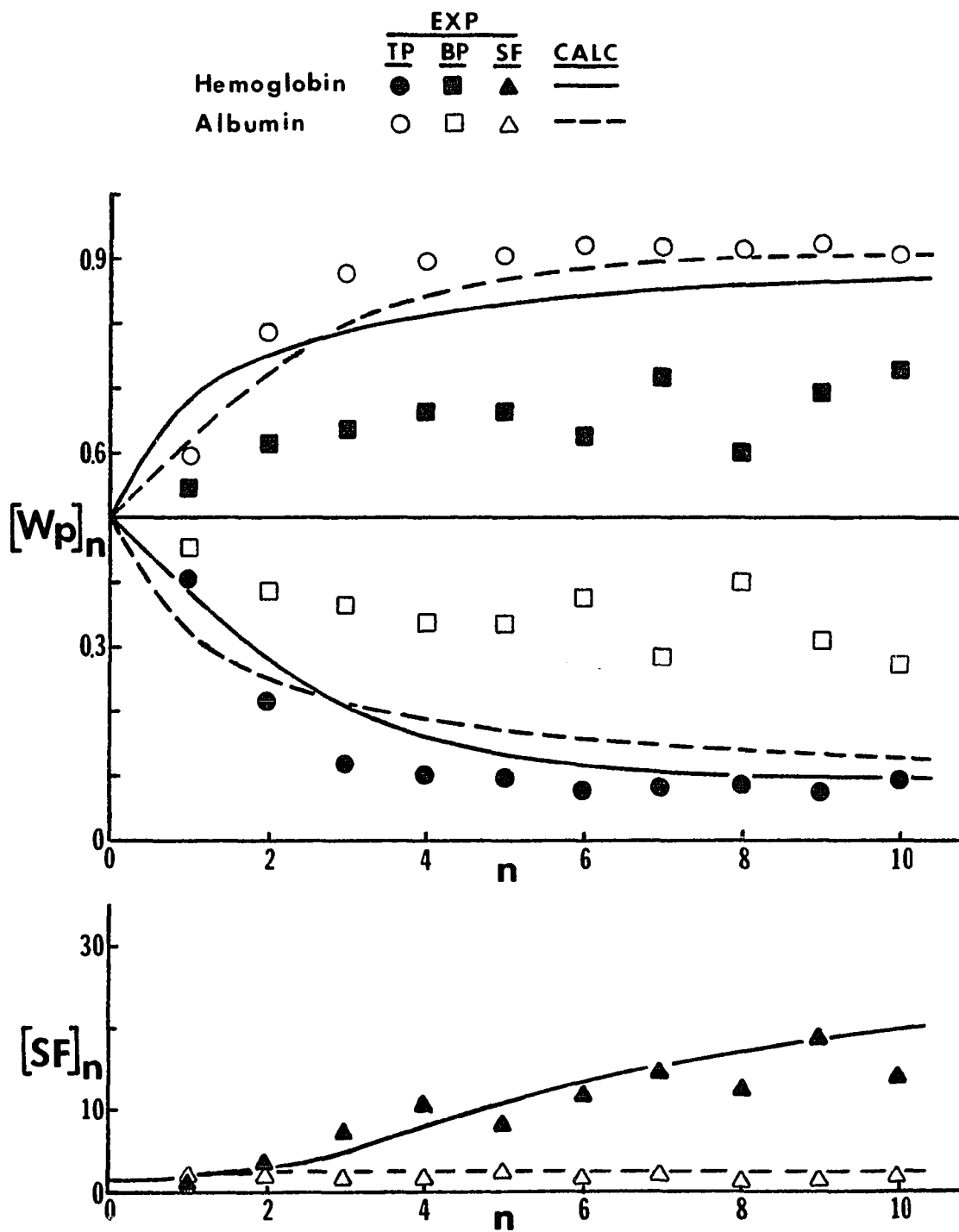


FIGURE 72. Calculated vs. Experimental Results

(Mode 7 - Run 57B: $F_B = 17.5$ cc & $F_T = 17.5$ cc)

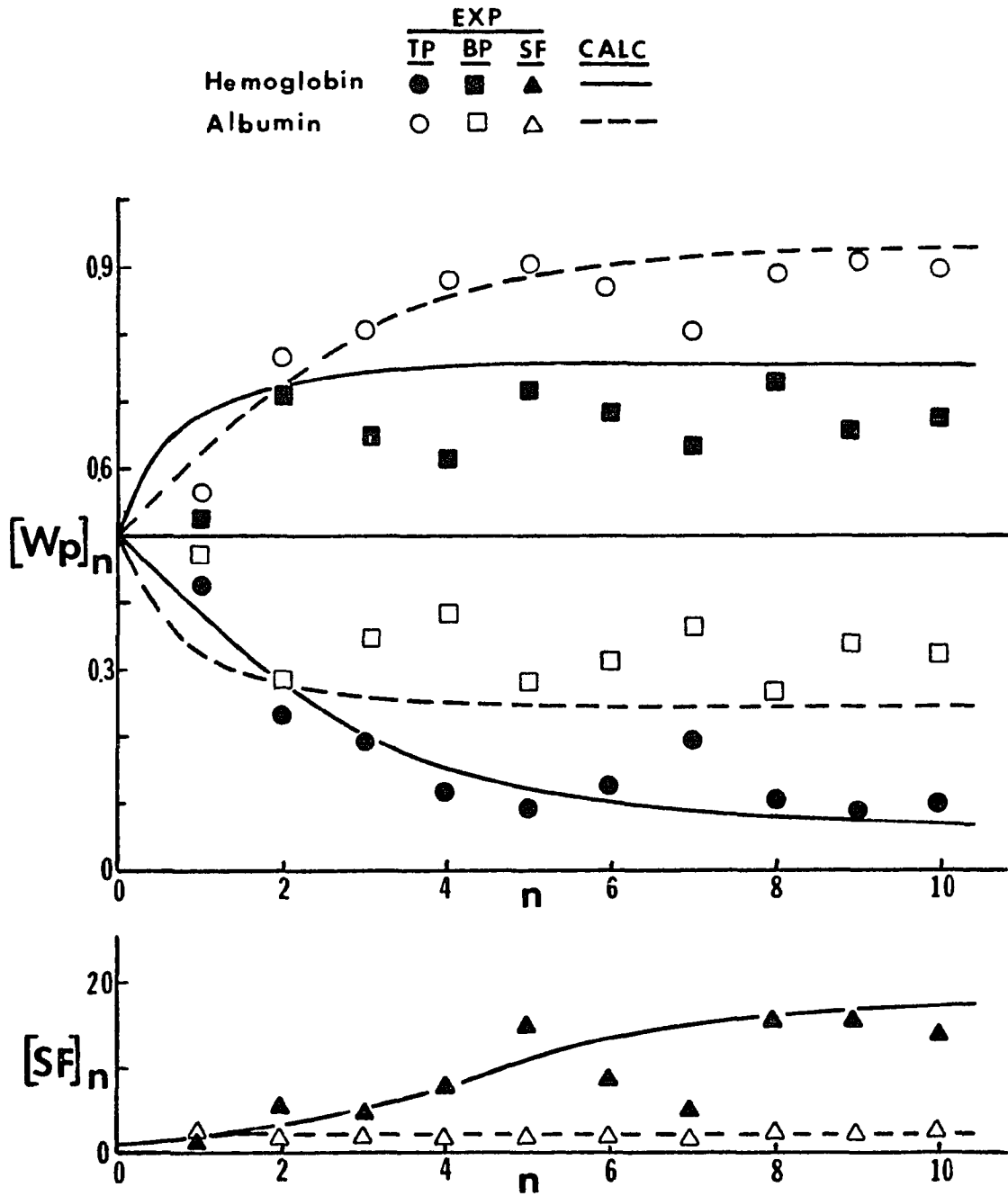


FIGURE 73. Calculated vs. Experimental Results
 (Mode 7 - Run 58B: $F_B = 17.5$ cc & $F_T = 52.5$ cc)

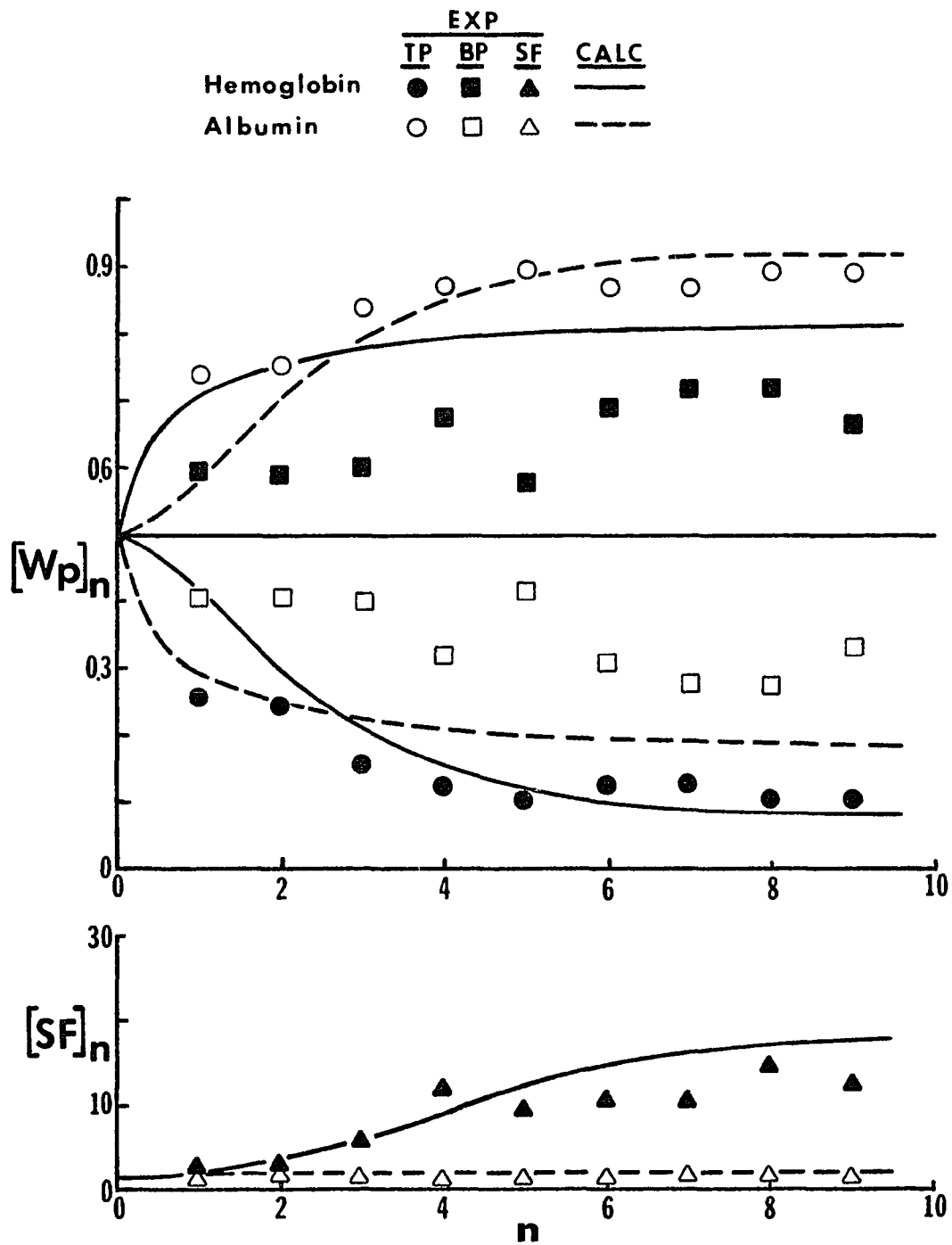


FIGURE 74. Calculated vs. Experimental Results
 (Mode 7 - Run 59B: $F_B = 52.5$ cc & $F_T = 52.5$ cc)

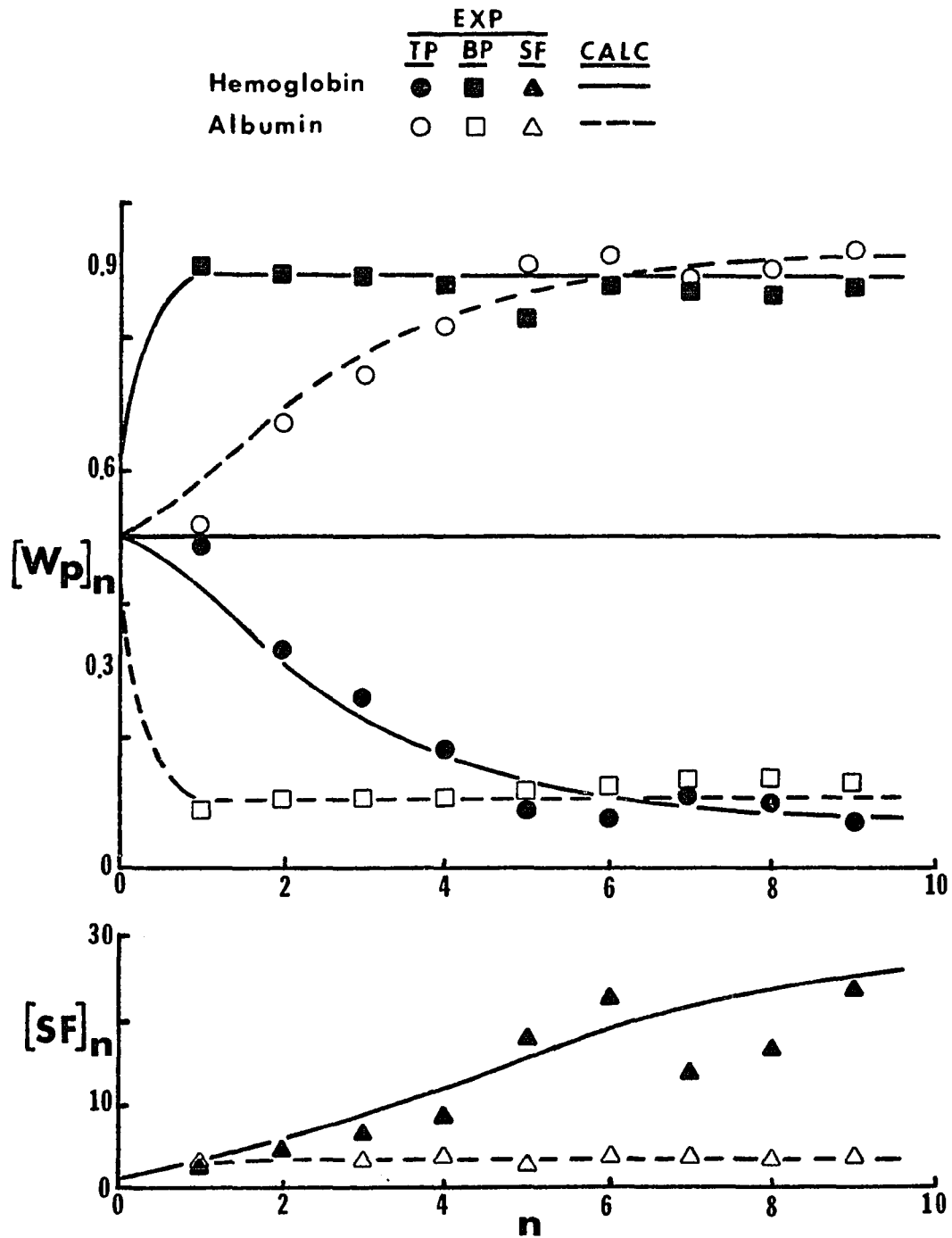


FIGURE 75. Calculated vs. Experimental Results
 (Mode 8 - Run 60A: $F_B = 35.0$ cc & $F_T = 35.0$ cc)

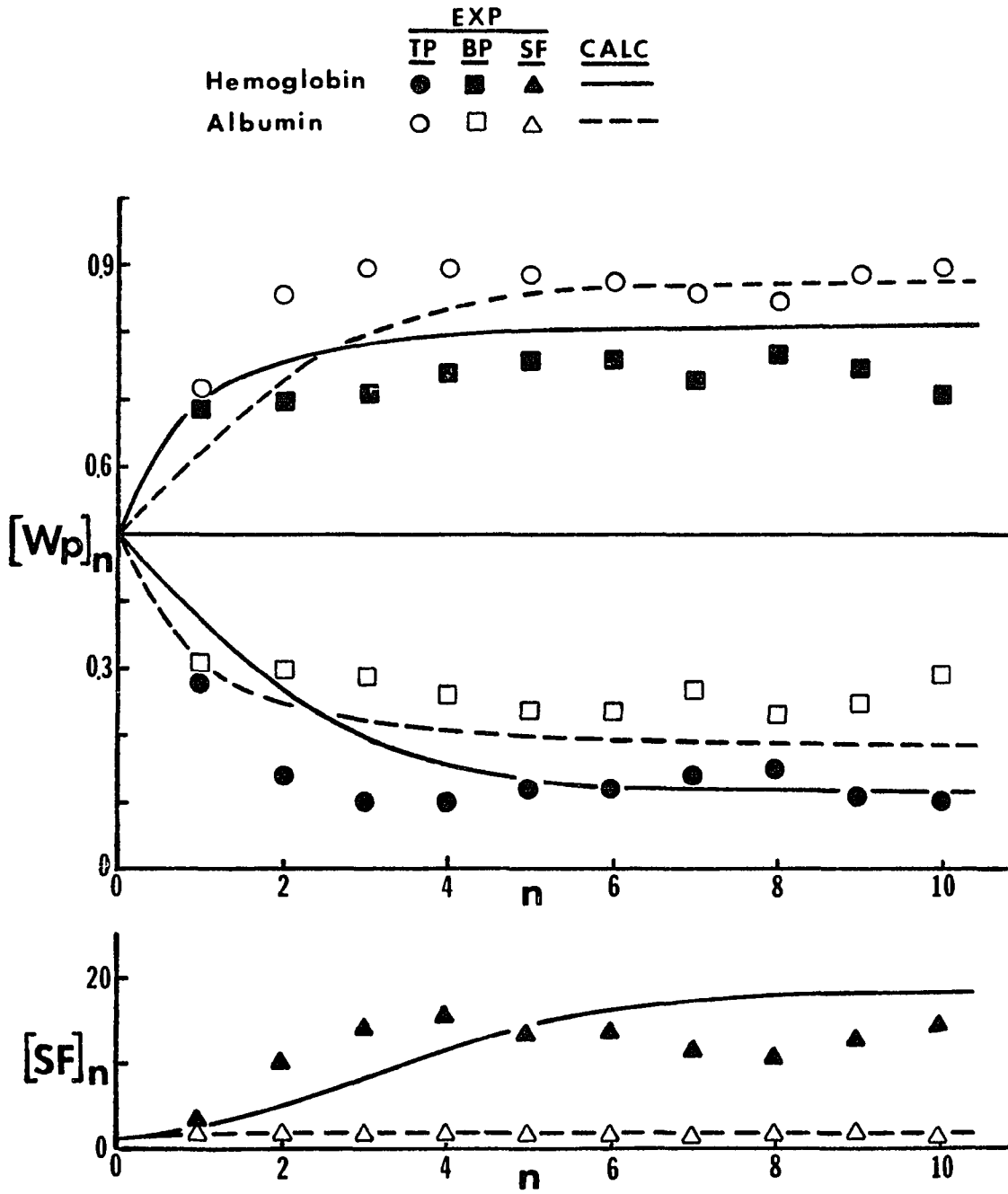


FIGURE 76. Calculated vs. Experimental Results
 (Mode 6 - Run 54B: $F_B = 35.0$ cc & $F_T = 35.0$ cc)

TABLE 12

Protein Recovery in the New Process

<u>Run</u>	<u>Mode</u>	<u>$\langle \delta_{\text{EXP}} \rangle (\%)$</u>		<u>$\langle \xi \rangle (\%)$</u>		
		<u>Al</u>	<u>Hb</u>	<u>Al</u>	<u>Hb</u>	<u>Protein</u>
54B	6	117.-	83.3	104.-	77.1	91.-
55A	7	128.-	71.6	110.-	94.1	102.-
56A	"	119.-	89.3	108.-	91.4	100.-
57B	"	61.3	49.1	44.6	52.3	48.5
58B	"	107.-	89.8	104.-	86.3	95.-
59B	"	87.4	57.6	85.3	60.3	72.8
60A	8	99.5	112.-	93.1	90.1	91.6

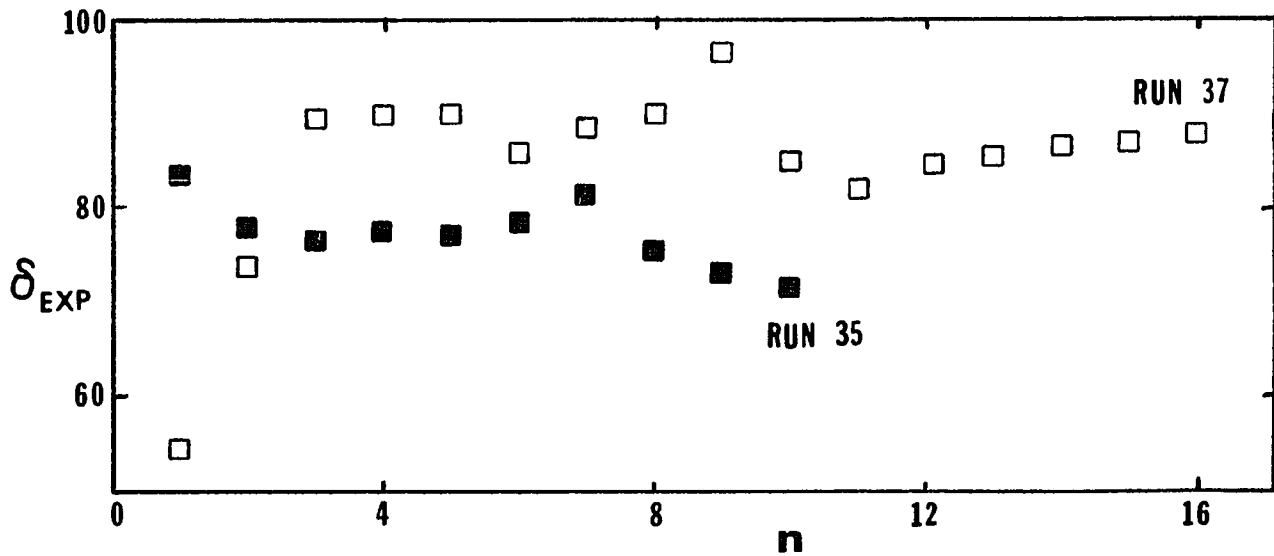
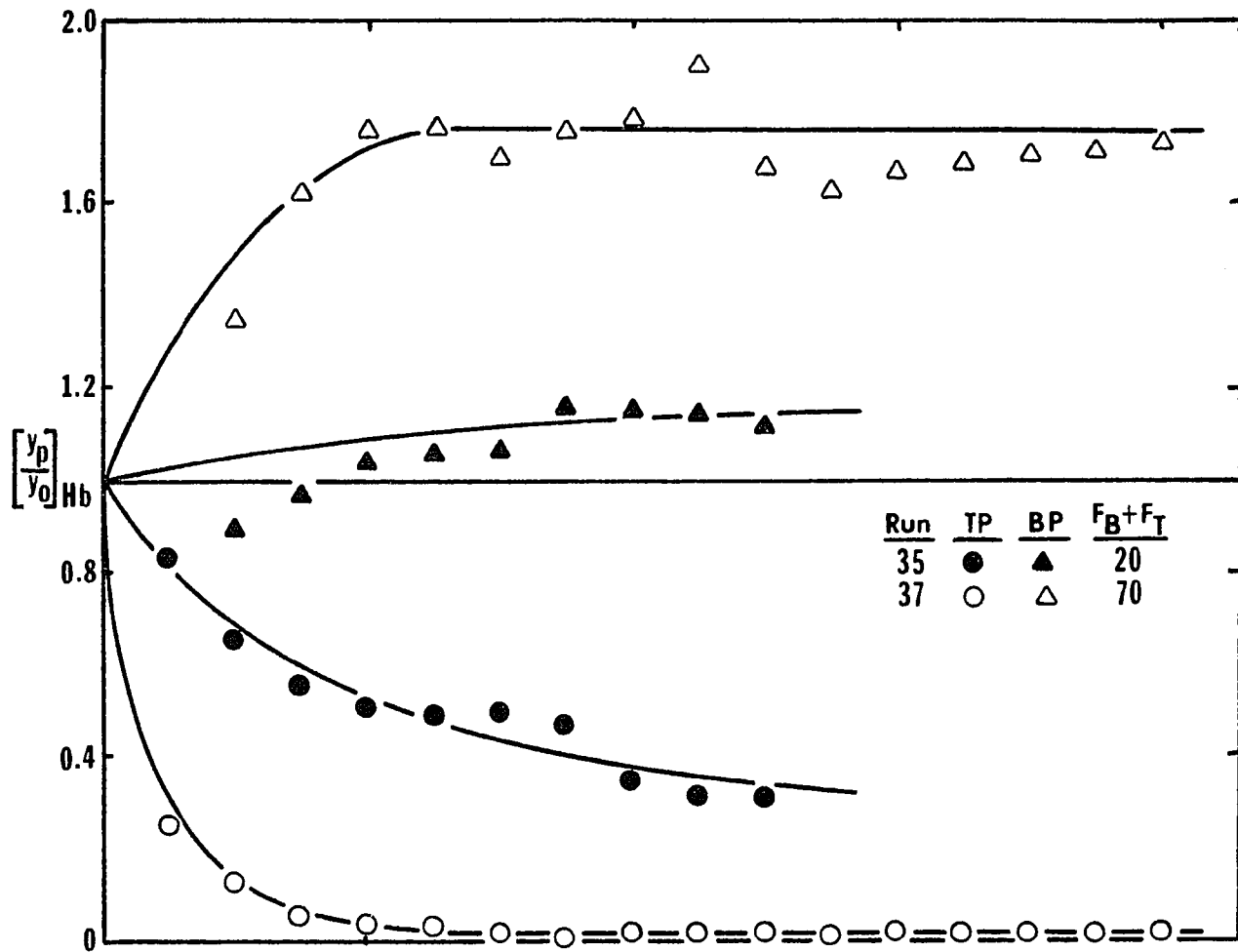


FIGURE 77. Effect of Low Feed Rate on Hemoglobin Recovery and Separation (Experimental)

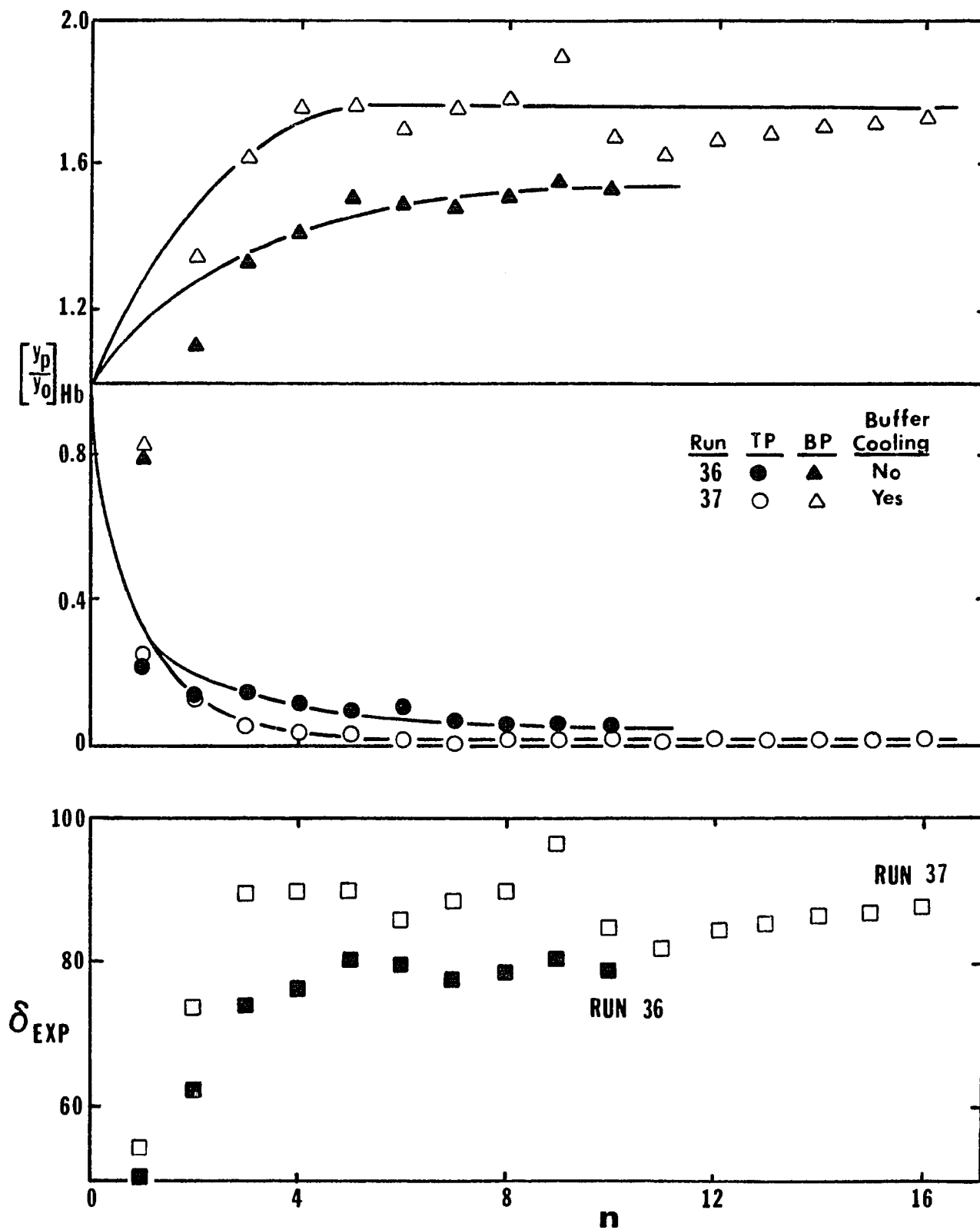


FIGURE 78. Effect of Buffer Cooling on Hemoglobin Recovery and Separation (Experimental)

Chapter 7

PRELIMINARY STUDIES: THE ENRICHMENT

PROCESS WITH ELECTRIC FIELD

The new process in Modes 5-10 is a separation process in that Protein A is stripped from the top product streams while Protein B is stripped from the bottom product streams. The preliminary work with the electric field (Modes 1-4) considered an enrichment process where Protein A or hemoglobin was stripped from the top streams and enriched in the bottom streams while Protein B concentrations remained unchanged. The graphical method in Chapter 2 calculated that the enrichment process has the potential for better separation when an electric field is applied to the pH parapump.

The first successful parapump with electric field is shown in Figure 79. Figures 79 and 80 were presented by Dr. H.T. Chen at the Gordon Conference in August, 1980. The parapump in Figure 79 is Mode 3 of operation. Mode 3 is identical to the four-stage pH parapump in Figure 8 (Chen et al., 1979a), except that Stage I is divided into two parts. The power is applied in Stages II and III and in the second part of Stage I.

The experimental results for Mode 3 are shown in Figure 80. The hemoglobin separation was increased from twelve to twenty-one by addition of the electric field. Runs 41 and 49 used 0.05M phosphate buffer plus 0.05M NaCl with $P_1 = 8.0$ and $P_2 = 6.0$ at a field strength of 120 volts. A similar pair of runs with 0.01M phosphate buffer (Runs 39

and 50, Appendix B) showed no effect of the electric field. The hemoglobin migration velocity was evidently negligible in the 0.01M phosphate buffer at 120 volts. The importance of the increased separation in Figure 80 is that it proved unequivocally that the electric field could actually improve the separation normally achieved in the pH parapump. The magnitude of the separation in this preliminary experiment is much less than the optimal separations achieved or predicted via the new process.

Mode 3 was then tested on the mixture (Figure 81). The hemoglobin separation was again improved, but the albumin separation was decreased, so that the overall separation was unchanged. This data pointed to the fact that the relative migration velocities of hemoglobin and albumin must both be considered in the final process. One preliminary experiment via Mode 4 attempted to use the electric field to enrich albumin. The albumin separation was also decreased in this experiment as seen in Run 49, Appendix B.

The first two modes of operation are shown in Figures 82 and 83. Modes 1 and 2 are identical to the four-stage pH parametric pumping process except that an electric field is applied in Stages I and III in Mode 1 and in Stages II and III in Mode 2. The top product in Mode 1 (Figure 82) was less concentrated in hemoglobin as predicted, but unfortunately the bottom product was also less concentrated leading to decreased hemoglobin recovery. Mode 2 in Figure 83 also gave poor recovery. The best enrichment data was obtained via Mode 3 .

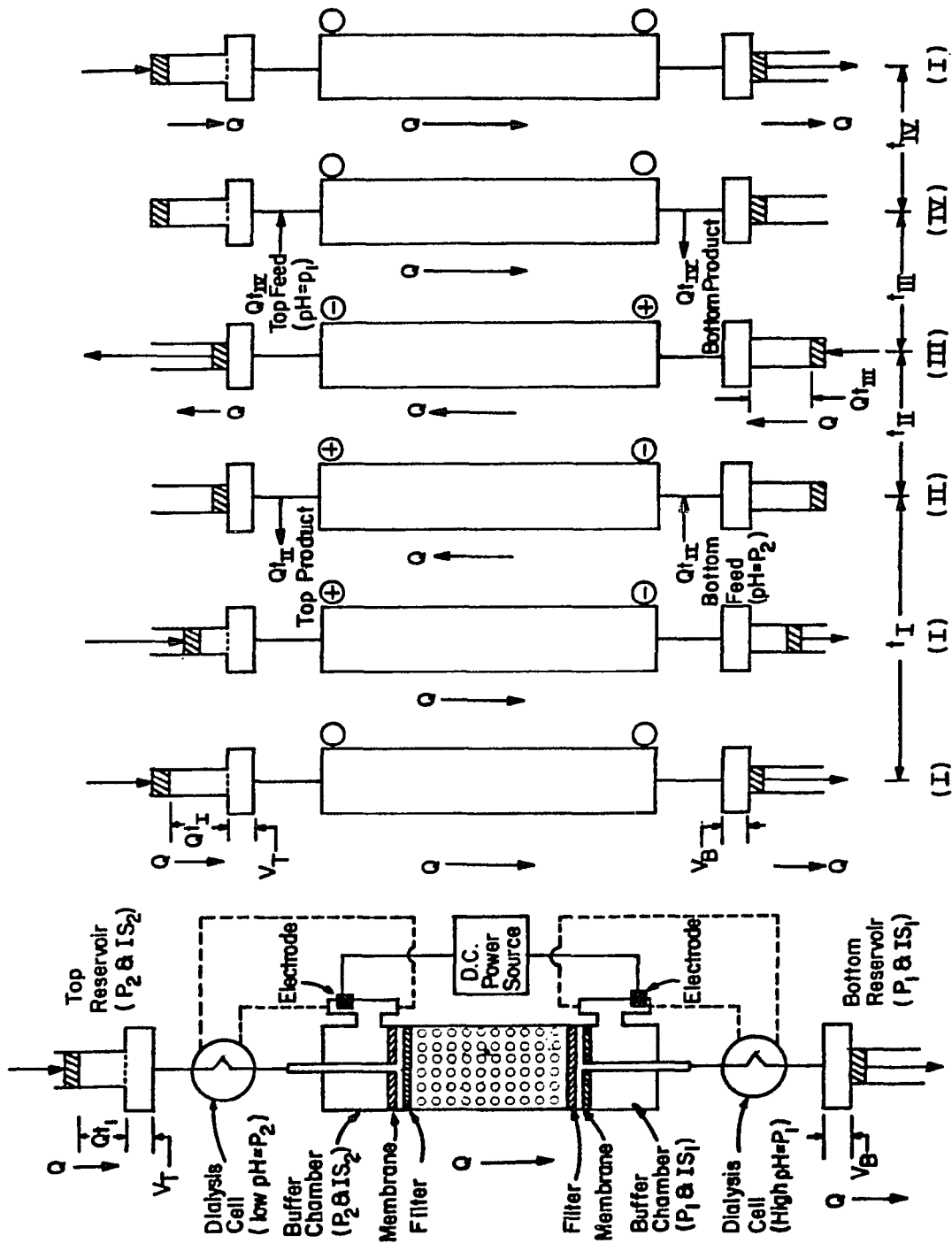


FIGURE 79. pH Parametric Pumping with Electric Field: The Enrichment Process

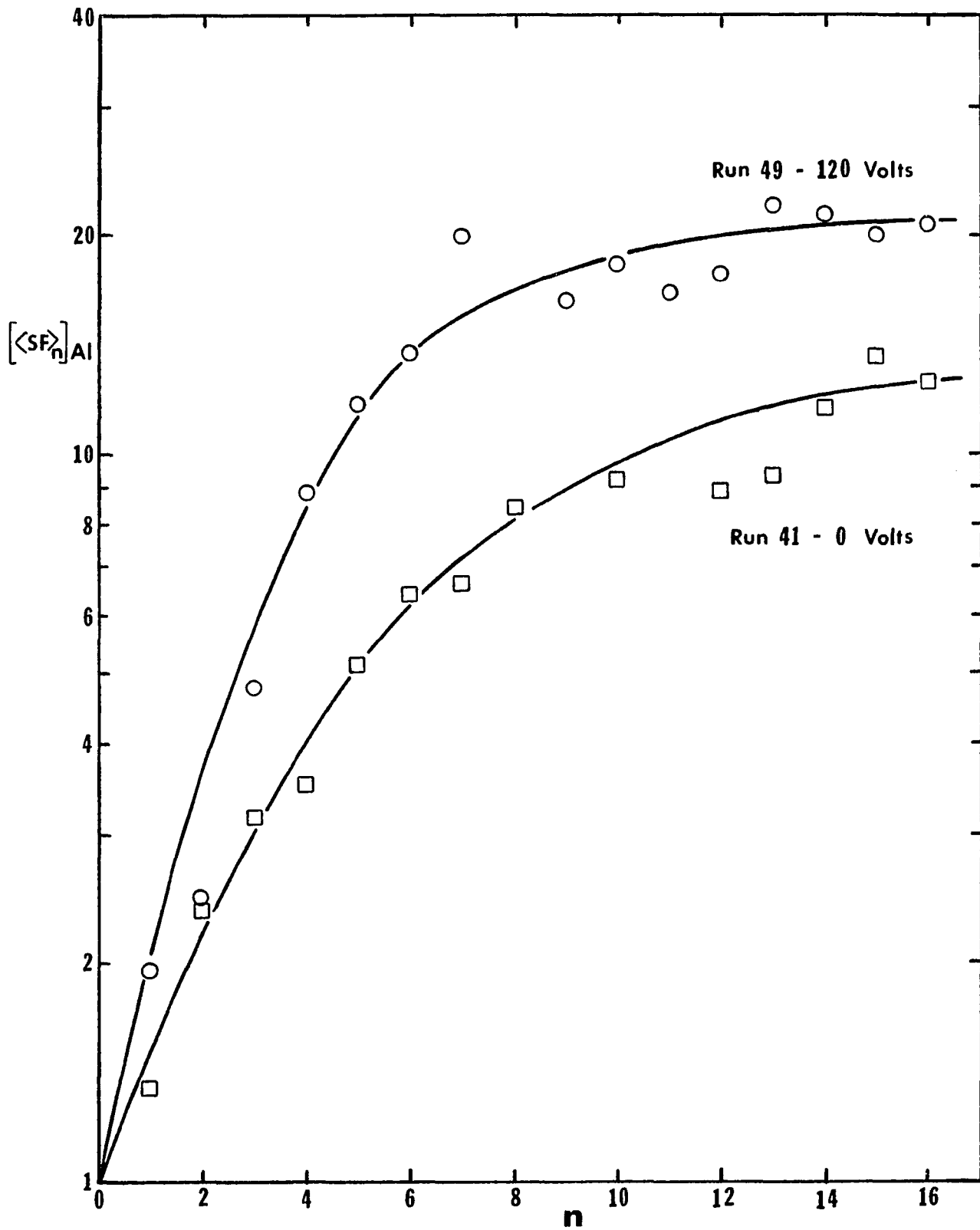


FIGURE 80. Experimental Results for the Enrichment Process with Electric Field (Mode 3)

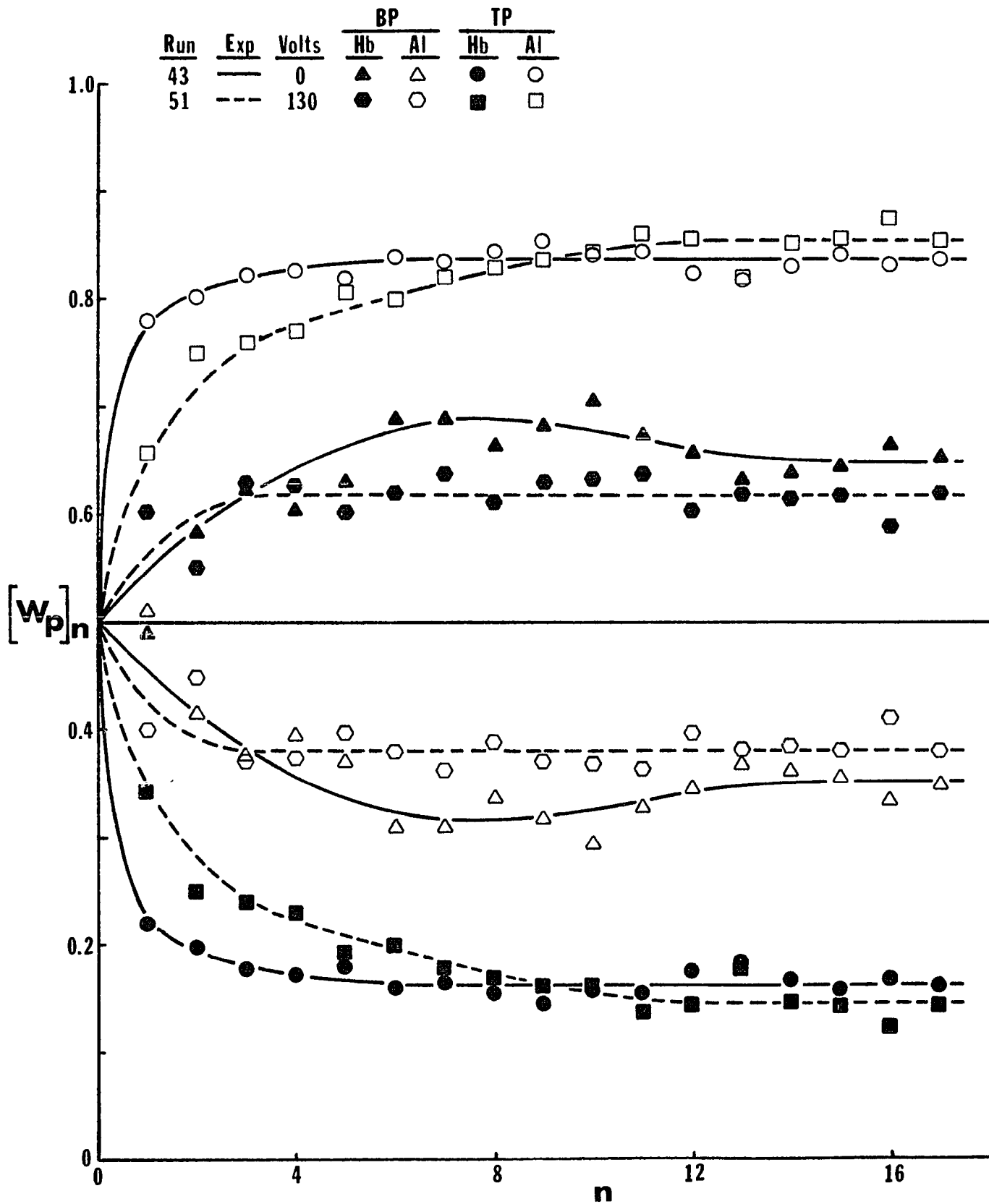


FIGURE 81. Experimental Results for the Mixture
via Mode 3 Operation

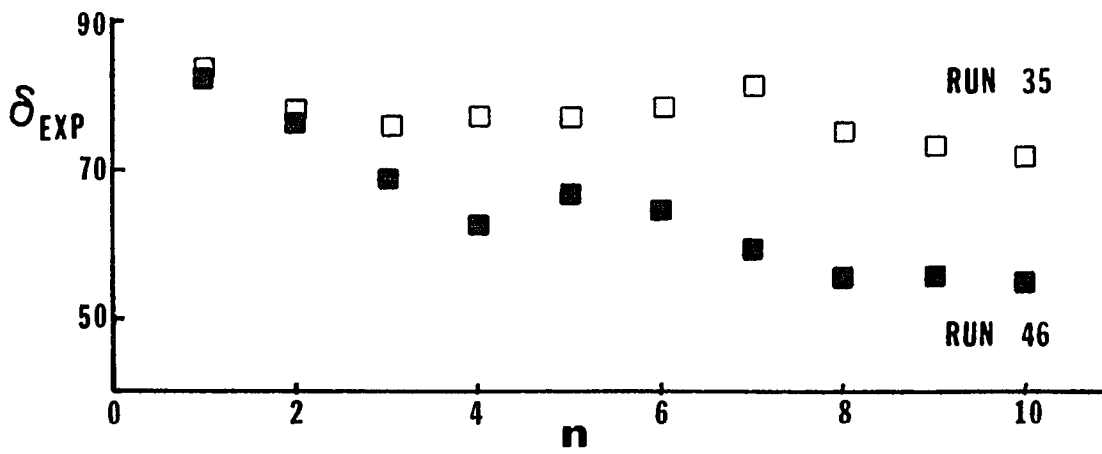
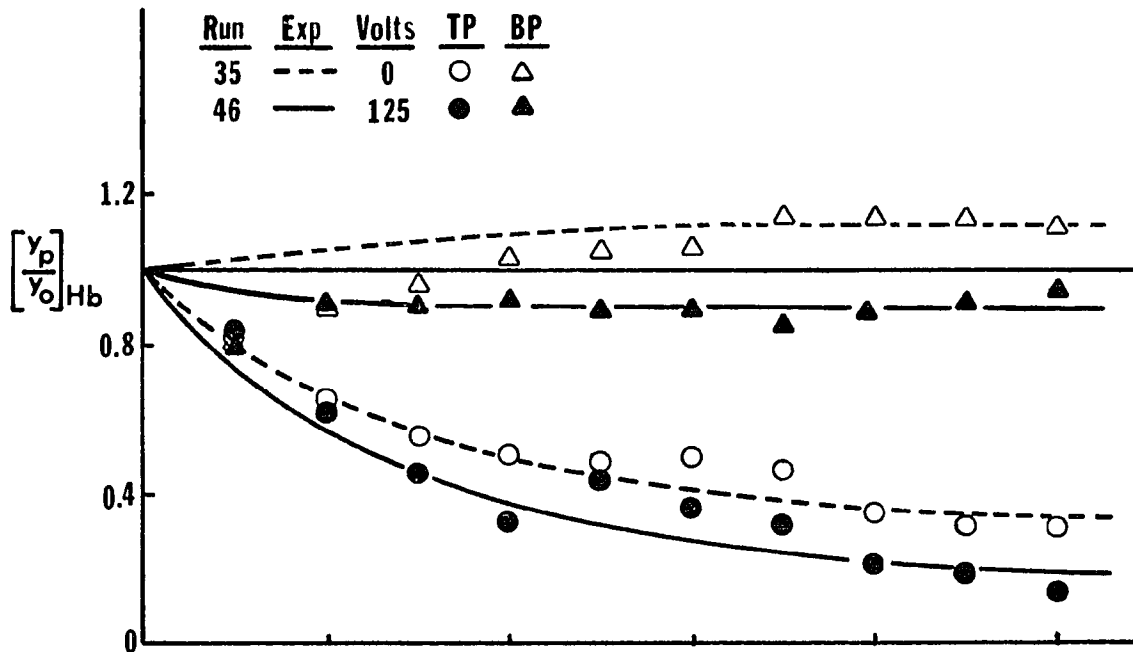
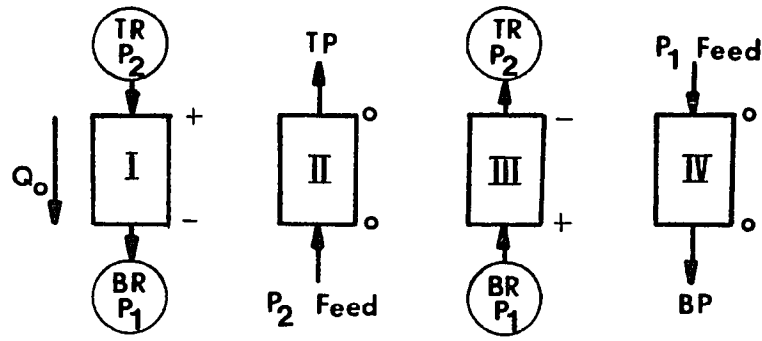


FIGURE 82. Experimental Enrichment of Hemoglobin via Mode 1 Operation

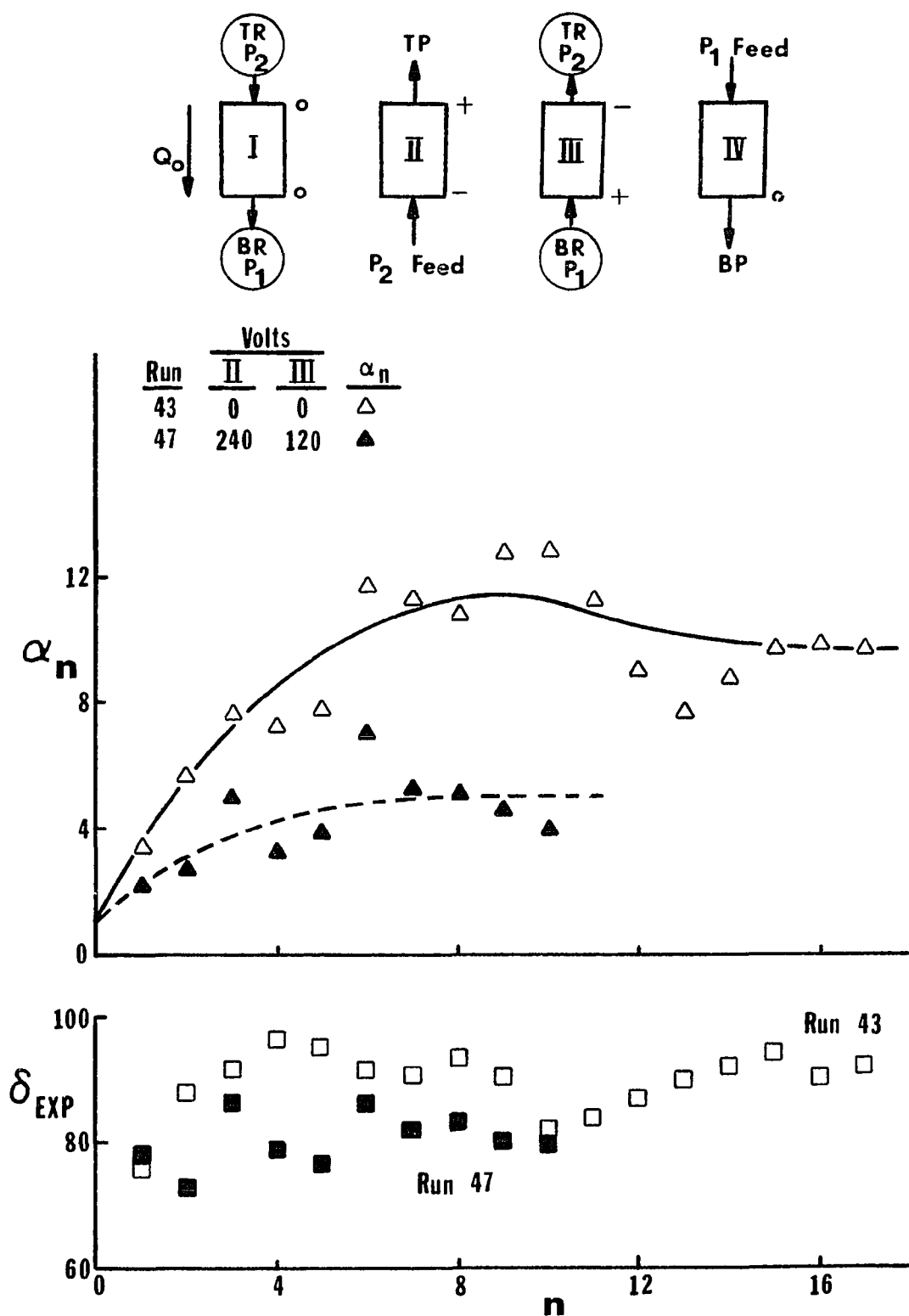


FIGURE 83. Experimental Enrichment of Hemoglobin via Mode 2 Operation

Chapter 8

CONCLUSIONS

A new separation process has been developed based on the principles of pH parametric pumping and electrophoresis. This is the first parametric pumping process to use electrophoresis as a basis for separation. The separation in the hybrid system is superior to that which can be obtained by single-column pH parametric pumping, electropolarization chromatography, or conventional polyacrylamide gel electrophoresis.

The maximum overall separation α which has been demonstrated experimentally via the new process is 120 (Mode 7 with $F_B = 3 F_T$). With equal top and bottom feeds or $F_B = F_T$, an α of 50 was obtained in Mode 7 and an α of 90 in Mode 8. This may be compared to the multicolumn pH parapump with an experimentally demonstrated α of 32 for the batch process and 21 for the semi-continuous process when F_B equals F_T . The proper combination of the two variables pH ($P_1 > I_A > P_2$) and electric field in a single ion exchange bed is, therefore, superior to the use of three pH levels ($P_1 > I_A > P_2 > I_B > P_3$) in two beds packed alternately with cation and anion exchangers.

The finite mass-transfer model for the pH parapump (Chen et al., 1981a) has been modified to simulate the new process. The model is solved by the finite difference method and the STOP & GO algorithm. The adsorptive properties of the ion exchanger are assumed to be unaffected by the electric field. The equations for the electrophoretic migration of the two

proteins in the mixture, are added to the GO step or the momentum transport step of the model. The electric field also affects the velocity of the pH wave, and the pH wave equations are included in the GO step. The pH wave velocity is linked to the desorptive concentration wave velocity, so the desorption process is indirectly affected by the electric field. The transport constants needed for the model were found by computer-fit to pulse experiments. The mathematical model simulates the experimental data in the new process quite well.

The new process was mathematically optimized. Mode 8 is the optimum mode of operation. The maximum separation is obtained when $F_T < 0.5V$, $F_B > 0.5V$, and $F_B \gg F_T$. The maximum separation is predicted when $0 < F_B + F_T < V$, but the experimental recovery in this range was low. A maximum production rate obviously dictates that $F_B + F_T \gg 0$. The optimum between maximum separation and maximum production rate occurs in the range $V < F_B + F_T < 2V$. The experimental recovery was much better in this intermediate range, and the predicted separation is still relatively high.

The new process was developed for an arbitrary mixture of hemoglobin and albumin, but may be applied to any mixture of proteins which have different isoelectric points. Protein separations are generally long tedious procedures involving many process steps. The pH parapump with electric field offers the potential for excellent separation via a comparatively simple process.

APPENDIX A
EXPERIMENTAL CALCULATIONS AND METHODS

TABLE 13Phosphate Buffer

Ref: Colowick & Kaplan, 1955

The phosphate buffer was made-up by mixing equal molar solutions of monobasic-phosphate, NaH_2PO_4 , and dibasic-phosphate, Na_2HPO_4 , until the desired pH was obtained. The correct proportions may be estimated from the data below.

<u>pH</u>	<u>NaH_2PO_4</u>	<u>Na_2HPO_4</u>	<u>pH</u>	<u>NaH_2PO_4</u>	<u>Na_2HPO_4</u>
5.7	93.5	6.5	7.5	16.0	84.0
5.8	92.0	8.0	7.6	13.0	87.0
5.9	90.0	10.0	7.7	10.5	90.5
6.0	87.7	12.3	7.8	8.5	91.5
6.1	85.0	15.0	7.9	7.0	93.0
6.2	81.5	18.5	8.0	5.3	94.7
6.3	77.5	22.5			
6.4	73.5	26.5			
6.5	68.5	31.5			
6.6	62.5	37.5			
6.7	56.5	43.5			
6.8	51.0	49.0			
6.9	45.0	55.0			
7.0	39.0	61.0			
7.1	33.0	67.0			
7.2	28.0	72.0			
7.3	23.0	77.0			
7.4	19.0	81.0			

TABLE 14

Tris-maleate / NaOH Buffer

Ref: Colowick & Kaplan, 1955

The Tris-maleate / NaOH buffer was made-up by mixing equal molar solutions of Tris-maleate and sodium hydroxide, until the desired pH was obtained. Tris-maleate solution of molarity π was made-up by dissolving π moles of THAM, i.e., tris(hydroxymethyl) amino methane, $C_4H_{11}NO_3$, and π moles of maleic acid, cis- $HOOCCH=CHCOOH$, in one liter of distilled water. The correct proportions for the buffer may be estimated from the data below.

<u>pH</u>	<u>Tris-</u> <u>maleate</u>	<u>NaOH</u>	<u>pH</u>	<u>Tris-</u> <u>maleate</u>	<u>NaOH</u>
5.2	50.0	7.0	8.0	50.0	69.0
5.4	"	10.8	8.2	"	75.0
5.6	"	15.5	8.4	"	81.0
5.8	"	20.5	8.6	"	86.5
6.0	"	26.0			
6.2	"	31.5			
6.4	"	37.0			
6.6	"	42.5			
6.8	"	45.0			
7.0	"	48.0			
7.2	"	51.0			
7.4	"	54.0			
7.6	"	58.0			
7.8	"	63.5			

Concentration Calculations

Hemoglobin

The hemoglobin concentration was measured on a Bausch and Lomb spectrophotometer at 403 μm . Readings were taken for both the high and low pH feeds. These readings are sensitive to pH. A correction curve was generated experimentally across the pH range of interest by Chen and co-workers (Figure 84). The maximum spectrometer readings on the hemoglobin samples are obtained in the pH=6.0 region. The product readings are corrected for pH and converted to dimensionless concentrations as follows:

$$r_{\text{Hb}} = \frac{R_{\text{S}}(403) / \theta_{\text{S}}}{R_{\text{F}}(403) / \theta_{\text{F}}} \quad (\text{A-1})$$

Where, $r_{\text{Hb}} = C_{\text{L}} / C_{\text{O}} =$ dimensionless hemoglobin concentration

R_{F} = feed reading

R_{S} = sample reading

θ_{F} = correction factor at feed pH (Figure 84)

θ_{S} = correction factor at sample pH (Figure 84)

Also, R_{B} = buffer reading

r_{Al} = dimensionless albumin concentration

Albumin

Total protein concentration was measured at 595 μm using BIO-RAD protein assay. The commercial dye was diluted according to the manufacturer's instructions. Three to five milliliters of dye were used to analyze an 0.1 cc sample. The buffer was also measured at both pH's, and the

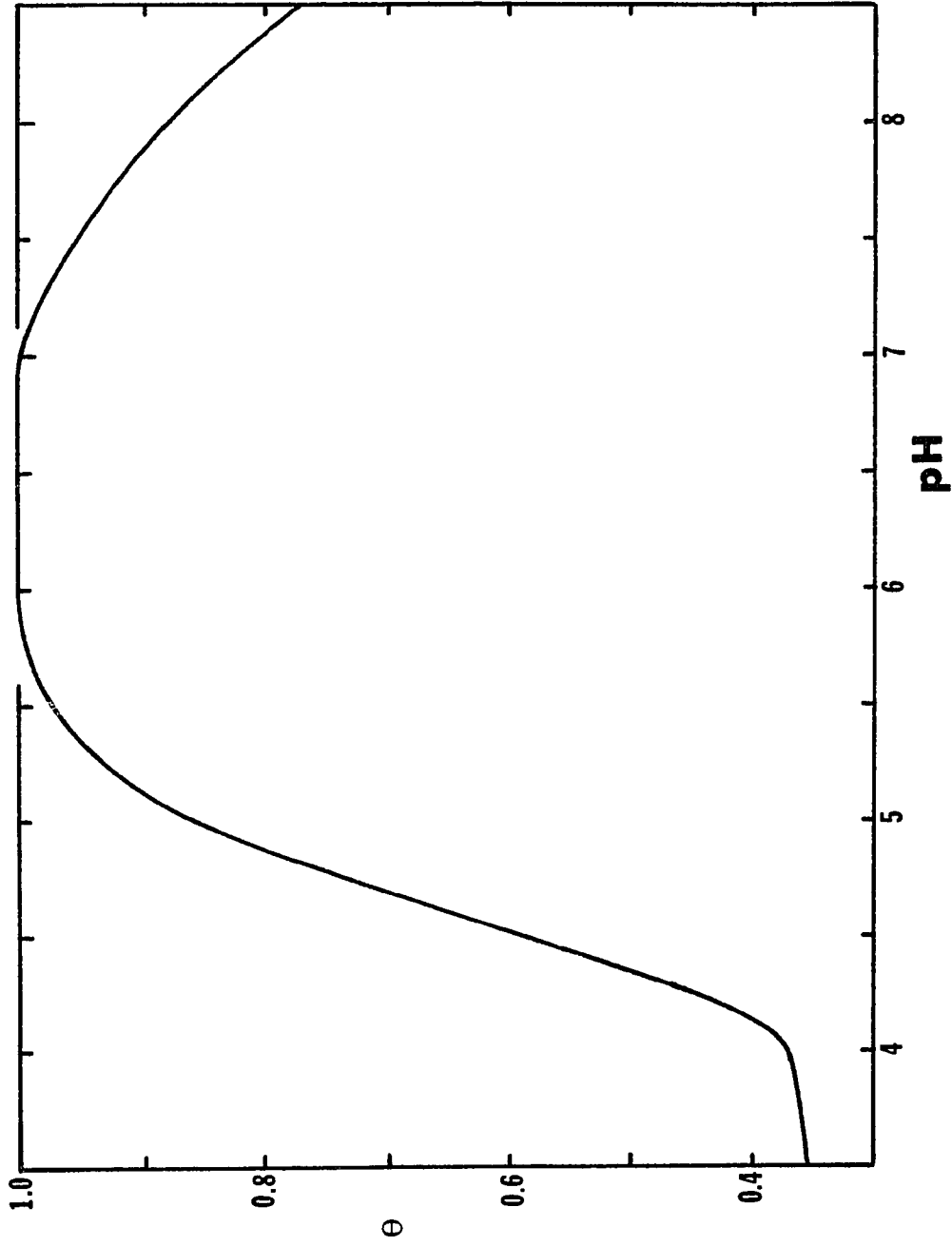


FIGURE 84. pH Correction Curve for Hemoglobin Concentration

spectrometer readings at 595 μm were corrected by subtracting the appropriate buffer readings.

$$[\text{Hb} + \text{Al}]_F = R_F(595) - R_B(595) \quad (\text{A-2})$$

$$[\text{Hb} + \text{Al}]_S = R_S(595) - R_B(595) \quad (\text{A-3})$$

The albumin concentrations are determined as follows:

$$r_{\text{Al}} = \frac{[\text{Al}]_S}{[\text{Al}]_F} \quad (\text{A-4})$$

Given, $[\text{Al}]_F = [\text{Hb}]_F$

Equation B-4 becomes,

$$\begin{aligned} r_{\text{Al}} &= \frac{[\text{Al} + \text{Hb}]_S - [\text{Hb}]_S}{[\text{Hb}]_F} = \frac{[\text{Al} + \text{Hb}]_S}{[\text{Hb}]_F} - \frac{[\text{Hb}]_S}{[\text{Hb}]_F} \\ &= \frac{2 [\text{Al} + \text{Hb}]_S}{2 [\text{Hb}]_F} - r_{\text{Hb}} = \frac{2 [\text{Al} + \text{Hb}]_S}{[\text{Al} + \text{Hb}]_F} - r_{\text{Hb}} \\ &= \frac{2 (R_S(595) - R_B(595))}{R_F(595) - R_B(595)} - r_{\text{Hb}} \quad (\text{A-5}) \end{aligned}$$

Dye Preparation

In the later runs, the protein dye was prepared from basic chemicals as follows (500 ml dye):

- (1) Dissolve 0.1 g Brilliant Blue G in 25 ml ethyl alcohol in a 500 ml beaker. Dilute to 275 ml of solution with 250 ml of distilled water.
- (2) Drop 50 ml H_3PO_4 into the former solution and dilute to 500 ml final solution with 175 ml distilled water.
- (3) Filter the solution through No. 4 filter paper.
- (4) Filter the dye solution twice through a vacuum filter. Buffer readings should be around 0.4.

This dye gives equivalent results to the BIO-RAD reagent.

APPENDIX B
EXPERIMENTAL DATA

RUN NO. 1Polyacrylamide Gel Electrophoresis - HemoglobinPreliminary Data

Column: LKB 7900 UNIPHOR (15 cm height)

Buffer: 0.05M Tris-Maleate + 0.05M NaOH @ pH = 8.6

Feed: 0.04 weight % hemoglobin + 3. weight % sucrose
in 0.01M Tris-Maleate/NaOH buffer @ pH = 8.6

Feed Volume: 20 cc

Gel: 20 ml 0.05M Tris-Maleate/NaOH buffer @ pH = 8.6
+ 5.2 weight % acrylamide monomer
+ 0.1375 weight % N,N'-methylene-bis-acrylamide
+ 0.15 weight % ammonia persulfate
+ 0.1 cc N,N,N',N'-tetramethylethylenediamine

Gel Height: 4 cm

Elution Buffer Flow Rate: 20 cc/hr

Power: 10 watts

Procedure: Run equipment as instructed in manual in order
to understand operation of column and principle
of electrophoresis. Note: Gel formula developed
experimentally by J. F. Chao.

Feed Reading: $R_{403} = 1.907$

<u>t(hr)</u>	<u>\bar{t}(hr)</u>	<u>Volt</u>	<u>mAmp.</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>	<u>y/y_o(adj.)</u>
0	0	310	33	---	---	---
2	1.00	381	25	---	---	---
3	2.50	384	28	0.001	0.0005	0.001
4	3.50	400	25	0.002	0.001	0.002
5	4.50	375	32	0.002	0.001	0.002
$5\frac{1}{2}$	5.25	---	--	0.009	0.005	0.01-
6	5.75	450	23	0.012	0.0063	0.012

RUN NO. 1 (cont.)

<u>t(hr)</u>	<u>\bar{t}(hr)</u>	<u>Volt</u>	<u>mAmp.</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>y/y_o(adj.)</u>
6½	6.25	300	35	0.025	0.013	0.026
7	6.75	---	--	0.021	0.011	0.022
7½	7.25	340	32	0.038	0.020	0.040
8	7.75	---	--	0.054	0.028	0.056
8½	8.25	---	--	0.058	0.030	0.060
9	8.75	420	24	0.147	0.0771	0.154
9½(9¼)	9.13	--	--	0.047	0.025	0.050

Note: POWER OFF WHILE PUMPING last 15 minutes. Following this sample, ALL EQUIPMENT OFF 61 hours.

9½	9.38	325	34	0.151	0.0792	0.158
10	9.75	---	--	0.130	0.0682	0.136
10½	10.25	---	--	0.254	0.133	0.266
11	10.75	304	36	0.312	0.164	0.328
11½	11.25	314	34	0.210	0.110	0.220
12	11.75	324	34	0.127	0.0666	0.133
12½	12.25	---	--	0.141	0.0739	0.148
13	12.75	---	--	0.066	0.035	0.070
13½	13.25	---	--	0.092	0.048	0.096
13.75	13.63	365	30	0.098	0.051	0.102
14	13.88	391	27	0.056	0.029	0.058

Note: y_{Hb}/y_o adjusted to an elution buffer flow rate of 10 cc/hr in order to compare with Runs 2 & 3.

Recovery: 49.8% (tail missing)

RUN NO. 2Polyacrylamide Gel Electrophoresis - AlbuminPreliminary Data

Column: LKB 7900 UNIPHOR (15 cm height)

Buffer: 0.05M Tris-Maleate + 0.05M NaOH @ pH = 8.6

Feed: 0.04 weight % albumin + 3. weight % sucrose
in 0.01M Tris-Maleate/NaOH buffer @ pH = 8.6

Feed Volume: 20 cc

Gel: 20 ml 0.05M Tris-Maleate/NaOH buffer @ pH = 8.6
+ 5.2 weight % acrylamide monomer
+ 0.1375 weight % N,N'-methylene-bis-acrylamide
+ 0.15 weight % ammonia persulfate
+ 0.1 cc N,N,N',N'-tetramethylethylenediamine

Gel Height: 4 cm

Elution Buffer Flow Rate: 10 cc/hr

Power: 10 watts

Procedure: Run equipment as instructed in manual in order to understand operation of column and principle of electrophoresis. Note: Gel formula developed experimentally by J. F. Chao.

Feed Reading: $R_{595} = 1.214$

Buffer Reading: $R_{595} = 0.444$

Note: 0.1 cc sample + 5 cc BIO-RAD dye reagent

<u>t(hr)</u>	<u>\bar{t}(hr)</u>	<u>Volt</u>	<u>mAmp.</u>	<u>R_{595}</u>	<u>y_{A1}/y_0</u>
0	0	300	35	---	---
$\frac{1}{2}$	0.25	---	--	0.453	0.01-
1	0.75	---	--	0.450	0.008
$1\frac{1}{2}$	1.25	---	--	0.454	0.013
2	1.75	---	--	0.454	0.013

RUN NO. 2 (cont.)

<u>t(hr)</u>	<u>\bar{t}(hr)</u>	<u>Volt</u>	<u>mAmp.</u>	<u>R₅₉₅</u>	<u>y_{A1}/y_o</u>
2½	2.25	---	--	0.453	0.01-
3	2.75	---	--	0.447	0.004
3½	3.25	349	30	0.454	0.013
4	3.75	507	20	0.492	0.062
4½	4.25	444	22	0.588	0.187
5	4.75	277	37	0.788	0.447

Note: ALL EQUIPMENT OFF 20 hours.

5½	5.25	294	35	0.758	0.408
6	5.75	---	--	0.832	0.504
6½	6.25	---	--	0.770	0.423
7	6.75	---	--	0.659	0.279
7½	7.25	---	--	0.587	0.186
8	7.75	---	--	0.567	0.160
8½	8.25	---	--	0.550	0.138
9½	9.00	---	--	0.524	0.104

Recovery: 76.8% (tail missing)

RUN NO. 3Polyacrylamide Gel Electrophoresis - MixturePreliminary Data

Column: LKB 7900 UNIPHOR (15 cm)

Buffer: 0.05M Tris-Maleate + 0.05M NaOH @ pH = 8.6

Feed: 0.04 weight % hemoglobin + 0.04 weight % albumin
+ 3. weight % sucrose in 0.01M Tris-Maleate/NaOH
buffer @ pH = 8.6

Feed Volume: 20 cc

Gel: 20 ml 0.05M Tris-Maleate/NaOH buffer @ pH = 8.6
+ 5.2 weight % acrylamide monomer
+ 0.1375 weight % N,N'-methylene-bis-acrylamide
+ 0.15 weight % ammonia persulfate
+ 0.1 cc N,N,N',N'-tetramethylethylenediamine

Gel Height: 4 cm

Elution Buffer Flow Rate: 10 cc/hr

Power: 10 watts

Procedure: Run equipment as instructed in manual in order
to understand operation of column and principle
of electrophoresis. Note: Gel formula developed
experimentally by J. F. Chao.

Feed Reading: $R_{403} = 1.950$

$R_{595} = 1.937$

Buffer Reading: $R_{595} = 0.453$

Note: 0.1 cc sample + 5 cc BIO-RAD dye reagent

<u>t(hr)</u>	<u>\bar{t}(hr)</u>	<u>Volt</u>	<u>mAmp.</u>	<u>R_{403}</u>	<u>R_{595}</u>	<u>y_{Hb}/y_0</u>	<u>y_{Al}/y_0</u>
0	0	301	35	---	---	---	---
$\frac{1}{2}$	0.25	295	36	0.001	0.464	0.0005	0.014
1	0.75	305	35	0.001	0.455	0.0005	0.002

RUN NO. 3 (cont.)

<u>t(hr)</u>	<u>\bar{t}(hr)</u>	<u>Volt</u>	<u>mAmp.</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
1½	1.25	286	36	0.000	0.450	0	0
2	1.75	270	38	0.001	0.454	0.0005	0.001
2½	2.25	---	--	0.001	0.449	0.0005	0
3	2.75	282	37	0.003	0.451	0.002	0
3½	3.25	274	39	0.005	0.449	0.003	0
4	3.75	---	--	0.005	0.460	0.003	0.006
4½	4.25	265	40	0.116	0.759	0.0595	0.353
5	4.75	265	40	0.290	1.085	0.149	0.703
5½ } 6 }	5.50	---	--	0.374	1.051	0.192	0.614
6½	6.25	257	39	0.252	0.846	0.129	0.401
7	6.75	---	--	0.318	0.765	0.163	0.257
7½	7.25	259	39	0.369	0.800	0.189	0.279
8	7.75	---	--	0.379	0.843	0.194	0.332
8½	8.25	256	40	0.275	0.685	0.141	0.172
9	8.75	---	--	0.214	0.662	0.110	0.172
9½	9.25	259	39	0.184	0.649	0.0944	0.170
10	9.75	---	--	0.213	0.646	0.109	0.151
10½	10.25	---	--	0.155	---	0.0795	---

Hemoglobin Recovery: 45.3% (tail missing)

Albumin Recovery: 106% (slightly high)

Note: Polyacrylamide gel electrophoresis has been optimized for this mixture by J. F. Chao.

RUN NO. 4Adsorption/Desorption Curves - Phosphate Buffer

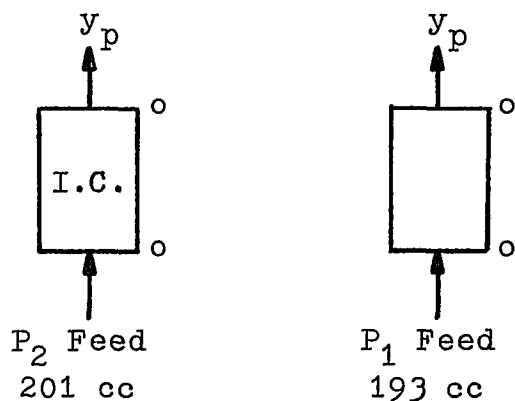
Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4

Feed: 0.005 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.68 cc/min

Power: None

Procedure: As shown. Optimize Reservoir displacement.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.0$)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	y_{Hb}/y_0
ADSORPTION (P_2 Feed Reading: $R_{403} = 0.133*2$)					
1	8.0	4.0	7.82	0.091*2	0.75-
2	13.4	10.7	7.82	0.089*2	0.74-
3	18.8	16.1	7.82	0.087*2	0.72-
4	24.1	21.4	7.82	0.085*2	0.70-
5	29.5	26.8	7.82	0.086*2	0.71-
6	34.8	32.1	7.82	0.087*2	0.72-
7	40.2	37.5	7.82	0.082*2	0.68-
8	45.6	42.9	7.82	0.078*2	0.64-

RUN NO. 4 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>
9	50.9	48.2	7.82	0.069*2	0.57-
10	56.3	53.6	7.82	0.054*2	0.45-
11	61.4	58.9	7.81	0.033*2	0.27-
12	67.0	64.3	7.80	0.018*2	0.15-
13	72.4	69.7	7.35	0.009*2	0.07-
14	77.7	75.0	6.90	0.005*2	0.04-
15	83.1	80.4	6.68	0.003*2	0.02-
16	88.4	85.7	6.50	0.003*2	0.02-
17	93.8	91.1	6.35	0.003*2	0.02-
18	99.2	96.5	6.30	0.003*2	0.02-
19	104.5	101.8	6.29	0.003*2	0.02-
20	109.9	107.2	6.27	0.003*2	0.02-
21	115.2	112.5	6.18	0.004*2	0.03-
22	120.6	117.9	6.28	0.012*2	0.090
23	126.0	123.3	6.10	0.003*2	0.02-
24	131.3	128.6	6.08	0.002*2	0.02-
25	136.7	134.0	6.08	0.002*2	0.02-
26	142.0	139.3	6.07	0.001*2	0.01-
27	147.4	144.7	6.05	0.001*2	0.01-
28	152.8	150.1	6.05	0.001*2	0.01-
29	158.1	155.4	6.05	0.002*2	0.02-
30	163.5	160.8	6.05	0.002*2	0.02-
31	168.8	166.1	6.05	0.002*2	0.02-
32	174.2	171.5	6.05	0.002*2	0.02-
33	179.6	176.9	6.05	0.002*2	0.02-

<u>RUN NO. 4 (cont.)</u>					
<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>
34	184.9	182.2	6.05	0.003*2	0.02-
35	190.3	187.6	6.05	0.003*2	0.02-
36	195.6	192.9	6.05	0.004*2	0.03-
37	201.0	198.3	6.05	0.004*2	0.03-
DESORPTION (P ₁ Feed Reading: R ₄₀₃ = 0.197)					
38	8.0	4.0	5.95	0.009	0.04-
39	16.1	12.1	5.95	0.010	0.045
40	24.1	20.1	5.95	0.011	0.049
41	32.2	28.1	5.95	0.014	0.062
42	40.2	36.2	5.95	0.013	0.058
43	48.2	44.2	5.95	0.011	0.049
44	56.3	52.3	5.92	0.012	0.053
45	64.3	60.3	5.92	0.011	0.049
46	72.4	68.3	5.92	0.012	0.053
47	80.4	76.4	6.05	0.195	0.869
48	88.4	84.4	6.62	0.942	4.20-
49	96.5	92.5	7.22	0.537	2.44-
50	104.5	100.5	7.32	0.261	1.19-
51	112.6	108.5	7.32	0.201	0.918
52	120.6	116.6	7.40	0.180	0.830
53	128.6	124.6	7.42	0.169	0.778
54	136.7	132.7	7.40	0.171	0.788
55	144.7	140.7	7.45	0.171	0.788
56	152.8	148.7	7.45	0.169	0.783
57	160.8	156.8	7.50	0.168	0.782

RUN NO. 4 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>
58	168.8	164.8	7.50	0.164	0.764
59	176.9	172.9	7.53	0.160	0.751
60	184.9	180.9	7.52	0.164	0.768
61	193.0	188.9	7.52	0.157	0.735

Recovery: 47.4%

RUN NO. 5Breakthrough Curve - Mixture

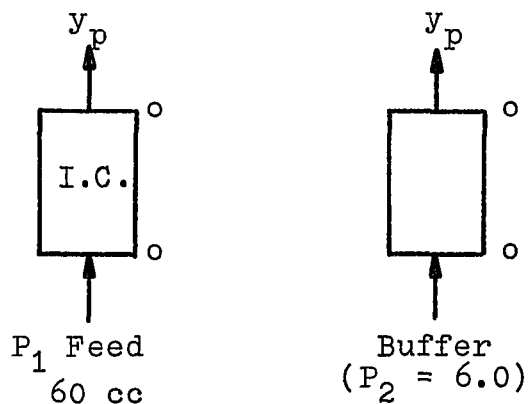
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: None

Procedure: As shown. Simulate Stage IV of pH Parapumping.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{403} = 0.367$ (pH = 8.50)

$R_{595} = 0.686$

Buffer Reading: $R_{595} = 0.379$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	6.12	---	---	---	---
2	7.50	11.25	6.10	---	---	---	---
3	7.50	18.75	6.10	---	---	---	---
4	7.50	26.25	6.08	---	---	---	---
5	3.75	31.88	6.08	0.000	0.368	0	0

RUN NO. 5 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
6	3.75	35.63	6.05	0.002	0.379	0.004	0
7	3.75	39.38	6.07	0.004	0.392	0.008	0.077
8	3.75	43.13	6.07	0.018	0.448	0.037	0.41-
9	3.75	46.88	6.05	0.037	0.501	0.077	0.718
10	3.75	50.63	6.08	0.050	0.536	0.10-	0.92-
11	3.75	54.38	6.05	0.062	0.549	0.13-	0.98-
12	3.75	58.13	6.05	0.072	0.545	0.15-	0.93-
Note: Change Feed to Buffer.							
13	3.75	61.88	6.05	0.077	0.570	0.16-	1.09-
14	3.75	65.63	6.05	0.084	0.564	0.17-	1.03-
15	3.75	69.38	6.10	0.095	0.563	0.20-	1.00-
16	3.75	73.13	6.20	0.092	0.540	0.19-	0.86-
17	3.75	76.88	6.22	0.089	0.545	0.18-	0.90-
18	3.75	80.63	6.25	0.090	0.540	0.19-	0.86-
19	3.75	84.38	6.28	0.092	0.528	0.19-	0.78-
20	3.75	88.13	6.28	0.091	0.536	0.19-	0.83-
21	3.75	91.88	6.28	0.091	0.529	0.19-	0.79-
22	3.75	95.63	6.28	0.086	0.550	0.18-	0.94-
23	3.75	99.38	6.37	0.341	0.610	0.706	0.80-
24	3.75	103.13	7.22	1.345	0.870	2.837	0.36-
25	3.75	106.88	7.95	1.441	0.952	3.367	0.37-
26	3.75	110.63	8.12	1.084	0.864	2.626	0.53-
27	3.75	114.38	8.22	0.520	0.652	1.29-	0.49-
28	3.75	118.13	8.25	0.194	0.491	0.485	0.245
29	7.50	123.75	8.15	0.028	0.404	0.068	0.095

RUN NO. 5 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
30	7.50	131.25	8.10	0.008	---	0.02-	---
31	7.50	138.75	7.92	0.005	---	0.01-	---

	<u>Recovery</u>	<u>Center of Mass</u>
Hemoglobin	86.5%	106.16 ± 1.88 cc
Albumin	100.5%	75.11 ± 1.88 cc
Total Protein	93.5%	
Difference in Centers of Mass		31.05 ± 3.75 cc

RUN NO. 6Breakthrough Curve - Mixture

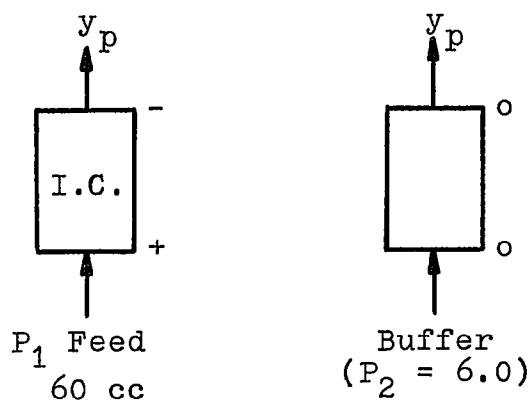
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Optimize electrode placement.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{403} = 0.367$ (pH = 8.50)

$R_{595} = 0.725$

Buffer Reading: $R_{595} = 0.377$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	6.50	---	---	---	---
2	7.50	11.25	6.30	---	---	---	---
3	7.50	18.75	6.30	---	---	---	---
4	7.50	26.25	6.28	---	---	---	---
5	3.75	31.88	6.30	---	---	---	---

RUN NO. 6 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
6	3.75	35.63	6.27	0.004	0.380	0.008	0.02-
7	3.75	39.38	6.25	0.006	0.386	0.01-	0.04-
8	3.75	43.13	6.28	0.012	0.385	0.025	0.02-
9	3.75	46.88	6.28	0.018	0.428	0.037	0.026
10	3.75	50.63	6.25	0.026	0.478	0.054	0.526
11	3.75	54.38	6.25	0.039	0.514	0.081	0.707
12	3.75	58.13	6.25	0.053	0.527	0.11-	0.75-
Note: POWER OFF. Change Feed to Buffer.							
13	3.75	61.88	6.27	0.063	0.542	0.13-	0.82-
14	3.75	65.63	6.10	0.075	0.576	0.16-	0.99-
15	3.75	69.38	6.05	0.085	0.570	0.18-	0.93-
16	3.75	73.13	6.12	0.091	0.571	0.19-	0.93-
17	3.75	76.88	6.20	0.099	0.571	0.21-	0.91-
18	3.75	80.63	6.30	0.100	0.556	0.207	0.82-
19	3.75	84.38	6.35	0.095	0.558	0.20-	0.85-
20	3.75	88.13	6.35	0.095	0.542	0.20-	0.75-
21	3.75	91.88	6.35	0.094	0.568	0.20-	0.90-
22	3.75	95.63	6.37	0.094	0.560	0.20-	0.86-
23	3.75	99.38	6.35	0.081	0.539	0.16-	0.77-
24	3.75	103.13	6.35	0.064	0.481	0.13-	0.47-
25	3.75	106.88	6.40	0.055	0.485	0.11-	0.51-
26	3.75	110.63	6.65	0.250	0.544	0.518	0.442
27	3.75	114.38	7.32	0.503	0.638	1.07-	0.43-
28	3.75	118.13	7.55	0.555	0.655	1.21-	0.39-
29	3.75	121.88	7.65	0.732	0.723	1.62-	0.37-

RUN NO. 6 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
30	3.75	125.63	7.72	0.768	0.718	1.71-	0.25-
31	3.75	129.38	7.75	0.784	0.724	1.76-	0.27-
32	3.75	133.13	7.72	0.647	0.660	1.44-	0.19-
33	3.75	136.88	7.68	0.463	0.587	1.03-	0.18-
34	3.75	140.63	7.70	0.201	0.491	0.449	0.206
35	3.75	144.38	7.58	0.060	0.401	0.13-	0.01-
36	3.75	148.13	7.32	0.020	0.387	0.04-	0.02-
37	7.50	153.75	7.00	0.009	---	0.02-	---

	<u>Recovery</u>	<u>Center of Mass</u>
Hemoglobin	85.0%	123.28 ± 1.88 cc
Albumin	95.9%	83.28 ± 1.88 cc
Total Protein	90.5%	
Difference in Centers of Mass		40.00 ± 3.75 cc

RUN NO. 7Breakthrough Curve - Mixture

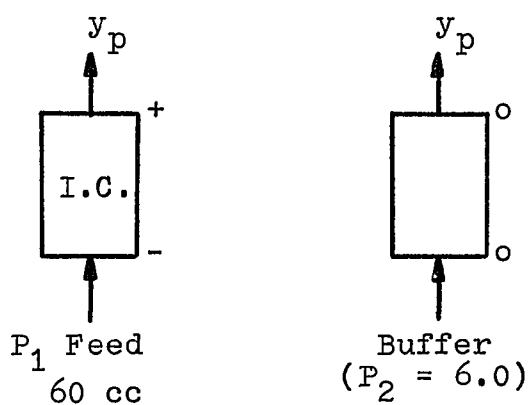
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Optimize electrode placement.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{403} = 0.369$ (pH = 8.52)

$R_{595} = 0.714$

Buffer Reading: $R_{595} = 0.379$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	6.57	---	---	---	---
2	7.50	11.25	6.10	---	---	---	---
3	7.50	18.75	6.05	---	---	---	---
4	7.50	26.25	6.05	---	---	---	---
5	3.75	31.88	6.05	0.005	0.399	0.01-	0.11-

RUN NO. 7 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
6	3.75	35.63	6.05	0.015	0.438	0.031	0.32-
7	3.75	39.38	6.05	0.034	0.482	0.071	0.544
8	3.75	43.13	6.05	0.053	0.546	0.11-	0.89-
9	3.75	46.88	6.05	0.067	0.537	0.14-	0.80-
10	3.75	50.63	6.05	0.074	0.541	0.15-	0.81-
11	3.75	54.38	6.10	0.079	0.542	0.16-	0.81-
12	3.75	58.13	6.20	0.082	0.544	0.17-	0.81-
Note: POWER OFF. Change Feed to Buffer.							
13	3.75	61.88	6.25	0.081	0.538	0.17-	0.78-
14	3.75	65.63	6.25	0.092	0.556	0.19-	0.87-
15	3.75	69.38	6.25	0.089	0.556	0.19-	0.87-
16	3.75	73.13	6.28	0.081	0.555	0.17-	0.88-
17	3.75	76.88	6.28	0.080	0.539	0.17-	0.79-
18	3.75	80.63	6.30	0.269	0.589	0.560	0.69-
19	3.75	84.38	6.55	0.868	0.812	1.81-	0.78-
20	3.75	88.13	7.34	1.462	0.973	3.130	0.42-
21	3.75	91.88	7.85	0.945	0.875	2.18-	0.78-
22	3.75	95.63	8.02	0.770	0.815	1.83-	0.77-
23	3.75	99.38	8.10	0.469	0.665	1.14-	0.57-
24	3.75	103.13	8.10	0.277	0.583	0.672	0.55-
25	7.50	108.75	8.08	0.137	0.481	0.331	0.278
26	7.50	116.25	8.08	0.041	0.406	0.099	0.062
27	7.50	123.75	7.98	0.015	0.387	0.035	0.01-

RUN NO. 7 (cont.)

	<u>Recovery</u>	<u>Center of Mass</u>
Hemoglobin	87.3%	89.71 \pm 1.88
Albumin	<u>91.0%</u>	<u>69.79 \pm 1.88</u>
Total Protein	89.2%	
Difference in Centers of Mass		19.92 \pm 3.75

RUN NO. 8Breakthrough Curve - Mixture

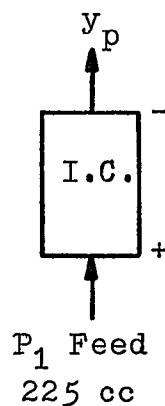
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts X 43 mAmp = 9 watts

Procedure: As shown. Simulate Stage IV of New Process.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{403} = 0.371$ (pH = 8.45)

$R_{595} = 0.761$

Buffer Reading: $R_{595} = 0.348$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	5.92	---	---	---	---
2	7.50	11.25	6.00	---	---	---	---
3	7.50	18.75	6.09	---	---	---	---
4	7.50	26.25	6.19	---	---	---	---
5	7.50	33.75	6.21	0.000	0.342	0	0

RUN NO. 8 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
6	7.50	41.25	6.23	0.009	0.355	0.02-	0.01-
7	7.50	48.75	6.23	0.026	0.452	0.055	0.449
8	7.50	56.25	6.25	0.045	0.508	0.10-	0.67-
9	7.50	63.75	6.22	0.056	0.511	0.12-	0.67-
10	7.50	71.25	6.28	0.071	0.542	0.15-	0.79-
11	7.50	78.75	6.40	0.082	0.560	0.17-	0.86-
12	7.50	86.25	6.53	0.086	0.556	0.18-	0.83-
13	7.50	93.75	6.59	0.081	0.542	0.17-	0.77-
14	7.50	101.25	6.65	0.078	0.538	0.17-	0.75-
15	7.50	108.75	6.69	0.077	0.537	0.16-	0.76-
16	7.50	116.25	6.85	0.321	0.674	0.678	0.90-
17	3.75	121.88	7.45	0.810	0.635*2	1.78-	1.00-
18	3.75	125.63	7.68	0.987	0.708*2	2.22-	1.27-
19	3.75	129.38	7.80	0.887	0.669*2	2.05-	1.06-
20	3.75	133.12	7.85	1.120	0.712*2	2.615	0.91-
21	7.50	138.75	7.90	1.299	0.665*3	3.060	1.55-
22	7.50	146.25	7.92	0.937	0.576*3	2.21-	1.10-
23	7.50	153.75	7.93	0.513	0.853	1.22-	1.23-
24	7.50	161.25	7.92	0.490	0.859	1.16-	1.31-
25	7.50	168.75	7.98	0.473	0.864	1.13-	1.37-
26	7.50	176.25	7.92	0.430	0.815	1.02-	1.24-
27	7.50	183.75	7.95	0.402	0.754	0.947	1.02-
28	7.50	191.25	7.92	0.403	0.756	0.953	1.02-
29	7.50	198.75	7.92	0.409	0.754	0.967	1.00-
30	7.50	206.25	7.92	0.403	0.741	0.953	0.95-

HEMOGLOBIN RECOVERY: 90.6%

ALBUMIN RECOVERY: 97.1%

RUN NO. 9Breakthrough Curve - Hemoglobin

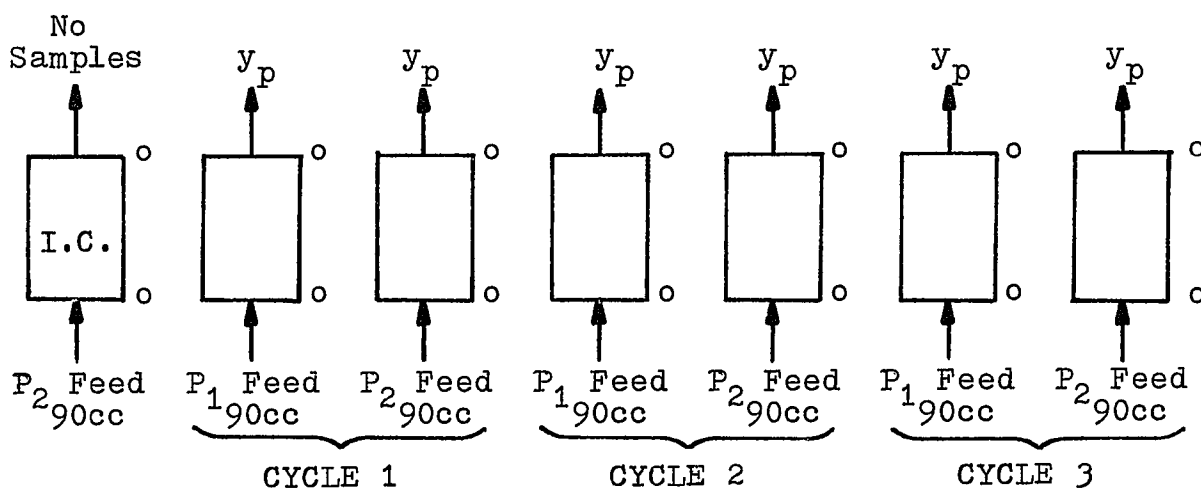
Buffer: 0.1M NaH_2PO_4 + 0.1M Na_2HPO_4

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: As shown. Optimize bulk velocity for New Process.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.0$)

	<u>pH</u>	<u>R_{403}</u>
P_2 Feed	5.98	0.460
P_1 Feed	7.98	0.392

RUN NO. 9 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>
CYCLE 1					
1	7.50	3.75	6.07	0.007	0.02-
2	7.50	11.25	6.02	0.007	0.02-
3	7.50	18.75	6.02	0.009	0.02-
4	7.50	26.25	6.00	0.012	0.026
5	7.50	33.75	6.00	0.012	0.026
6	7.50	41.25	6.00	0.014	0.030
7	7.50	48.75	6.00	0.013	0.028
8	7.50	56.25	6.00	0.017	0.037
9	7.50	63.75	6.02	0.134	0.292
10	3.75	67.50	6.22	1.890	4.113
11	3.75	71.25	6.65	1.391*3	9.082
12	3.75	75.00	7.15	1.233*3	7.985
13	3.75	78.75	7.55	0.702*3	4.58-
14	3.75	82.50	7.80	1.034	2.543
15	3.75	86.25	7.90	0.634	1.59-
16	7.50	93.75	7.98	0.469	1.20-
17	7.50	101.25	7.98	0.414	1.06-
18	7.50	108.75	7.98	0.402	1.03-
19	7.50	116.25	7.98	0.398	1.02-
20	7.50	123.75	7.98	0.389	0.994
21	7.50	131.25	7.98	0.369	0.943
22	7.50	138.75	7.98	0.304	0.777
23	7.50	146.25	7.98	0.169	0.432
24	7.50	153.75	7.75	0.078	0.20-
25	7.50	161.25	6.90	0.021	0.054
26	7.50	168.75	6.38	0.010	0.026
27	7.50	176.25	6.12	0.008	0.02-
CYCLE 2					
28	7.50	3.75	6.13	0.006	0.01-
29	7.50	11.25	6.02	0.013	0.028
30	7.50	18.75	6.02	0.009	0.02-
31	7.50	26.25	6.00	0.009	0.02-
32	7.50	33.75	6.00	0.014	0.030
33	7.50	41.25	6.00	0.014	0.030
34	7.50	48.75	6.00	0.018	0.039
35	7.50	56.25	6.00	0.015	0.033
36	7.50	63.75	6.00	0.140	0.305
37	3.75	67.50	6.22	0.578*3	3.77-
38	3.75	71.25	6.67	1.392*3	9.088
39	3.75	75.00	7.18	1.211*3	7.906
40	3.75	78.75	7.58	0.689*3	4.50-
41	3.75	82.50	7.81	1.023	2.522
42	3.75	86.25	7.90	0.645	1.62-
43	7.50	93.75	7.95	0.482	1.20-
44	7.50	101.25	8.00	0.428	1.10-
45	7.50	108.75	7.99	0.419	1.07-

RUN NO. 9 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>
46	7.50	116.25	7.99	0.411	1.05-
47	7.50	123.75	7.99	0.402	1.03-
48	7.50	131.25	7.99	0.397	1.01-
49	7.50	138.75	7.98	0.397	0.963
50	7.50	146.25	7.98	0.198	0.431
51	7.50	153.75	7.90	0.084	0.21-
52	7.50	161.25	7.00	0.033	0.072
53	7.50	168.75	6.52	0.007	0.02-
54	7.50	176.25	6.20	0.005	0.01-
CYCLE 3					
55	7.50	3.75	6.12	0.007	0.02-
56	7.50	11.25	6.05	0.005	0.01-
57	7.50	18.75	6.00	0.007	0.02-
58	7.50	26.25	6.02	0.008	0.02-
59	7.50	33.75	6.01	0.008	0.02-
60	7.50	41.25	6.00	0.010	0.022
61	7.50	48.75	6.01	0.012	0.026
62	7.50	56.25	6.01	0.015	0.033
63	7.50	63.75	6.01	0.140	0.305
64	3.75	67.50	6.23	1.714	3.726
65	3.75	71.25	6.62	1.180*3	7.696
66	3.75	75.00	7.08	1.121*3	7.319
67	3.75	78.75	7.48	0.663*3	4.33-
68	3.75	82.50	7.78	1.043	2.54-
69	3.75	86.25	7.90	0.647	1.63-
70	7.50	93.75	7.96	0.467	1.19-
71	7.50	101.25	8.00	0.410	1.05-
72	7.50	108.75	7.98	0.395	1.01-
73	7.50	116.25	7.99	0.392	1.00-
74	7.50	123.75	7.99	0.383	0.978
75	7.50	131.25	8.00	0.363	0.929
76	7.50	138.75	8.00	0.295	0.755
77	7.50	146.25	8.00	0.157	0.402
78	7.50	153.75	7.78	0.053	0.13-
79	7.50	161.25	6.88	0.012	0.026
80	7.50	168.75	6.40	0.005	0.01-
81	7.50	176.25	6.15	0.004	0.01-

RUN NO. 9 (cont.)

<u><V_e></u>	<u>y_{Hb}/y_o</u>			<u>Average</u>	<u>pH (avg.)</u>
	<u>CYCLE 1</u>	<u>CYCLE 2</u>	<u>CYCLE 3</u>		
3.75	0.02	0.01	0.02	0.02	6.11
11.25	0.02	0.03	0.01	0.02	6.03
18.75	0.02	0.02	0.02	0.02	6.01
26.25	0.03	0.02	0.02	0.02	6.01
33.75	0.03	0.03	0.02	0.03	6.00
41.25	0.03	0.03	0.02	0.03	6.00
48.75	0.03	0.04	0.03	0.03	6.00
56.25	0.04	0.03	0.03	0.03	6.00
63.75	0.29	0.31	0.31	0.30	6.01
67.50	4.11	3.77	3.73	3.87	6.22
71.25	9.08	9.09	7.70	8.62	6.65
75.00	7.99	7.91	7.32	7.74	7.14
78.75	4.58	4.50	4.33	4.47	7.54
82.50	2.54	2.52	2.54	2.53	7.80
86.25	1.59	1.62	1.63	1.61	7.90
93.75	1.20	1.20	1.19	1.20	7.96
101.25	1.06	1.10	1.05	1.07	7.99
108.75	1.03	1.07	1.01	1.04	7.98
116.25	1.02	1.05	1.00	1.02	7.99
123.75	0.99	1.03	0.98	1.00	7.99
131.25	0.94	1.01	0.93	0.96	7.99
138.75	0.78	0.96	0.76	0.83	7.99
146.25	0.43	0.43	0.40	0.42	7.99
153.75	0.20	0.21	0.13	0.18	7.81
161.25	0.05	0.07	0.03	0.05	6.93
168.25	0.03	0.02	0.01	0.02	6.43
176.25	0.02	0.01	0.01	0.01	6.16

Recovery: CYCLE 1 - 96.6%
 CYCLE 2 - 97.4%
 CYCLE 3 - 89.9%

Average - 94.6%

RUN NO. 10

Breakthrough Curve - Hemoglobin

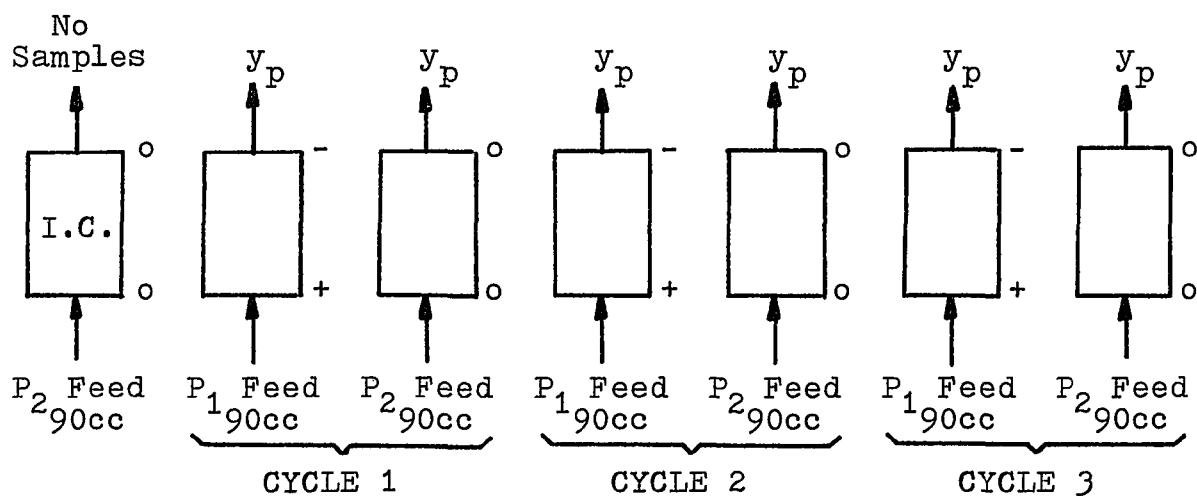
Buffer: 0.1M NaH_2PO_4 + 0.1M Na_2HPO_4

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: 120 volts

Procedure: As shown. Optimize bulk velocity for New Process.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.0$)

	<u>pH</u>	<u>R₄₀₃</u>
P ₂ Feed	6.00	0.454
P ₁ Feed	8.00	0.383

RUN NO. 10 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>
CYCLE 1					
1	7.50	3.75	6.05	0.010	0.022
2	7.50	11.25	6.06	0.012	0.024
3	7.50	18.75	6.07	0.013	0.029
4	7.50	26.25	6.12	0.016	0.035
5	7.50	33.75	6.12	0.019	0.042
6	7.50	41.25	6.12	0.021	0.046
7	7.50	48.75	6.12	0.025	0.055
8	7.50	56.25	6.12	0.051	0.11-
9	7.50	63.75	6.15	0.476	1.05-
10	3.75	67.50	6.37	1.664	3.669
11	3.75	71.25	6.60	0.901*3	5.96-
12	3.75	75.00	6.90	1.268*3	8.388
13	3.75	78.75	7.37	0.925*3	6.12-
14	3.75	82.50	7.70	1.238	3.051
15	3.75	86.25	7.90	0.657	1.68-
16	7.50	93.75	7.98	0.438	1.14-
17	7.50	101.25	7.99	0.393	1.02-
18	7.50	108.75	8.00	0.395	1.03-
19	7.50	116.25	8.00	0.390	1.02-
20	7.50	123.75	8.00	0.378	0.987
21	7.50	131.25	7.99	0.346	0.903
22	7.50	138.75	7.99	0.251	0.655
23	7.50	146.25	7.97	0.115	0.298
24	7.50	153.75	7.42	0.044	0.11-
25	7.50	161.25	6.71	0.035	0.077
26	7.50	168.75	6.32	0.041	0.090
27	7.50	176.25	6.20	0.022	0.048
CYCLE 2					
28	7.50	3.75	6.18	0.018	0.040
29	7.50	11.25	6.18	0.011	0.024
30	7.50	18.75	6.16	0.015	0.033
31	7.50	26.25	6.09	0.013	0.029
32	7.50	33.75	6.10	0.016	0.035
33	7.50	41.25	6.09	0.018	0.040
34	7.50	48.75	6.10	0.023	0.051
35	7.50	56.25	6.08	0.024	0.053
36	7.50	63.75	6.12	0.299	0.659
37	3.75	67.50	6.32	1.827	4.029
38	3.75	71.25	6.68	1.096*3	7.250
39	3.75	75.00	7.07	1.111*3	7.350
40	3.75	78.75	7.39	0.732*3	4.84-
41	3.75	82.50	7.63	1.187	2.900
42	3.75	86.25	7.83	0.690	1.74-
43	7.50	93.75	7.92	0.469	1.21-
44	7.50	101.25	7.98	0.408	1.06-
45	7.50	108.75	7.98	0.391	1.02-

RUN NO. 10 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	y_{Hb}/y_o
46	7.50	116.25	7.98	0.393	1.02-
47	7.50	123.75	7.98	0.381	0.992
48	7.50	131.25	7.98	0.349	0.909
49	7.50	138.75	7.98	0.256	0.667
50	7.50	146.25	7.90	0.125	0.320
51	7.50	153.75	7.15	0.043	0.10-
52	7.50	161.25	6.72	0.019	0.042
53	7.50	168.75	6.27	0.006	0.01-
54	7.50	176.25	6.09	0.013	0.029
CYCLE 3					
55	7.50	3.75	6.08	0.008	0.02-
56	7.50	11.25	6.08	0.009	0.02-
57	7.50	18.75	6.09	0.008	0.02-
58	7.50	26.25	6.10	0.013	0.029
59	7.50	33.75	6.11	0.015	0.033
60	7.50	41.25	6.12	0.015	0.033
61	7.50	48.75	6.12	0.018	0.040
62	7.50	56.25	6.12	0.021	0.046
63	7.50	63.75	6.17	0.512	1.13-
64	3.75	67.50	6.42	0.648*3	4.29-
65	3.75	71.25	6.70	0.952*3	6.30-
66	3.75	75.00	7.03	1.025*3	6.781
67	3.75	78.75	7.38	0.783*3	5.18-
68	3.75	82.50	7.67	1.320	3.246
69	3.75	86.25	7.83	0.719	1.82-
70	7.50	93.75	7.94	0.470	1.12-
71	7.50	101.25	7.97	0.411	1.05-
72	7.50	108.75	7.97	0.401	1.03-
73	7.50	116.25	7.97	0.402	1.03-
74	7.50	123.75	7.97	0.387	0.992
75	7.50	131.25	7.97	0.364	0.933
76	7.50	138.75	7.98	0.275	0.716
77	7.50	146.25	7.98	0.132	0.344
78	7.50	153.75	7.41	0.046	0.11-
79	7.50	161.25	6.78	0.015	0.033
80	7.50	168.75	6.33	0.009	0.02-
81	7.50	176.25	6.12	0.008	0.02-

RUN NO. 10 (cont.)

<u><V_e></u>	<u>y_{Hb}/y_o</u>				<u>pH (avg.)</u>
	<u>CYCLE 1</u>	<u>CYCLE 2</u>	<u>CYCLE 3</u>	<u>Average</u>	
3.75	0.02	0.04	0.02	0.03	6.10
11.25	0.02	0.02	0.02	0.02	6.11
18.75	0.03	0.03	0.02	0.03	6.11
26.25	0.04	0.03	0.03	0.03	6.10
33.75	0.04	0.04	0.03	0.04	6.11
41.25	0.05	0.04	0.03	0.04	6.11
48.75	0.06	0.05	0.04	0.05	6.11
56.25	0.11	0.05	0.05	0.07	6.11
63.75	1.05	0.66	1.13	0.95	6.15
67.50	3.67	4.03	4.29	4.00	6.37
71.25	5.96	7.25	6.30	6.50	6.66
75.00	8.39	7.35	6.78	7.51	7.00
78.75	6.12	4.84	5.18	5.38	7.38
82.50	3.05	2.90	3.25	3.07	7.67
86.25	1.68	1.74	1.82	1.75	7.85
93.75	1.14	1.21	1.12	1.16	7.95
101.25	1.02	1.06	1.05	1.04	7.98
108.75	1.03	1.02	1.03	1.03	7.98
116.25	1.02	1.02	1.03	1.02	7.98
123.75	0.99	0.99	0.99	0.99	7.98
131.25	0.90	0.91	0.93	0.91	7.98
138.75	0.66	0.67	0.72	0.68	7.98
146.25	0.30	0.32	0.34	0.32	7.95
153.75	0.11	0.10	0.11	0.11	7.33
161.25	0.08	0.04	0.03	0.05	6.74
168.75	0.09	0.01	0.02	0.04	6.31
176.25	0.05	0.03	0.02	0.03	6.14

Recovery: CYCLE 1 - 96.8%
 CYCLE 2 - 93.3%
 CYCLE 3 - 94.7%

Average - 94.9%

RUN NO. 11Breakthrough Curve - Hemoglobin

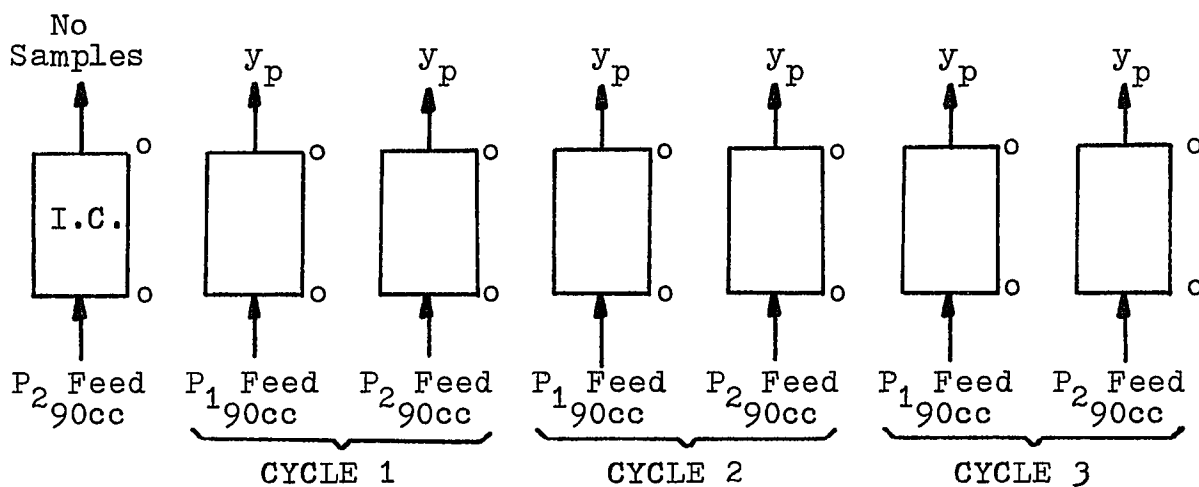
Buffer: 0.1M NaH_2PO_4 + 0.1M Na_2HPO_4

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 1.0 cc/min

Power: None

Procedure: As shown. Optimize bulk velocity for New Process.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.0$)

	<u>pH</u>	<u>R_{403}</u>
P_2 Feed	6.02	0.439
P_1 Feed	8.00	0.396

RUN NO. 11 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>
CYCLE 1					
1	7.50	3.75	6.08	0.006	0.01-
2	7.50	11.25	6.08	0.011	0.026
3	7.50	18.75	6.03	0.011	0.026
4	7.50	26.25	6.05	0.014	0.032
5	7.50	33.75	6.05	0.015	0.034
6	7.50	41.25	6.08	0.017	0.039
7	7.50	48.75	6.05	0.018	0.041
8	7.50	56.25	6.05	0.025	0.057
9	7.50	63.75	6.13	1.064	2.423
10	3.75	67.50	6.69	1.008*5	11.48-
11	3.75	71.25	7.25	1.130*3	7.722
12	3.75	75.00	7.65	1.284	3.041
13	3.75	78.75	7.82	0.622	1.52-
14	3.75	82.50	7.90	0.458	1.13-
15	3.75	86.25	7.92	0.419	1.04-
16	7.50	93.75	7.95	0.411	1.03-
17	7.50	101.25	7.98	0.404	1.02-
18	7.50	108.75	8.00	0.399	1.01-
19	7.50	116.25	8.00	0.399	1.01-
20	7.50	123.75	8.00	0.386	0.945
21	7.50	131.25	8.00	0.371	0.937
22	7.50	138.75	8.00	0.265	0.669
23	7.50	146.25	8.00	0.106	0.268
24	7.50	153.75	7.30	0.017	0.038
25	7.50	161.25	6.42	0.004	0.009
26	7.50	168.75	6.19	0.005	0.01-
27	7.50	176.25	6.10	0.004	0.009
CYCLE 2					
28	7.50	3.75	6.08	0.008	0.02-
29	7.50	11.25	6.05	0.007	0.02-
30	7.50	18.75	6.05	0.012	0.027
31	7.50	26.25	6.05	0.013	0.030
32	7.50	33.75	6.05	0.016	0.036
33	7.50	41.25	6.05	0.018	0.041
34	7.50	48.75	6.05	0.021	0.048
35	7.50	56.25	6.05	0.026	0.059
36	7.50	63.75	6.12	1.088	2.478
37	3.75	67.50	6.72	1.168*5	13.30-
38	3.75	71.25	7.32	1.118*3	7.640
39	3.75	75.00	7.64	1.131	2.676
40	3.75	78.75	7.82	0.584	1.32-
41	3.75	82.50	7.88	0.462	1.14-
42	3.75	86.25	7.90	0.421	1.04-
43	7.50	93.75	7.92	0.376	0.931
44	7.50	101.25	7.98	0.407	1.03-
45	7.50	108.75	7.98	0.405	1.02-

RUN NO. 11 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>
46	7.50	116.25	8.00	0.402	1.02-
47	7.50	123.75	8.00	0.387	0.977
48	7.50	131.25	7.98	0.375	0.945
49	7.50	138.75	8.00	0.280	0.707
50	7.50	146.25	7.98	0.105	0.265
51	7.50	153.75	7.42	0.025	0.057
52	7.50	161.25	6.43	0.006	0.01-
53	7.50	168.75	6.22	0.006	0.01-
54	7.50	176.25	6.07	0.006	0.01-
CYCLE 3					
55	7.50	3.75	6.03	0.010	0.023
56	7.50	11.25	6.03	0.010	0.023
57	7.50	18.75	6.03	0.012	0.027
58	7.50	26.25	6.03	0.013	0.030
59	7.50	33.75	6.03	0.015	0.034
60	7.50	41.25	6.03	0.016	0.036
61	7.50	48.75	6.02	0.017	0.039
62	7.50	56.25	6.02	0.021	0.048
63	7.50	63.75	6.10	0.580	1.32-
64	3.75	67.50	6.60	1.024*5	11.66-
65	3.75	71.25	7.20	1.411*3	9.868
66	3.75	75.00	7.59	1.705	3.883
67	3.75	78.75	7.80	0.692	1.68-
68	3.75	82.50	7.92	0.467	1.16-
69	3.75	86.25	7.92	0.418	1.04-
70	7.50	93.75	7.95	0.415	1.04-
71	7.50	101.25	7.95	0.408	1.02-
72	7.50	108.75	7.98	0.407	1.03-
73	7.50	116.25	7.98	0.398	1.00-
74	7.50	123.75	8.00	0.388	0.980
75	7.50	131.25	8.00	0.379	0.957
76	7.50	138.75	8.00	0.290	0.732
77	7.50	146.25	8.00	0.114	0.288
78	7.50	153.75	7.62	0.026	0.061
79	7.50	161.25	6.42	0.010	0.022
80	7.50	168.75	6.15	0.008	0.018
81	7.50	176.25	6.08	0.008	0.018

RUN NO. 11 (cont.)

<u><V_e></u>	<u>y_{Hb}/y_o</u>			<u>Average</u>	<u>pH (avg.)</u>
	<u>CYCLE 1</u>	<u>CYCLE 2</u>	<u>CYCLE 3</u>		
3.75	0.01	0.02	0.02	0.02	6.06
11.25	0.03	0.02	0.02	0.02	6.05
18.75	0.03	0.03	0.03	0.03	6.04
26.25	0.03	0.03	0.03	0.03	6.04
33.75	0.03	0.04	0.03	0.03	6.04
41.25	0.04	0.04	0.04	0.04	6.05
48.75	0.04	0.05	0.04	0.04	6.04
56.25	0.06	0.06	0.05	0.06	6.04
63.75	2.42	2.48	1.32	2.07	6.12
67.50	11.48	13.30	11.66	12.15	6.67
71.25	7.72	7.64	9.87	8.41	7.26
75.00	3.04	2.68	3.88	3.20	7.63
78.75	1.52	1.32	1.68	1.51	7.81
82.50	1.13	1.14	1.16	1.14	7.90
86.25	1.04	1.04	1.04	1.04	7.92
93.75	1.03	0.93	1.04	1.00	7.94
101.25	1.02	1.03	1.02	1.02	7.97
108.75	1.01	1.02	1.03	1.02	7.99
116.25	1.01	1.02	1.00	1.01	7.99
123.75	0.95	0.98	0.98	0.97	8.00
131.25	0.94	0.95	0.96	0.95	7.99
138.75	0.67	0.71	0.73	0.70	8.00
146.25	0.27	0.27	0.29	0.27	7.99
153.75	0.04	0.06	0.06	0.05	7.45
161.25	0.01	0.01	0.02	0.01	6.42
168.75	0.01	0.01	0.02	0.01	6.19
176.25	0.01	0.01	0.02	0.01	6.03

Recovery: CYCLE 1 - 94.2%
 CYCLE 2 - 97.1%
 CYCLE 3 - 97.5%

Average - 96.3%

RUN NO. 12Breakthrough Curve - Hemoglobin

Buffer: $0.1M NaH_2PO_4 + 0.1M Na_2HPO_4$

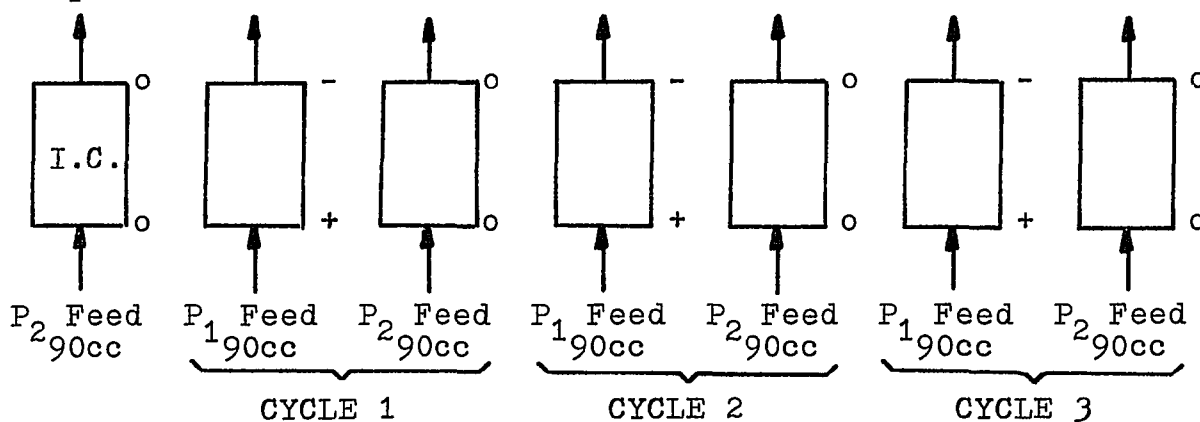
Feed: 0.01 weight % hemoglobin ($P_2 = 6.0, P_1 = 8.0$)

Flow Rate: 1.0 cc/min

Power: 120 volts

Procedure: As shown. Optimize bulk velocity for New Process.

No
Samples



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.0$)

	<u>pH</u>	<u>R₄₀₃</u>
P_2 Feed	6.02	0.459
P_1 Feed	8.00	0.396

RUN NO. 12 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>
CYCLE 1					
1	7.50	3.75	6.05	0.010	0.022
2	7.50	11.25	6.20	0.010	0.022
3	7.50	18.75	6.25	0.011	0.024
4	7.50	26.25	6.25	0.018	0.039
5	7.50	33.75	6.25	0.019	0.041
6	7.50	41.25	6.27	0.021	0.046
7	7.50	48.75	6.27	0.021	0.046
8	7.50	56.25	6.27	0.027	0.059
9	7.50	63.75	6.23	0.041	0.089
10	3.75	67.50	6.27	0.353	0.770
11	3.75	71.25	6.46	0.996*3	6.52-
12	3.75	75.00	6.92	1.231*5	13.23-
13	3.75	78.75	7.44	0.883*3	5.78-
14	3.75	82.50	7.82	0.811	1.976
15	3.75	86.25	7.98	0.469	1.182
16	7.50	93.75	8.00	0.429	1.08-
17	7.50	101.25	8.02	0.436	1.11-
18	7.50	108.75	8.02	0.423	1.07-
19	7.50	116.25	8.03	0.424	1.07-
20	7.50	123.75	8.02	0.413	1.05-
21	7.50	131.25	8.02	0.388	0.983
22	7.50	138.75	8.02	0.271	0.687
23	7.50	146.25	8.00	0.098	0.247
24	7.50	153.75	7.18	0.018	0.040
25	7.50	161.25	6.32	0.005	0.01-
26	7.50	168.75	6.10	0.007	0.02-
27	7.50	176.25	6.08	0.008	0.02-
CYCLE 2					
28	7.50	3.75	6.10	0.009	0.02-
29	7.50	11.25	6.22	0.009	0.02-
30	7.50	18.75	6.25	0.012	0.026
31	7.50	26.25	6.25	0.015	0.033
32	7.50	33.75	6.27	0.018	0.039
33	7.50	41.25	6.28	0.020	0.044
34	7.50	48.75	6.25	0.026	0.057
35	7.50	56.25	6.28	0.025	0.055
36	7.50	63.75	6.28	0.034	0.074
37	3.75	67.50	6.30	0.791	1.73-
38	3.75	71.25	6.48	1.009*3	6.602
39	3.75	75.00	6.90	1.046*5	11.41-
40	3.75	78.75	7.45	0.935*3	6.12-
41	3.75	82.50	7.88	0.909	2.25-
42	3.75	86.25	7.93	0.484	1.20-
43	7.50	93.75	7.90	0.411	1.15-
44	7.50	101.25	8.00	0.433	1.09-
45	7.50	108.75	8.00	0.430	1.09-

RUN NO. 12 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>
46	7.50	116.25	8.01	0.420	1.06-
47	7.50	123.75	8.00	0.412	1.04-
48	7.50	131.25	8.01	0.380	0.961
49	7.50	138.75	8.00	0.377	0.952
50	7.50	146.25	7.99	0.112	0.282
51	7.50	153.75	6.92	0.021	0.046
52	7.50	161.25	6.32	0.006	0.01-
53	7.50	168.75	6.12	0.007	0.02-
54	7.50	176.25	6.10	0.008	0.02-
CYCLE 3					
55	7.50	3.75	6.10	0.008	0.02-
56	7.50	11.25	6.22	0.014	0.031
57	7.50	18.75	6.25	0.019	0.041
58	7.50	26.25	6.28	0.010	0.035
59	7.50	33.75	6.29	0.021	0.046
60	7.50	41.25	6.30	0.025	0.055
61	7.50	48.75	6.30	0.029	0.063
62	7.50	56.25	6.30	0.035	0.076
63	7.50	63.75	6.30	0.039	0.085
64	3.75	67.50	6.30	0.064	0.140
65	3.75	71.25	6.30	1.073	2.340
66	3.75	75.00	6.60	1.181*3	7.727
67	3.75	78.75	7.00	1.008*5	10.99-
68	3.75	82.50	7.45	0.871*3	5.70-
69	3.75	86.25	7.80	0.819	1.99-
70	7.50	93.75	7.98	0.471	1.19-
71	7.50	101.25	8.00	0.440	1.11-
72	7.50	108.75	8.01	0.438	1.11-
73	7.50	116.25	8.00	0.426	1.08-
74	7.50	123.75	8.03	0.418	1.06-
75	7.50	131.25	8.03	0.403	1.02-
76	7.50	138.75	8.00	0.308	0.777
77	7.50	146.25	8.00	0.113	0.285
78	7.50	153.75	7.00	0.031	0.21-
79	7.50	161.25	6.30	0.011	0.024
80	7.50	168.75	6.12	0.008	0.02-
81	7.50	176.25	6.10	0.012	0.03-

RUN NO. 12 (cont.)

<u>< V_e ></u>	<u>y_{Hb}/y_o</u>			<u>Average</u>	<u>pH (avg.)</u>
	<u>CYCLE 1</u>	<u>CYCLE 2</u>	<u>CYCLE 3</u>		
3.75	0.02	0.02	0.02	0.02	6.08
11.25	0.02	0.02	0.03	0.02	6.21
18.75	0.02	0.03	0.04	0.03	6.25
26.25	0.04	0.03	0.04	0.04	6.26
33.75	0.04	0.04	0.05	0.04	6.27
41.25	0.05	0.04	0.06	0.05	6.28
48.75	0.05	0.06	0.06	0.06	6.27
56.25	0.06	0.06	0.08	0.07	6.28
63.75	0.09	0.07	0.09	0.08	6.27
67.50	0.77	1.73	0.14	0.88	6.29
71.25	6.52	6.60	2.34	5.15	6.41
75.00	13.23	11.41	7.73	10.79	6.81
78.75	5.78	6.12	10.99	7.63	7.30
82.50	1.98	2.25	5.70	3.31	7.72
86.25	1.18	1.20	1.99	1.46	7.90
93.75	1.08	1.15	1.19	1.14	7.96
101.25	1.11	1.09	1.11	1.10	8.01
108.75	1.07	1.09	1.11	1.09	8.01
116.25	1.07	1.06	1.08	1.07	8.01
123.75	1.05	1.04	1.06	1.05	8.02
131.25	0.98	0.96	1.02	0.99	8.02
138.75	0.69	0.95	0.78	0.81	8.01
146.25	0.25	0.28	0.29	0.27	8.00
153.75	0.04	0.05	0.21	0.10	7.03
161.25	0.01	0.01	0.02	0.01	6.31
168.25	0.02	0.02	0.02	0.02	6.11
176.25	0.02	0.02	0.03	0.02	6.09

Recovery: CYCLE 1 - 93.6%
 CYCLE 2 - 94.7%
 CYCLE 3 - 95.0%

Average - 94.4%

RUN NO. 13Adsorption/Desorption Curves - Tris-Maleate Buffer

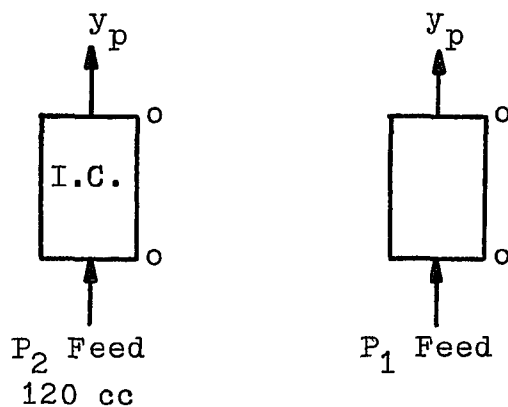
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 6.0$, $P_1 = 8.5$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: As shown. Optimize reservoir displacement.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.5$)

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.98	0.424	0.741
P_2 Buffer	5.98	---	0.335
P_1 Feed	8.51	0.401	0.745
P_1 Buffer	8.51	---	0.335

RUN NO. 13 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	8.10	0.391	---	0.875	---
2	7.50	11.25	8.23	0.411	0.749	0.948	1.07-
3	7.50	18.75	8.38	0.398	---	0.956	---
4	7.50	26.25	8.40	0.387	0.741	0.933	1.05-
5	7.50	33.75	8.40	0.363	---	0.876	---
6	7.50	41.25	8.40	0.258	0.671	0.622	1.02-
7	7.50	48.75	8.43	0.141	---	0.344	---
8	7.50	56.25	8.43	0.072	0.561	0.18-	0.93-
9	7.50	63.75	8.40	0.047	---	0.11-	---
10	7.50	71.25	8.30	0.042	0.565	0.099	1.02-
11	7.50	78.75	8.15	0.041	---	0.093	---
12	7.50	86.25	7.75	0.042	0.557	0.088	1.00-
13	7.50	93.75	7.00	0.040	---	0.095	---
14	7.50	101.25	6.57	0.040	0.555	0.094	1.02-
15	7.50	108.75	6.35	0.042	---	0.099	---
16	7.50	116.25	6.23	0.042	0.545	0.099	0.96-
Note: Change P_2 Feed to P_1 Feed.							
17	7.50	3.75	6.15	0.042	---	0.099	---
18	7.50	11.25	6.10	0.041	0.562	0.097	1.05-
19	7.50	18.75	6.10	0.042	---	0.099	---
20	7.50	26.25	6.08	0.042	0.557	0.099	1.02-
21	7.50	33.75	6.08	0.047	---	0.11-	---
22	7.50	41.25	6.05	0.059	0.566	0.14-	1.03-
23	7.50	48.75	6.05	0.074	---	0.18-	---
24	7.50	56.25	6.05	0.087	0.583	0.21-	1.04-

RUN NO. 13 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
25	7.50	63.75	6.08	0.91	---	0.22-	---
26	7.50	71.25	6.18	0.092	0.575	0.22-	0.99-
27	7.50	78.75	6.30	0.92	---	0.22-	---
28	7.50	86.25	6.25	0.92	0.569	0.22-	0.96-
29	7.50	93.75	6.25	0.103	---	0.243	---
30	7.50	101.25	6.81	1.471*2	0.959*2	6.947	XXX
31	7.50	108.75	8.13	1.759*2	0.995*2	7.919	XXX
32	7.50	116.25	8.32	1.039*2	0.821*2	4.913	XXX
33	7.50	123.75	8.33	1.102	1.053	2.612	0.890
34	7.50	131.25	8.33	0.537	---	1.27-	---
35	7.50	138.75	8.30	0.449	0.779	1.06-	1.11-
36	7.50	146.25	8.25	0.445	---	1.02-	---
37	7.50	153.75	8.20	0.432	0.763	0.987	1.10-
38	7.50	161.25	8.15	0.439	---	0.994	---
39	7.50	168.75	8.10	0.432	0.759	0.967	1.10-
40	7.50	176.25	8.15	0.383	---	0.867	---

Recovery: Hemoglobin - 95.6%

Albumin - 102%

Total Protein - 98%

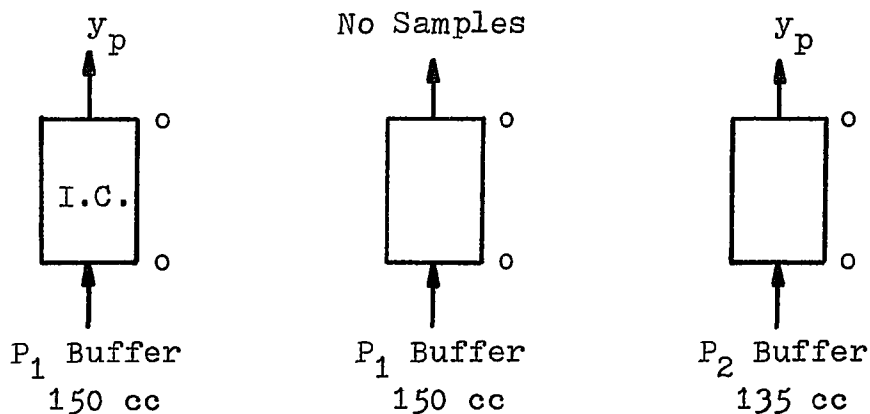
RUN NO. 14pH Wave Velocity - Tris-Maleate Buffer

Buffer: 0.05M Tris-Maleate + 0.05M NaOH ($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: As shown. Measure pH wave velocity without feed (buffer only) in order to determine whether adsorption/desorption affects the velocity.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 5.8$)

<u>Vol, cc</u>	<u><V_e></u>	<u>#</u>	<u>pH</u>	<u>#</u>	<u>pH</u>
7.50	3.75	1	5.98	41	8.31
15.00	11.25	2	5.93	42	8.38
22.50	18.75	3	5.92	43	8.42
30.00	26.25	4	5.92	44	8.40
37.50	33.75	5	5.92	45	8.41
45.00	41.25	6	5.92	46	8.42
52.50	48.75	7	5.92	47	8.42
60.00	56.25	8	5.92	48	8.41
67.50	63.75	9	5.93	49	8.41

RUN NO. 14 (cont.)

<u>Vol, cc</u>	<u><V_e></u>	<u>#</u>	<u>pH</u>	<u>#</u>	<u>pH</u>
75.00	71.25	10	6.02	50	8.32
82.50	78.75	11	6.08	51	8.21
90.00	86.25	12	6.09	52	7.63
97.50	93.75	13	6.10	53	6.83

Note: ISOELECTRIC POINT for Adsorbed Protein (HEMOGLOBIN) is at this point, i.e., pH = 6.7.

105.00	101.25	14	6.82	54	6.45
112.50	108.75	15	7.82	55	6.28
120.00	116.25	16	7.91	56	6.18
127.50	123.75	17	7.92	57	6.12
135.00	131.25	18	7.92	58	6.06
142.50	138.75	19	7.92	--	--
150.00	146.25	20	7.91	--	--

Note: Sample #'s 21-40 (150 cc) not recorded.

RUN NO. 15Adsorption Curve - Tris-Maleate Buffer

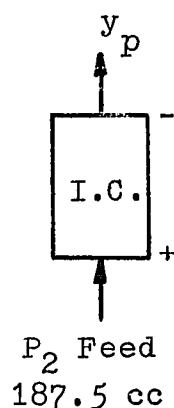
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Investigate effect of electric field
in Stage I.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.5$)

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.75	0.409	0.844
P_2 Buffer	5.75	---	0.430
P_1 Feed	8.52	0.370	0.856
P_1 Buffer	8.52	---	0.433

<u>#</u>	<u>Vol, cc</u>	<u>$\langle V_e \rangle$</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{HB}/y_o</u>	<u>y_{Al}/y_o</u>
1	7.50	3.75	8.30	0.331	---	0.840	---
2	7.50	11.25	8.35	0.335	0.817	0.863	0.96-

RUN NO. 15 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
3	7.50	18.75	8.41	0.335	---	0.876	---
4	7.50	26.25	8.50	0.340	0.816	0.919	0.89-
5	7.50	33.75	8.50	0.335	---	0.905	---
6	7.50	41.25	8.50	0.295	0.809	0.797	0.97-
7	7.50	48.75	8.51	0.162	---	0.440	---
8	7.50	56.25	8.50	0.070	0.668	0.19-	0.92-
9	7.50	63.75	8.50	0.052	---	0.17-	---
10	7.50	71.25	8.50	0.046	0.618	0.12-	0.75-
11	7.50	78.75	8.40	0.045	---	0.12-	---
12	7.50	86.25	7.95	0.045	0.635	0.12-	0.84-
13	7.50	93.75	7.60	0.045	---	0.12-	---
14	7.50	101.25	7.40	0.044	0.649	0.12-	0.90-
15	7.50	108.75	7.30	0.047	---	0.12-	---
16	7.50	116.25	7.12	0.048	0.638	0.12-	0.85-
17	7.50	123.75	6.87	0.047	---	0.12-	---
18	7.50	131.25	6.70	0.045	0.634	0.12-	0.83-
19	7.50	138.75	6.53	0.048	---	0.12-	---
20	7.50	146.25	6.42	0.049	0.654	0.12-	0.93-
21	7.50	153.75	6.30	0.048	---	0.12-	---
22	7.50	161.25	6.19	0.050	0.652	0.12-	0.92-
23	7.50	168.75	6.10	0.052	---	0.12-	---
24	7.50	176.25	6.00	0.052	0.650	0.13-	0.90-
25	7.50	183.75	5.93	0.051	---	0.13-	---

RUN NO. 16Adsorption Curve - Tris-Maleate Buffer

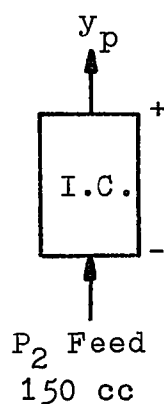
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Investigate effect of electric field
in Stage I.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.5$)

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.83	0.441	0.832
P_2 Buffer	5.83	---	0.382
P_1 Feed	8.68	0.350	0.856
P_1 Buffer	8.68	---	0.390

RUN NO. 16 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>
1	7.50	3.75	8.40	0.345	0.798	0.904	0.85-
2	7.50	11.25	8.40	0.373	0.879	0.978	1.12-
3	7.50	18.75	8.40	0.419	0.919	1.10-	1.17-
4	7.50	26.25	8.50	0.398	0.890	1.07-	1.07-
5	7.50	33.75	8.45	0.266	0.745	0.708	0.82-
6	7.50	41.25	8.33	0.132	0.635	0.340	0.71-
7	7.50	48.75	8.25	0.062	0.600	0.16-	0.75-
8	7.50	56.25	8.10	0.054	0.625	0.13-	0.88-
9	7.50	63.75	8.05	0.054	0.619	0.13-	0.85-
10	7.50	71.25	8.00	0.054	0.646	0.13-	0.97-
11	7.50	78.75	7.40	0.048	---	0.10-	---
12	7.50	86.25	6.25	0.044	0.622	0.099	0.97-
13	7.50	93.75	6.10	0.044	0.618	0.099	0.95-
14	7.50	101.25	6.05	0.048	0.627	0.11-	0.98-
15	7.50	108.75	6.08	0.049	---	0.11-	---
16	7.50	116.25	6.08	0.050	0.633	0.11-	1.00-
17	7.50	123.75	6.09	0.053	---	0.12-	---
18	7.50	131.25	6.09	0.053	0.633	0.12-	1.00-
19	7.50	138.75	6.09	0.056	---	0.13-	---
20	7.50	146.25	6.09	0.057	0.633	0.13-	0.99-

RUN NO. 17Breakthrough Curve - Mixture

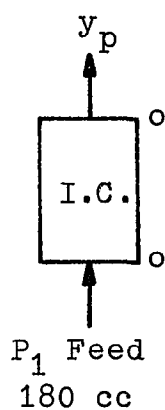
Buffer: 0.20M Tris-Maleate + 0.20M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 2.5 cc/min

Power: None

Procedure: As shown. Simulate Stage IV operation with high ionic strength solutions.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 5.8$)

Feed Reading: $R_{403} = 0.372$ (pH = 8.52)

$R_{595} = 0.842$

Buffer Reading: $R_{595} = 0.385$ (pH = 5.78)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	5.78	---	---	---	---
2	7.50	11.25	5.80	---	---	---	---
3	7.50	18.75	5.79	---	---	---	---
4	7.50	26.25	5.79	---	---	---	---

RUN NO. 17 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{HB}/y_o	y_{Al}/y_o
5	7.50	33.75	5.80	0.001	0.388	0.002	0.01-
6	7.50	41.25	5.79	0.001	0.402	0.002	0.072
7	7.50	48.75	5.79	0.008	0.475	0.02-	0.38-
8	7.50	56.25	5.80	0.019	0.567	0.039	0.758
9	7.50	63.75	5.79	0.041	0.623	0.085	0.96-
10	7.50	71.25	5.79	0.082	0.645	0.17-	0.97-
11	7.50	78.75	5.90	0.397	0.769	0.820	0.86-
12	7.50	86.25	6.95	1.054	1.058	2.177	0.771
13	7.50	93.75	7.82	0.512	0.850	1.17-	0.87-
14	7.50	101.25	7.95	0.399	0.813	0.928	0.95-
15	7.50	108.75	7.95	0.402	0.813	0.936	0.94-
16	7.50	116.25	7.98	0.407	0.835	0.955	1.01-
17	7.50	123.75	8.00	0.405	0.840	0.953	1.04-
18	7.50	131.25	8.02	0.408	0.825	0.963	0.97-
19	7.50	138.75	8.12	0.396	0.802	0.956	0.87-
20	7.50	146.25	8.35	0.378	0.820	0.967	0.94-
21	7.50	153.75	8.48	0.366	0.822	0.975	0.94-
22	7.50	161.25	8.50	0.364	0.829	0.974	0.97-
23	7.50	168.75	8.50	0.362	0.809	0.968	0.89-
24	7.50	176.25	8.50	0.363	0.835	0.971	1.00-

Recovery: Hemoglobin - 83.5%

Albumin - 84.7%

Total Protein - 84.1%

RUN NO. 18Breakthrough Curve - Mixture

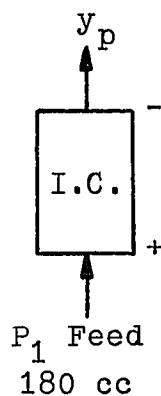
Buffer: 0.20M Tris-Maleate + 0.20M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts X 162 mAmp = 34 watts

Procedure: As shown. Prove detrimental effects of high power operation on protein recovery.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 5.8$)

Feed Reading: $R_{403} = 0.361$ (pH = 8.55)

$R_{595} = 0.811$

Buffer Reading: $R_{595} = 0.387$ (pH = 5.78)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	5.79	---	---	---	---
2	7.50	11.25	6.02	---	---	---	---
3	7.50	18.75	6.15	---	---	---	---
4	7.50	26.25	6.15	---	---	---	---

RUN NO. 18 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
5	7.50	33.75	6.12	0.004	0.386	0.008	0
6	7.50	41.25	6.13	0.000	0.384	0	0
7	7.50	48.75	6.12	0.000	0.406	0	0.090
8	7.50	56.25	6.13	0.004	0.453	0.008	0.30-
9	7.50	63.75	6.15	0.011	0.515	0.023	0.581
10	7.50	71.25	6.12	0.027	0.555	0.057	0.735
11	7.50	78.75	6.12	0.088	0.563	0.19-	0.645
12	7.50	86.25	6.32	0.195	0.635	0.410	0.76-
13	7.50	93.75	6.72	0.264	0.671	0.556	0.78-
14	7.50	101.25	7.52	0.418	0.769	0.923	0.88-
15	7.50	108.75	8.38	0.384	0.772	1.01-	0.80-
16	7.50	116.25	8.48	0.268	0.717	0.726	0.83-
17	7.50	123.75	8.48	0.265	0.738	0.718	0.94-
18	7.50	131.25	8.48	0.272	0.754	0.737	0.99-
19	7.50	138.75	8.50	0.291	0.754	0.792	0.94-
20	7.50	146.25	8.50	0.273	0.757	0.743	1.00-
21	7.50	153.75	8.48	0.270	0.746	0.731	0.96-
22	7.50	161.25	8.51	0.261	0.745	0.714	0.98-
23	7.50	168.75	8.48	0.262	0.749	0.710	1.00-
24	7.50	176.25	8.50	0.289	0.772	0.787	1.03-

Recovery: Hemoglobin - 54.7%

Albumin - 79.2%

Total Protein - 67.0%

RUN NO. 19Breakthrough Curve - Mixture

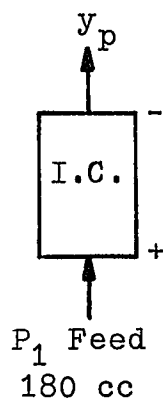
Buffer: 0.10M Tris-Maleate + 0.10M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts X 82 mAmp = 17 watts

Procedure: As shown. Prove detrimental effects of high power operation on protein recovery.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{403} = 0.370$ (pH = 8.50)

$R_{595} = 0.809$

Buffer Reading: $R_{595} = 0.384$ (pH = 5.95)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	6.22	---	---	---	---
2	7.50	11.25	6.20	---	---	---	---
3	7.50	18.75	6.08	---	---	---	---
4	7.50	26.25	6.02	0.000	0.388	0	0.02-

RUN NO. 19 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
5	7.50	33.75	6.00	0.000	0.374	0	0
6	7.50	41.25	5.99	0.001	0.398	0.002	0.064
7	7.50	48.75	6.00	0.004	0.495	0.008	0.51-
8	7.50	56.25	6.02	0.019	0.571	0.040	0.840
9	7.50	63.75	6.02	0.030	0.591	0.063	0.911
10	7.50	71.25	6.02	0.045	0.591	0.094	0.880
11	7.50	78.75	6.15	0.066	0.594	0.14-	0.85-
12	7.50	86.25	6.39	0.405	0.712	0.845	0.70-
13	7.50	93.75	7.68	0.992	0.909	2.22-	0.25-
14	7.50	101.25	7.84	0.367	0.773	0.844	0.99-
15	7.50	108.75	7.87	0.292	0.722	0.675	0.92-
16	7.50	116.25	7.90	0.262	0.710	0.609	0.93-
17	7.50	123.75	7.93	0.263	0.727	0.619	1.00-
18	7.50	131.25	8.02	0.261	0.696	0.622	0.85-
19	7.50	138.75	8.12	0.264	0.704	0.643	0.86-
20	7.50	146.25	8.22	0.260	0.722	0.648	0.94-
21	7.50	153.75	8.30	0.257	0.701	0.656	0.84-
22	7.50	161.25	8.38	0.255	0.689	0.665	0.77-
23	7.50	168.75	8.42	0.246	0.702	0.649	0.85-
24	7.50	176.25	8.40	0.243	0.693	0.637	0.82-

Recovery: Hemoglobin - 59.3%

Albumin - 82.1%

Total Protein - 70.7%

RUN NO. 20

Breakthrough Curve - Mixture

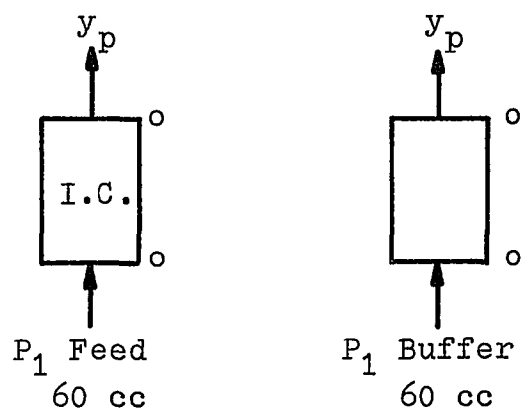
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: None

Procedure: As shown. Simulate Stages V & VI.



Initial Conditions (I.C.) in the column: Buffer ($P_1 = 8.5$)

Feed Reading: $R_{403} = 0.365$ (pH = 8.52)

$R_{595} = 0.725$

Buffer Reading: $R_{595} = 0.374$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	8.42	---	---	---	---
2	7.50	11.25	8.51	---	---	---	---
3	7.50	18.75	8.50	---	---	---	---
4	7.50	26.25	8.51	---	---	---	---

RUN NO. 20 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>
5	3.75	31.88	8.51	0.005	0.381	0.01-	0.03-
6	3.75	35.63	8.52	0.014	0.387	0.038	0.036
7	3.75	39.38	8.52	0.049	0.447	0.13-	0.28-
8	3.75	43.13	8.52	0.124	0.521	0.340	0.499
9	3.75	46.88	8.52	0.216	0.623	0.593	0.83-
10	3.75	50.63	8.52	0.283	0.668	0.776	0.90-
11	3.75	54.38	8.52	0.324	0.671	0.889	0.81-
12	3.75	58.13	8.52	0.343	0.700	0.941	0.92-
Note: Change Feed to Buffer.							
13	3.75	61.88	8.52	0.350	0.709	0.960	0.95-
14	3.75	65.63	8.52	0.354	0.691	0.971	0.84-
15	3.75	69.38	8.52	0.356	0.698	0.977	0.87-
16	3.75	73.13	8.52	0.354	0.705	0.971	0.92-
17	3.75	76.88	8.52	0.359	0.698	0.985	0.86-
18	3.75	80.63	8.52	0.359	0.715	0.985	0.96-
19	3.75	84.38	8.52	0.362	0.721	0.993	0.99-
20	3.75	88.13	8.52	0.360	0.697	0.988	0.86-
21	7.50	93.75	8.51	0.354	0.698	0.971	0.88-
22	7.50	101.25	8.51	0.259	0.598	0.711	0.57-
23	7.50	108.75	8.52	0.097	0.450	0.27-	0.17-
24	7.50	116.25	8.52	0.026	0.396	0.071	0.06-

Recovery: Hemoglobin - 97.5%

Albumin - 93.0%

Total Protein - 95.3%

RUN NO. 21Breakthrough Curve - Mixture

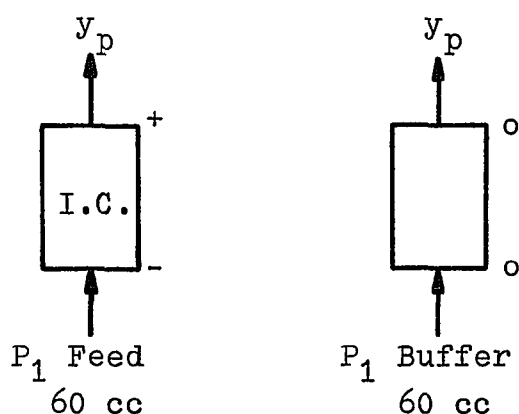
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
@ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Investigate effect of electric field
in Stages V or VI.



Initial Conditions (I.C.) in the column: Buffer ($P_1 = 8.5$)

Feed Reading: $R_{403} = 0.365$ (pH = 8.52)

$R_{595} = 0.725$

Buffer Reading: $R_{595} = 0.374$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
1	7.50	3.75	8.32	---	---	---	---
2	7.50	11.25	8.70	---	---	---	---
3	7.50	18.75	8.60	---	---	---	---
4	7.50	26.25	8.58	0.003	0.369	0.008	0

RUN NO. 21 (cont.)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o
5	3.75	31.88	8.55	0.015	0.390	0.042	0.049
6	3.75	35.63	8.55	0.053	0.465	0.15-	0.37-
7	3.75	39.38	8.50	0.115	0.524	0.313	0.543
8	3.75	43.13	8.52	0.173	0.574	0.475	0.67-
9	3.75	46.88	8.58	0.212	0.597	0.585	0.69-
10	3.75	50.63	8.52	0.249	0.620	0.683	0.72-
11	3.75	54.38	8.40	0.291	0.660	0.769	0.86-
12	3.75	58.13	8.30	0.338	0.669	0.871	0.81-
Note: POWER OFF. Change Feed to Buffer.							
13	3.75	61.88	8.19	0.371	0.685	0.929	0.85-
14	3.75	65.63	8.11	0.358	0.677	0.880	0.85-
15	3.75	69.38	8.21	0.333	0.632	0.836	0.64-
16	3.75	73.13	8.32	0.325	0.640	0.840	0.68-
17	3.75	76.88	8.34	0.321	0.635	0.835	0.65-
18	3.75	80.63	8.32	0.324	0.649	0.838	0.73-
19	3.75	84.38	8.31	0.324	0.640	0.836	0.68-
20	3.75	88.13	8.32	0.322	0.643	0.832	0.70-
21	7.50	93.75	8.37	0.318	0.635	0.833	0.66-
22	7.50	101.25	8.40	0.276	0.610	0.730	0.62-
23	7.50	108.75	8.39	0.128	0.469	0.337	0.21-
24	7.50	116.25	8.40	0.030	0.394	0.079	0.04-

Recovery: Hemoglobin - 91.7%

Albumin - 84.5%

Total Protein - 88.1%

RUN NO. 22Breakthrough Curve - Hemoglobin

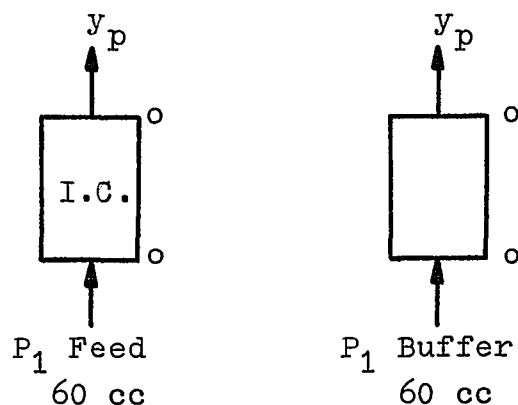
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin @ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: None

Procedure: As shown. Simulate Stages V & VI.



Initial Conditions (I.C.) in the column: Buffer ($P_1 = 8.5$)

Feed Reading: $R_{403} = 0.329$ (pH = 8.62)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{403}	y_{Hb}/y_o
1	7.50	3.75	8.26	0.032	0.091
2	7.50	11.25	8.61	0.012	0.037
3	7.50	18.75	8.63	0.010	0.032
4	7.50	26.25	8.64	0.019	0.060
5	3.75	31.88	8.64	0.012	0.038
6	3.75	35.63	8.64	0.011	0.035

RUN NO. 22 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{HB}/y_O</u>
7	3.75	39.38	8.68	0.036	0.11-
8	3.75	43.13	8.67	0.105	0.333
9	3.75	46.88	8.68	0.190	0.615
10	3.75	50.63	8.68	0.248	0.803
11	3.75	54.38	8.68	0.270	0.874
12	3.75	58.13	8.68	0.285	0.923
Note: Change Feed to Buffer.					
13	3.75	61.88	8.64	0.308	0.984
14	3.75	65.63	8.62	0.325	1.03-
15	3.75	69.38	8.61	0.318	1.00-
16	3.75	73.13	8.62	0.311	0.980
17	3.75	76.88	8.69	0.310	0.977
18	3.75	80.63	8.68	0.311	0.973
19	3.75	84.38	8.63	0.314	0.989
20	3.75	88.13	8.67	0.312	0.996
21	7.50	93.75	8.72	0.309	1.01-
22	7.50	101.25	8.71	0.223	0.724
23	7.50	108.75	8.72	0.075	0.24-
24	7.50	116.25	8.72	0.018	0.058

Recovery: Hemoglobin - 101%

RUN NO. 23Breakthrough Curve - Hemoglobin

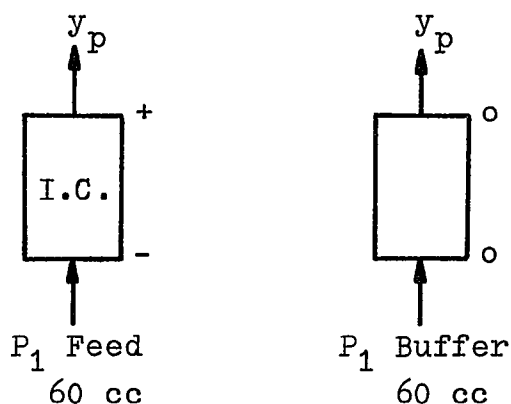
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin @ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Investigate effect of electric field in Stages V or VI.



Initial Conditions (I.C.) in the column: Buffer ($P_1 = 8.5$)

Feed Reading: $R_{403} = 0.336$ (pH = 8.50)

<u>#</u>	<u>Vol, cc</u>	<u>$\langle V_e \rangle$</u>	<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>
1	7.50	3.75	8.20	---	---
2	7.50	11.25	8.40	---	---
3	7.50	18.75	8.40	---	---
4	7.50	26.25	8.50	0.012	0.036
5	7.50	33.75	8.40	0.035	0.10-
6	7.50	41.25	8.40	0.163	0.472

RUN NO. 23 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>
7	3.75	46.88	8.42	0.226	0.656
8	3.75	50.63	8.38	0.252	0.725
9	3.75	54.38	8.25	0.292	0.812
10	3.75	58.13	8.20	0.320	0.879
Note: POWER OFF. Change Feed to Buffer.					
11	3.75	61.88	8.35	0.349	0.994
12	3.75	65.63	8.20	0.346	0.950
13	3.75	69.38	8.20	0.317	0.870
14	3.75	73.13	8.25	0.312	0.867
15	3.75	76.88	8.28	0.305	0.859
16	3.75	80.63	8.23	0.307	0.838
17	3.75	84.38	8.30	0.303	0.853
18	3.75	88.13	8.42	0.303	0.880
19	7.50	93.75	8.35	0.297	0.847
20	7.50	101.25	8.38	0.259	0.745
21	7.50	108.75	8.40	0.107	0.311
22	7.50	116.25	8.42	0.027	0.079

Recovery: Hemoglobin - 96.0%

RUN NO. 24

Breakthrough Curve - Albumin

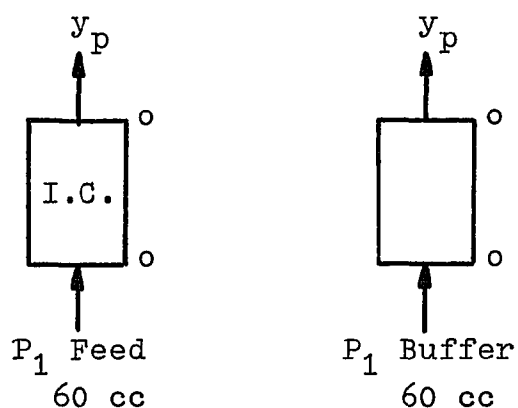
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin @ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: None

Procedure: As shown. Simulate Stages V & VI.



Initial Conditions (I.C.) in the column: Buffer ($P_1 = 8.5$)

Feed Reading: $R_{595} = 0.581$ (pH = 8.62)

Buffer Reading: $R_{595} = 0.404$

<u>#</u>	<u>Vol, cc</u>	<u>$\langle V_e \rangle$</u>	<u>pH</u>	<u>R_{595}</u>	<u>y_{A1}/y_o</u>
1	7.50	3.75	8.72	0.398	0
2	7.50	11.25	8.70	0.394	0
3	7.50	18.75	8.68	0.394	0
4	7.50	26.25	8.62	0.401	0
5	3.75	31.88	8.68	0.425	0.12-

RUN NO. 24 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{Al}/y_o</u>
6	3.75	35.63	8.62	0.427	0.13-
7	3.75	39.38	8.62	0.479	0.42-
8	3.75	43.13	8.62	0.522	0.667
9	3.75	46.88	8.62	0.543	0.785
10	3.75	50.63	8.62	0.543	0.785
11	3.75	54.38	8.62	0.549	0.819
12	3.75	58.13	8.59	0.550	0.825
Note: Change Feed to Buffer.					
13	3.75	61.88	8.59	0.573	0.955
14	3.75	65.63	8.61	0.570	0.938
15	3.75	69.38	8.62	0.568	0.927
16	3.75	73.13	8.62	0.582	1.00-
17	3.75	76.88	8.61	0.565	0.910
18	3.75	80.63	8.60	0.581	1.00-
19	3.75	84.38	8.59	0.580	0.994
20	3.75	88.13	8.60	0.592	1.06-
21	7.50	93.75	8.59	0.566	0.915
22	7.50	101.25	8.59	0.479	0.42-
23	7.50	108.75	8.58	0.400	0
24	7.50	116.25	8.58	0.388	0

Albumin Recovery: 93.9%

RUN NO. 25Breakthrough Curve - Albumin

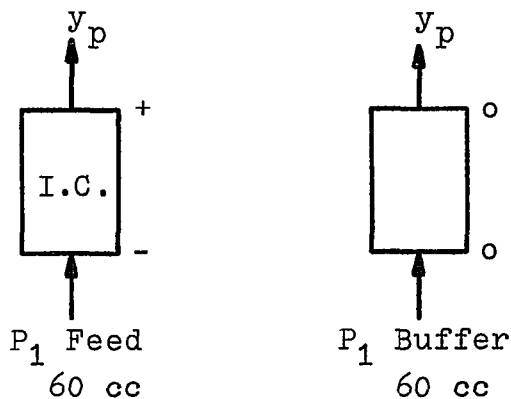
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin @ $P_1 = 8.5$

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Investigate effect of electric field in Stages V or VI.



Initial Conditions (I.C.) in the column: Buffer ($P_1 = 8.5$)

Feed Reading: $R_{595} = 0.575$

Buffer Reading: $R_{595} = 0.388$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{595}	y_{Al}/y_o
1	7.50	3.75	8.60	---	---
2	7.50	11.25	8.65	---	---
3	7.50	18.75	8.60	---	---
4	7.50	26.25	8.58	0.396	0.04-
5	3.75	31.88	8.55	0.447	0.32-

RUN NO. 25 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{A1}/y₀</u>
6	3.75	35.63	8.55	0.504	0.620
7	3.75	39.38	8.55	0.526	0.738
8	3.75	43.13	8.53	0.543	0.829
9	3.75	46.88	8.53	0.552	0.877
10	3.75	50.63	8.48	0.571	0.979
11	3.75	54.38	8.35	0.568	0.963
12	3.75	58.13	8.35	0.577	1.01-
Note. POWER OFF. Change Feed to Buffer.					
13	3.75	61.88	8.30	0.578	1.02-
14	3.75	65.63	8.12	0.556	0.898
15	3.75	69.38	8.38	0.543	0.829
16	3.75	73.13	8.45	0.550	0.866
17	3.75	76.88	8.42	0.537	0.797
18	3.75	80.63	8.42	0.550	0.866
19	3.75	84.38	8.42	0.537	0.797
20	3.75	88.13	8.42	0.527	0.743
21	7.50	93.75	8.43	0.541	0.818
22	7.50	101.25	8.42	0.460	0.39-
23	7.50	108.75	8.43	0.402	0.075
24	7.50	116.25	8.43	0.390	0.01-

Albumin Recovery: 98.8%

RUN NO. 26Breakthrough Curve - Albumin

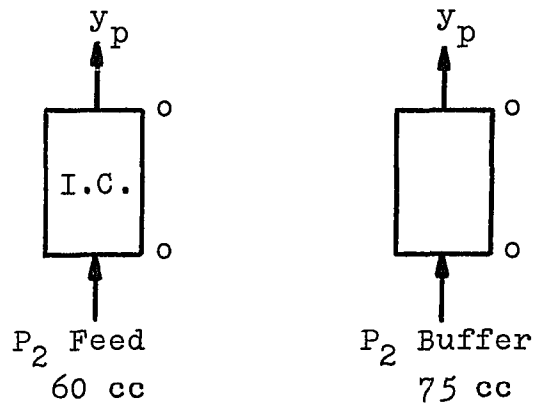
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin @ $P_2 = 6.0$

Flow Rate: 0.5 cc/min

Power: None

Procedure: As shown. Simulate Stages II & III.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{595} = 0.576$ (pH = 5.98)

Buffer Reading: $R_{595} = 0.404$ (pH = 5.95)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{595}	y_{Al}/y_o
1	7.50	3.75	6.15	---	---
2	7.50	11.25	6.12	---	---
3	7.50	18.75	6.12	---	---
4	7.50	26.25	6.10	0.413	0.05-
5	3.75	31.88	6.10	0.405	0.006

RUN NO. 26 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{Al}/y_o</u>
6	3.75	35.63	6.10	0.419	0.087
7	3.75	39.38	6.10	0.453	0.29-
8	3.75	43.13	6.08	0.509	0.610
9	3.75	46.88	6.08	0.540	0.791
10	3.75	50.63	6.05	0.564	0.930
11	3.75	54.38	6.05	0.572	0.977
12	3.75	58.13	6.05	0.569	0.959
Note: Change Feed to Buffer.					
13	7.50	63.75	6.05	0.573	0.983
14	3.75	69.38	6.05	0.570	0.965
15	3.75	73.13	6.05	0.575	0.994
16	3.75	76.88	6.05	0.569	0.959
17	3.75	80.63	6.05	0.578	1.01-
18	3.75	84.38	6.10	0.573	0.983
19	3.75	88.13	6.10	0.577	1.01-
20	7.50	93.75	6.08	0.52	0.860
21	7.50	101.25	6.10	0.495	0.53-
22	7.50	108.75	6.08	0.421	0.10-
23	7.50	116.25	6.08	0.407	0.02-
24	7.50	123.75	6.08	0.404	0
25	7.50	131.25	6.08	0.410	0.03-

Albumin Recovery: 98.2%

RUN NO. 27Breakthrough Curve - Albumin

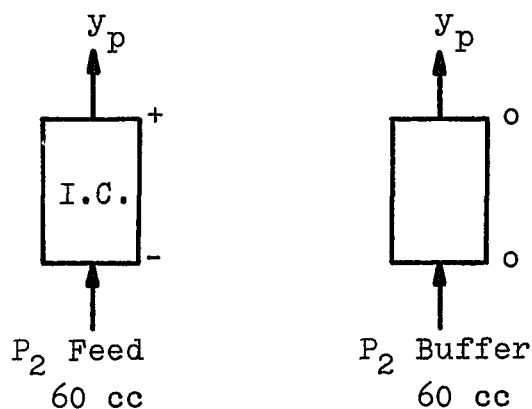
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin @ $P_2 = 6.0$

Flow Rate: 0.5 cc/min

Power: 210 volts

Procedure: As shown. Investigate effect of electric field in Stages II or III.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{595} = 0.585$ (pH = 5.98)

Buffer Reading: $R_{595} = 0.414$ (pH = 6.10)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{595}	y_{A1}/y_0
1	7.50	3.75	6.12	---	---
2	7.50	11.25	6.12	---	---
3	7.50	18.75	6.12	---	---
4	7.50	26.25	6.12	0.410	0
5	3.75	31.88	6.20	0.435	0.12-

RUN NO. 27 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{Al}/y_o</u>
6	3.75	35.63	6.27	0.475	0.35-
7	3.75	39.38	6.27	0.507	0.54-
8	3.75	43.13	6.20	0.516	0.600
9	3.75	46.88	6.20	0.512	0.572
10	3.75	50.63	6.12	0.545	0.767
11	3.75	54.38	5.95	0.525	0.649
12	3.75	58.13	6.00	0.551	0.802
Note: POWER OFF. Change Feed to Buffer.					
13	7.50	63.75	6.05	0.561	0.861
14	3.75	69.38	6.03	0.556	0.832
15	3.75	73.13	6.12	0.571	0.918
16	3.75	76.88	6.10	0.568	0.903
17	3.75	80.63	6.10	0.564	0.879
18	3.75	84.38	6.10	0.560	0.856
19	3.75	88.13	6.05	0.573	0.932
20	7.50	93.75	6.05	0.566	0.891
21	7.50	101.25	6.05	0.533	0.696
22	7.50	108.75	6.12	0.426	0.065
23	7.50	116.25	6.12	0.412	0

Albumin Recovery: 92.2%

RUN NO. 28Breakthrough Curve - Albumin

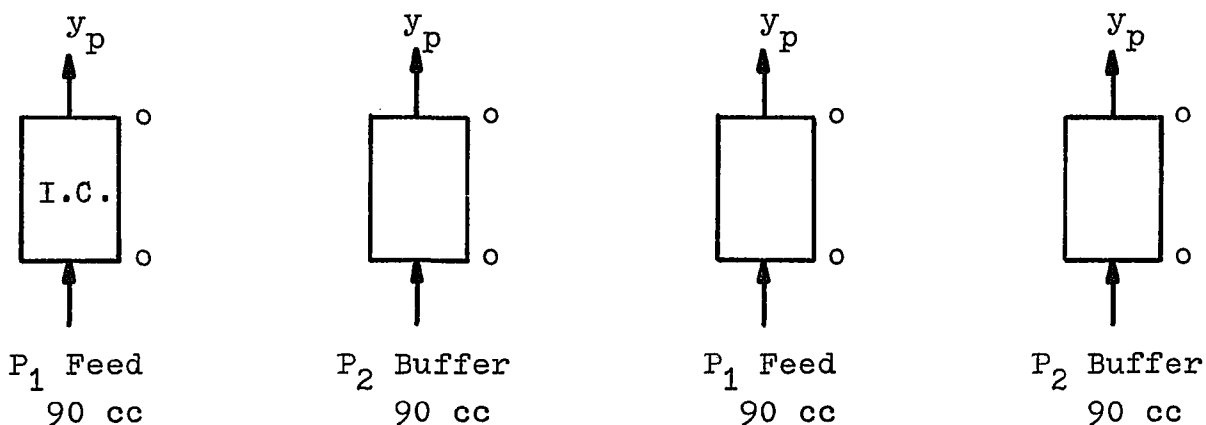
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin @ $P_1 = 8.0$

Flow Rate: 1.0 cc/min

Power: None

Procedure: As shown. Examine shape of wave front due to eddy diffusivity.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{595} = 0.598$

Buffer Reading: $R_{595} = 0.412$ (pH = 6.0)

$R_{595} = 0.412$ (pH = 8.0)

#	<u>Vol, cc</u>	<u>$\langle V_e \rangle$</u>	<u>pH</u>	<u>R_{595}</u>	<u>y_{Al}/y_o</u>
1	7.50	3.75	6.10	-	-
2	7.50	11.25	6.08	-	-
3	7.50	18.75	6.05	-	-
4	7.50	26.25	6.05	0.418	0.03-
5	7.50	33.75	6.05	0.425	0.070

RUN NO. 28 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u>$\langle V_e \rangle$</u>	<u>pH</u>	<u>R_{595}</u>	<u>y_{A1}/y_0</u>
6	7.50	41.25	6.08	0.471	0.32-
7	7.50	48.75	6.08	0.553	0.760
8	7.50	56.25	6.10	0.586	0.938
9	7.50	63.75	6.18	0.592	0.970
10	7.50	71.25	6.80	0.605	1.04-
11	7.50	78.75	7.80	0.601	1.02-
12	7.50	86.25	8.00	0.599	1.01-

Note: Change Feed to Buffer

13	7.50	93.75	8.02	0.599	1.01-
14	7.50	101.25	8.00	0.602	1.02-
15	7.50	108.75	8.02	0.599	1.01-
16	7.50	116.25	8.05	0.605	1.04-
17	7.50	123.75	8.05	0.611	1.07-
18	7.50	131.25	8.05	0.549	0.738
19	7.50	138.75	8.05	0.468	0.30-
20	7.50	146.25	8.05	0.425	0.070
21	7.50	153.75	7.60	0.415	0.02-
22	7.50	161.25	6.75	0.407	0
23	7.50	168.75	6.48	-	-
24	7.50	176.25	6.32	-	-

Note: Add Feed - Cycle 2

25	7.50	3.75	6.25	-	-
26	7.50	11.25	6.20	-	-
27	7.50	18.75	6.11	-	-
28	7.50	26.25	6.10	0.410	0

RUN NO. 28 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{A1}/y₀</u>
29	7.50	33.75	6.10	0.414	0
30	7.50	41.25	6.08	0.471	0.32-
31	7.50	48.75	6.08	0.555	0.771
32	7.50	56.25	6.10	0.575	0.879
33	7.50	63.75	6.10	0.599	1.01-
34	7.50	71.25	6.78	0.604	1.04-
35	7.50	78.75	7.75	0.595	0.986
36	7.50	86.25	8.00	0.607	1.05-
Note: Change Feed to Buffer					
37	7.50	93.75	8.02	0.580	0.906
38	7.50	101.25	8.02	0.578	0.895
39	7.50	108.75	8.05	0.601	1.02-
40	7.50	116.25	8.05	0.606	1.05-
41	7.50	123.75	8.05	0.593	0.976
42	7.50	131.25	8.05	0.552	0.755
43	7.50	138.75	8.05	0.567	0.296
44	7.50	146.25	8.05	0.417	0.03-
45	7.50	153.75	7.55	0.421	0.05-
46	7.50	161.25	6.75	0.415	0.02-
47	7.50	168.75	6.48	-	-
48	7.50	176.25	6.32	-	-

Recovery: CYCLE 1 - 103.6%

CYCLE 2 - 100.5%

RUN NO. 29Breakthrough Curve - Albumin

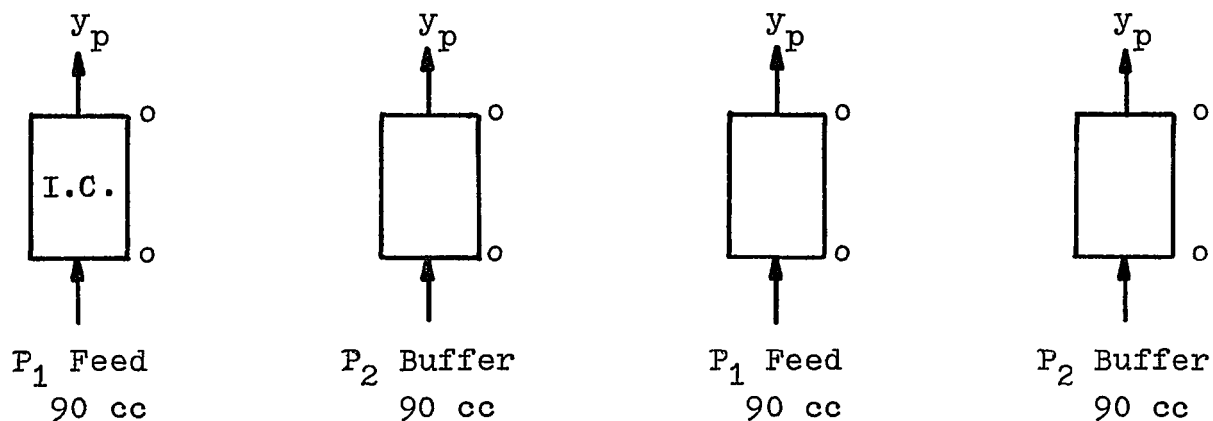
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin @ $P_1 = 8.0$

Flow Rate: 7.5 cc/min

Power: None

Procedure: As shown. Examine shape of wave front due to eddy diffusivity at relatively high flow rate.



Initial Conditions (I.C.) in the column: Buffer ($P_2 = 6.0$)

Feed Reading: $R_{595} = 0.588$

Buffer Reading: $R_{595} = 0.405$

#	Vol, cc	$\langle V_e \rangle$	pH	R_{595}	y_{Al}/y_o
1	7.50	3.75	6.05	-	-
2	7.50	11.25	6.05	-	-
3	7.50	18.75	6.05	-	-
4	7.50	26.25	6.05	0.408	0.02-
5	7.50	33.75	6.05	0.425	0.11-
6	7.50	41.25	6.05	0.485	0.44-

RUN NO. 29 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u>$\langle V_e \rangle$</u>	<u>pH</u>	<u>R_{595}</u>	<u>y_{A1}/y_0</u>
7	7.50	48.75	6.05	0.527	0.667
8	7.50	56.25	6.05	0.554	0.814
9	7.50	63.75	6.08	0.589	1.01-
10	7.50	71.25	6.50	0.596	1.04-
11	7.50	78.75	7.10	0.578	0.945
12	7.50	86.25	7.72	0.590	1.01-

Note: Change Feed to Buffer

13	7.50	93.75	7.90	0.604	1.09-
14	7.50	101.25	7.93	0.590	1.01-
15	7.50	108.75	7.92	0.587	0.994
16	7.50	116.25	7.92	0.590	1.01-
17	7.50	123.75	7.92	0.580	0.956
18	7.50	131.25	7.92	0.513	0.590
19	7.50	138.75	7.92	0.470	0.36-
20	7.50	146.25	7.85	0.455	0.27-
21	7.50	153.75	7.35	0.433	0.15-
22	7.50	161.25	6.82	0.412	0.04-
23	7.50	168.75	6.52	0.406	0.005
24	7.50	176.25	6.38	-	-

Note: Add Feed - Cycle 2

25	7.50	3.75	6.30	-	-
26	7.50	11.25	6.23	-	-
27	7.50	18.75	6.18	-	-
28	7.50	26.25	6.18	0.405	-
29	7.50	33.75	6.11	0.421	0.087

RUN NO. 29 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{Al}/y_o</u>
30	7.50	41.25	6.09	0.485	0.44-
31	7.50	48.75	6.10	0.533	0.699
32	7.50	56.25	6.08	0.555	0.820
33	7.50	63.75	6.03	0.558	0.836
34	7.50	71.25	6.52	0.605	1.09-
35	7.50	78.75	7.20	0.591	1.02-
36	7.50	86.25	7.73	0.605	1.09-
Note: Change Feed to Buffer					
37	7.50	93.75	7.89	0.590	1.01-
38	7.50	101.25	7.91	0.595	1.04-
39	7.50	108.75	7.92	0.577	0.940
40	7.50	116.25	7.92	0.606	1.10-
41	7.50	123.75	7.92	0.570	0.901
42	7.50	131.25	7.91	0.493	0.48-
43	7.50	138.75	7.90	0.462	0.31-
44	7.50	146.25	7.73	0.432	0.15-
45	7.50	153.75	7.22	0.423	0.098
46	7.50	161.25	6.79	0.423	0.098
47	7.50	168.75	6.52	0.404	0
48	7.50	176.25	6.37	-	-

Recovery: CYCLE 1 - 104.4%

CYCLE 2 - 101.7%

RUN NO. 30Breakthrough Curve - Albumin

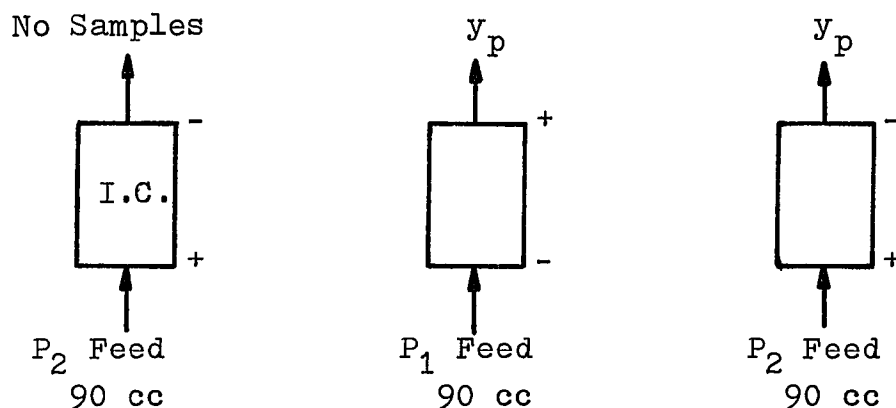
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 1 cc/min

Power: 9 watts

Procedure: As shown. Attempt to develop albumin peak.



Initial Conditions (I.C.) in the column: Feed ($P_1 = 8.0$)

Feed Reading: $R_{595} = 0.581$ (pH = 7.98)

$R_{595} = 0.581$ (pH = 6.01)

Buffer Reading: $R_{595} = 0.386$ (pH = 8.00)

$R_{595} = 0.381$ (pH = 6.01)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{595}	y_{A1}/y_o
1	7.50	3.75	6.32	0.565	0.918
2	7.50	11.25	6.19	0.576	0.974
3	7.50	18.75	6.10	0.564	0.913
4	7.50	26.25	6.08	0.592	1.06-

RUN NO. 30 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{A1}/y₀</u>
5	7.50	33.75	6.00	0.592	1.06-
6	7.50	41.25	6.00	0.597	1.08-
7	7.50	48.75	5.97	0.578	0.985
8	7.50	56.25	5.95	0.578	0.985
9	7.50	63.75	6.55	0.580	0.995
10	7.50	71.25	7.25	0.571	0.950
11	7.50	78.75	7.80	0.568	0.935
12	7.50	86.25	7.90	0.569	0.940
Note: Change P ₁ Feed to P ₂ Feed. SWITCH ELECTRODES.					
13	7.50	93.75	7.92	0.569	0.940
14	7.50	101.25	7.92	0.557	0.880
15	7.50	108.75	7.92	0.556	0.875
16	7.50	116.25	7.92	0.557	0.880
17	7.50	123.75	7.95	0.554	0.865
18	7.50	131.25	7.95	0.554	0.865
19	7.50	138.75	7.98	0.558	0.885
20	7.50	146.25	7.98	0.558	0.885
21	7.50	153.75	7.90	0.577	0.980
22	7.50	161.25	7.30	0.572	0.955
23	7.50	168.75	6.85	0.552	0.851
24	7.50	176.25	6.62	0.542	0.800

Albumin Recovery: 93.6%

RUN NO. 31Breakthrough Curve - Albumin

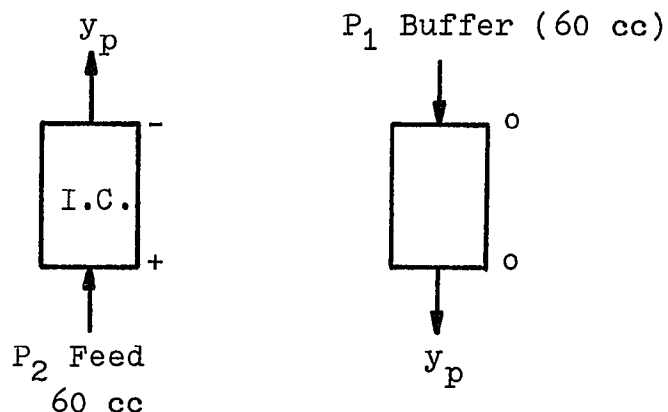
Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % albumin @ $P_2 = 6.0$

Flow Rate: 1 cc/min

Power: 9 watts

Procedure: As shown. Investigate whether albumin retained in column by electric field. YES!!!



Initial Conditions (I.C.) in the column: Buffer ($P_1 = 8.0$)

Feed Reading: $R_{595} = 0.598$ (pH = 5.98)

Buffer Reading: $R_{595} = 0.419$ (pH = 8.00)

#	Vol, cc	$\langle V_e \rangle$	pH	R_{595}	y_{Al}/y_o
1	7.50	3.75	8.00	---	---
2	7.50	11.25	7.92	---	---
3	7.50	18.75	7.95	---	---
4	7.50	26.25	8.00	0.423	0.02-
5	7.50	33.75	7.98	0.422	0.02-
6	7.50	41.25	8.00	0.464	0.25-

RUN NO. 31 (cont.)

<u>#</u>	<u>Vol, cc</u>	<u><V_e></u>	<u>pH</u>	<u>R₅₉₅</u>	<u>y_{A1}/y₀</u>
7	7.50	48.75	8.00	0.546	0.711
8	7.50	56.25	8.00	0.579	0.896
Note: POWER OFF. CHANGE DIRECTION OF FLOW. Change Feed to Buffer.					
9	3.75	61.88	6.03	0.602	1.03-
10	3.75	65.63	6.00	0.609	1.06-
11	3.75	69.38	5.98	0.603	1.03-
12	3.75	73.13	5.98	0.603	1.03-
13	3.75	76.88	5.98	0.606	1.05-
14	3.75	80.63	5.95	0.605	1.04-
15	3.75	84.38	5.97	0.609	1.06-
16	3.75	88.13	5.97	0.604	1.04-
17	7.50	93.75	6.00	0.594	0.980
18	7.50	101.25	6.02	0.524	0.588
19	7.50	108.75	6.40	0.460	0.23-
20	7.50	116.25	7.20	0.421	0.01-

Albumin Recovery: 98.5%

RUN NO. 32Voltage Curves

Buffer: 0.05M Tris-maleate + 0.05M NaOH (pH = 8.6)

<u>Volts</u>	<u>m Amps</u>	<u>Watts</u>	<u>Volts</u>	<u>m Amps</u>	<u>Watts</u>
10	1.5	0.015	230	59.0	13.6-
20	3.5	0.070	240	61.0	14.6-
30	6.0	0.18-	250	64.0	16.0-
40	9.0	0.36-			
50	11.0	0.55-			
60	13.0	0.78-			
70	16.0	1.1-			
80	18.0	1.4-			
90	21.0	1.9-			
100	24.0	2.40			
110	26.0	2.86			
120	29.0	3.48			
130	31.0	4.03			
140	34.0	4.76			
150	36.0	5.40			
160	39.0	6.24			
170	42.0	7.14			
180	44.0	7.92			
190	47.0	8.93			
200	50.0	10.0-			
210	53.0	11.1-			
220	56.0	12.3-			

RUN NO. 33Voltage CurvesBuffer: Phosphate, $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$ (pH = 8.0)

<u>Volts</u>	<u>0.05M</u>		<u>0.10M</u>		<u>0.20M</u>	
	<u>m Amps</u>	<u>Watts</u>	<u>m. Amps</u>	<u>Watts</u>	<u>m Amps</u>	<u>Watts</u>
10	3.5	0.035	3.5	0.035	9.5	0.095
20	7.0	0.14-	9.5	0.19-	21.0	0.42-
30	11.5	0.35-	15.0	0.45-	32.0	0.96-
40	15.0	0.60-	20.0	0.80-	45.0	1.8-
50	21.0	1.1-	26.0	1.3-	57.0	2.9-
60	24.5	1.4-	32.5	2.0-	69.0	4.1-
70	28.0	2.0-	39.5	2.8-	82.5	5.8-
80	33.5	2.7-	45.5	3.6-	95.0	7.6-
90	38.5	3.5-	52.5	4.7-	108.0	9.7-
100	42.5	4.25	59.0	5.90	122.5	12.3-
110	47.5	5.23	66.5	7.32	138.0	15.2-
120	51.5	6.18	74.0	8.88	152.5	18.3-
130	56.5	7.35	81.0	10.5-	168.5	21.9-
140	61.0	8.54	89.0	12.5-	187.0	26.2-
150	66.0	9.90	96.0	14.4-	204.5	30.7-
160	71.0	11.4-	104.0	16.6-	220.5	35.3-
170	76.0	12.9-	110.5	18.8-		
180	81.0	14.6-	119.5	21.5-		
190	86.0	16.3-				
200	92.0	18.4-				
210	98.0	20.6-				
220	104.0	22.9-				

RUN NO. 34

Voltage Curves

Buffer: Phosphate + NaCl (pH = 8.0)

<u>Volts</u>	<u>0.05M + 0.05M</u>		<u>0.10M + 0.10M</u>	
	<u>m Amps</u>	<u>Watts</u>	<u>m Amps</u>	<u>Watts</u>
10	2.5	0.025	Not Stable	-
20	10.5	0.21-	16.0	0.32
30	16.0	0.48-	25.0	0.75
40	22.5	0.90-	36.5	1.5-
50	28.0	1.4-	47.5	2.4-
60	35.0	2.1-	58.0	3.5-
70	41.0	2.9-	69.0	4.8-
80	48.5	3.9-	80.0	6.4-
90	57.0	5.1-	91.5	8.2-
100	64.0	6.40	102.5	10.3-
110	70.5	7.76	113.5	12.5-
120	78.5	9.42	121.5	14.6-
130	86.5	11.3-	134.0	17.4-
140	94.5	13.2-	147.8	20.7-
150	102.5	15.4-		
160	111.5	17.8-		
170	119.5	20.3-		
180	129.5	23.3-		
190	138.5	26.3-		
200	147.5	29.5-		
210	161.5	33.9-		
220	172.5	38.0-		

RUN NO. 35Four-Stage pH ParapumpBuffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4 Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

 F_T : 10 cc F_B : 10 cc V_T : 85 cc V_B : 60 cc $Q_o t_I = Q_o t_{III}$: 72.5 ccFeed Reading: $R_{403} = 0.504$ (pH = 6.05) $R_{403} = 0.439$ (pH = 8.00)

Cycle		pH	R_{403}	y_{Hb}/y_o	$[\text{SF}]_{\text{Hb}}$	δ_{EXP}
1	TP	6.07	0.429	0.842		
1	BP	8.15	0.352	0.830	0.986	83.6
2	TP	6.08	0.332	0.659		
2	BP	7.99	0.396	0.900	1.37	78.0
3	TP	6.05	0.283	0.562		
3	BP	8.15	0.412	0.971	1.73	76.7
4	TP	6.08	0.257	0.510		
4	BP	8.10	0.441	1.04-	2.04	77.5
5	TP	6.05	0.246	0.489		
5	BP	8.03	0.462	1.06-	2.16	77.2
6	TP	6.10	0.251	0.499		
6	BP	8.12	0.457	1.07-	2.14	78.4

RUN NO. 35 (cont.)

<u>Cycle</u>		<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>	<u>SF_{Hb}</u>	<u>EXP</u>
7	TP	6.10	0.238	0.474		
7	BP	8.17	0.487	1.16-	2.44	81.6
8	TP	6.08	0.175	0.349		
8	BP	8.05	0.501	1.15-	3.30	75.1
9	TP	6.15	0.161	0.321		
9	BP	8.15	0.484	1.14-	3.55	73.1
10	TP	6.12	0.156	0.310		
10	BP	8.05	0.486	1.12-	3.61	71.5

Average Recovery: 77.3%

(No external buffer cooling)

RUN NO. 36Four-Stage pH Parapump

Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_o t_I = Q_o t_{III}$: 72.5 cc

Feed Reading: $R_{403} = 0.441$ (pH = 6.02)

$R_{403} = 0.432$ (pH = 7.95)

<u>Cycle</u>	<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>	<u>δ_{EXP}</u>
1 TP	6.05	0.095	0.22-		
1 BP	8.20	0.325	0.786	3.6	50.5
2 TP	6.10	0.063	0.14-		
2 BP	8.10	0.462	1.10-	7.9	62.2
3 TP	6.03	0.067	0.15-		
3 BP	8.05	0.563	1.33-	8.9	74.1
4 TP	6.05	0.052	0.12-		
4 BP	8.00	0.608	1.41-	12.-	76.4
5 TP	6.05	0.043	0.098		
5 BP	8.10	0.635	1.51	15.-	80.4
6 TP	6.08	0.047	0.11-		
6 BP	8.12	0.625	1.49-	14.-	79.9

RUN NO. 36 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>	<u>[SF]_{Hb}</u>	<u>δ_{EXP}</u>
7 TP	6.00	0.032	0.073		
7 BP	8.00	0.633	1.48-	20.-	77.7
8 TP	6.00	0.027	0.062		
8 BP	8.12	0.632	1.51-	24.-	78.6
9 TP	6.00	0.026	0.059		
9 BP	8.10	0.648	1.55-	26.-	80.5
10 TP	6.00	0.021	0.049		
10 BP	8.08	0.653	1.53-	31.-	79.0

Average Recovery: 73.9%

(No external buffer cooling)

RUN NO. 37Four-Stage pH ParapumpBuffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4 Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

 F_T : 35 cc F_B : 35 cc V_T : 85 cc V_B : 60 cc $Q_o t_I = Q_o t_{III}$: 72.5 ccFeed Reading: $R_{403} = 0.471$ (pH = 6.05) $R_{403} = 0.425$ (pH = 8.00)

<u>Cycle</u>	<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>	<u>δ_{EXP}</u>
1 TP	6.05	0.124	0.263		
1 BP	8.10	0.344	0.828	3.15	54.5
2 TP	6.10	0.062	0.13-		
2 BP	8.10	0.561	1.35-	10.-	74.0
3 TP	6.05	0.027	0.057		
3 BP	8.12	0.675	1.63-	29.-	89.5
4 TP	6.05	0.019	0.040		
4 BP	8.10	0.732	1.76-	44.-	90.0
5 TP	6.05	0.020	0.041		
5 BP	8.10	0.731	1.76-	43.-	90.0
6 TP	6.00	0.010	0.020		
6 BP	7.95	0.728	1.70-	85.-	86.0

RUN NO. 37 (cont.)

<u>Cycle</u>		<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>	<u>[SF]_{Hb}</u>	<u>δ_{EXP}</u>
7	TP	6.05	0.006	0.013		
7	BP	8.08	0.734	1.76-	140	88.5
8	TP	6.05	0.007	0.016		
8	BP	8.18	0.723	1.78-	110	90.0
9	TP	6.05	0.008	0.017		
9	BP	8.05	0.801	1.91-	110	96.5
10	TP	6.05	0.008	0.017		
10	BP	8.10	0.698	1.68-	99	85.0
11	TP	6.05	0.006	0.014		
11	BP	8.05	0.686	1.63-	120	82.0
12	TP	6.05	0.007	0.016		
12	BP	8.12	0.693	1.67-	100	84.5
13	TP	6.01	0.009	0.019		
13	BP	8.05	0.710	1.69-	89	85.5
14	TP	6.05	0.008	0.017		
14	BP	8.05	0.720	1.71-	100	86.5
15	TP	6.00	0.010	0.021		
15	BP	8.05	0.722	1.72-	82	87.0
16	TP	6.00	0.009	0.020		
16	BP	8.00	0.741	1.74-	87	88.0

Average Recovery: 84.8%

(External buffer system is cooled in this and all other runs unless noted otherwise.)

RUN NO. 38Four-Stage pH Parapump

Buffer: 0.10M NaH_2PO_4 + 0.10M Na_2HPO_4

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_o t_I = Q_o t_{III}$: 72.5 cc (Cycles 1-6)

65.0 cc (Cycles 7-15)

Feed Reading: $R_{403} = 0.512$ (pH = 6.00)

$R_{403} = 0.436$ (pH = 8.00)

<u>Cycle</u>	<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>
1 TP	6.00	0.333	0.652	
1 BP	8.20	0.303	0.729	1.12
2 TP	6.08	0.330	0.646	
2 BP	8.00	0.371	0.852	1.32
3 TP	6.10	0.341	0.667	
3 BP	8.15	0.390	0.926	1.39
4 TP	6.05	0.318	0.622	
4 BP	8.20	0.394	0.947	1.53
5 TP	6.05	0.286	0.559	
5 BP	8.20	0.459	1.10-	1.98

RUN NO. 38 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_O</u>	<u>[SF]_{Hb}</u>
6 TP	6.05	0.279	0.547	
6 BP	8.10	0.473	1.11-	2.03
Note: Displacement too high. Decrease to 65 cc.				
7 TP	6.05	0.303	0.593	
7 BP	8.00	0.595	1.37-	2.31
8 TP	6.17	0.238	0.465	
8 BP	8.12	0.555	1.31-	2.81
9 TP	6.12	0.157	0.307	
9 BP	8.00	0.625	1.44-	4.69
10 TP	6.10	0.136	0.265	
10 BP	8.05	0.648	1.51-	5.68
11 TP	6.05	0.081	0.16-	
11 BP	8.05	0.670	1.56-	9.9-
12 TP	6.05	0.068	0.13-	
12 BP	8.08	0.690	1.61-	12.-
13 TP	6.03	0.069	0.13-	
13 BP	8.02	0.721	1.66-	12.-
14 TP	6.10	0.063	0.12-	
14 BP	8.11	0.680	1.60-	13.-
15 TP	6.08	0.053	0.10-	
15 BP	7.98	0.699	1.60-	15.-

Average Recovery: 84.3%

RUN NO. 39

Four-Stage pH Parapump

Buffer: 0.10M NaH_2PO_4 + 0.10M Na_2HPO_4

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_o t_I = Q_o t_{III}$: 65.0 cc

Feed Reading: $R_{403} = 0.512$ (pH = 6.03)

$R_{403} = 0.456$ (pH = 8.00)

Cycle		pH	R_{403}	y_{Hb}/y_o	$[\text{SF}]_{\text{Hb}}$
1	TP	6.02	0.318	0.621	
1	BP	8.06	0.398	0.883	1.42
2	TP	6.02	0.254	0.496	
2	BP	8.02	0.505	1.11-	2.24
3	TP	6.09	0.212	0.414	
3	BP	8.12	0.573	1.29-	3.11
4	TP	6.09	0.175	0.342	
4	BP	8.00	0.642	1.41-	4.11
5	TP	6.12	0.156	0.305	
5	BP	8.10	0.642	1.44-	4.71
6	TP	6.10	0.101	0.196	
6	BP	8.15	0.667	1.51-	7.72

RUN NO. 39 (cont.)

<u>Cycle</u>		<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>
7	TP	6.10	0.106	0.206	
7	BP	8.05	0.697	1.54-	7.49
8	TP	6.10	0.084	0.16-	
8	BP	8.10	0.689	1.54-	9.4-
9	TP	6.10	0.077	0.15-	
9	BP	8.05	0.720	1.59-	11.-
10	TP	6.02	0.069	0.14-	
10	BP	7.05	0.728	1.58-	12.-
11	TP	6.10	0.075	0.15-	
11	BP	8.10	0.722	1.62-	11.-
12	TP	6.09	0.064	0.12-	
12	BP	8.10	0.703	1.56-	13.-
13	TP	6.02	0.057	0.11-	
13	BP	8.04	0.715	1.58-	14.-
14	TP	6.03	0.051	0.10-	
14	BP	7.90	0.799	1.72-	17.-
15	TP	6.05	0.058	0.11-	
15	BP	8.20	0.658	1.53-	13.-
16	TP	6.10	0.048	0.094	
16	BP	8.05	0.699	1.55-	16.-

Average Recovery: 84.9%

RUN NO. 40

Four-Stage pH Parapump

Buffer: 0.20M NaH_2PO_4 + 0.20M Na_2HPO_4

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_0 t_I = Q_0 t_{III}$: 65.0 cc

Feed Reading: $R_{403} = 0.420$ (pH = 6.00)

$R_{403} = 0.399$ (pH = 8.00)

<u>Cycle</u>		<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_0</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>
1	TP	6.08	0.414	0.986	
1	BP	7.88	0.447	1.09-	1.11
2	TP	6.16	0.339	0.808	
2	BP	7.85	0.490	1.19-	1.47
3	TP	6.18	0.295	0.702	
3	BP	7.78	0.500	1.20-	1.71
4	TP	6.20	0.279	0.663	
4	BP	7.80	0.504	1.21-	1.83
5	TP	6.20	0.253	0.601	
5	BP	7.85	1.507	1.23-	2.05
6	TP	6.20	0.271	0.644	
6	BP	7.82	0.516	1.25-	1.94

RUN NO. 40 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>
7 TP	6.18	0.263	0.638	
7 BP	7.80	0.504	1.21-	1.90
8 TP	6.15	0.235	0.560	
8 BP	7.85	0.518	1.26-	2.24
9 TP	6.13	0.255	0.607	
9 BP	7.82	0.514	1.24-	2.04
10 TP	6.12	0.266	0.632	
10 BP	7.80	0.504	1.21-	1.92
11 TP	6.15	0.266	0.633	
11 BP	7.82	0.513	1.24-	1.96
12 TP	6.11	0.265	0.632	
12 BP	7.78	0.511	1.23-	1.95
13 TP	6.12	0.261	0.621	
13 BP	7.99	0.484	1.21-	1.95

Average Recovery: 94.2%

RUN NO. 41Four-Stage pH Parapump

Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4 + 0.05M NaCl

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_o t_I = Q_o t_{III}$: 70.0 cc

Feed Reading: $R_{403} = 0.428$ (pH = 5.80)

$R_{403} = 0.449$ (pH = 7.92)

<u>Cycle</u>	<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>
1 TP	6.12	0.249	0.582	
1 BP	8.00	0.347	0.789	1.35
2 TP	6.03	0.190	0.443	
2 BP	8.15	0.446	1.05-	2.37
3 TP	6.05	0.157	0.367	
3 BP	8.10	0.501	1.17-	3.18
4 TP	6.00	0.155	0.361	
4 BP	8.00	0.558	1.27-	3.51
5 TP	6.00	0.112	0.262	
5 BP	8.00	0.594	1.35-	5.15
6 TP	6.00	0.091	0.21-	
6 BP	8.00	0.617	1.37-	6.5-

RUN NO. 41 (cont.)

<u>Cycle</u>		<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>
7	TP	6.02	0.091	0.21-	
7	BP	8.03	0.621	1.41-	6.6
8	TP	6.00	0.074	0.17-	
8	BP	7.90	0.655	1.46-	8.5
9	TP	6.00	0.047	0.11-	
9	BP	7.98	0.641	1.45-	13.-
10	TP	6.00	0.067	0.16-	
10	BP	7.89	0.653	1.45-	9.2
11	TP	6.00	0.033	0.078	
11	BP	8.00	0.665	1.51-	19.-
12	TP	6.02	0.074	0.17-	
12	BP	8.05	0.673	1.54-	8.9
13	TP	6.00	0.071	0.17-	
13	BP	8.00	0.680	1.54-	9.4
14	TP	5.95	0.058	0.14-	
14	BP	8.00	0.696	1.58-	12.-
15	TP	6.00	0.049	0.12-	
15	BP	8.00	0.692	1.57-	14.-
16	TP	6.02	0.054	0.13-	
16	BP	7.95	0.693	1.58-	13.-

Average Recovery: 80.5%

RUN NO. 42Four-Stage pH Parapump

Buffer: 0.10M NaH_2PO_4 + 0.10M Na_2HPO_4 + 0.10M NaCl

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_o t_I = Q_o t_{III}$: 65.0 cc

Feed Reading: $R_{403} = 0.428$ (pH = 6.00)

$R_{403} = 0.368$ (pH = 8.00)

<u>Cycle</u>	<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>
1 TP	6.00	0.392	0.916	
1 BP	8.12	0.317	0.882	0.963
2 TP	6.03	0.344	0.805	
2 BP	8.08	0.387	1.07-	1.33-
3 TP	6.03	0.258	0.602	
3 BP	7.95	0.413	1.11-	1.84-
4 TP	6.02	0.195	0.456	
4 BP	8.03	0.423	1.15-	2.52-
5 TP	6.02	0.195	0.456	
5 BP	8.08	0.443	1.23-	2.69-
6 TP	6.02	0.203	0.475	
6 BP	8.12	0.465	1.30-	2.74-

RUN NO. 42 (cont.)

<u>Cycle</u>		<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>
7	TP	6.02	0.143	0.322	
7	BP	8.01	0.481	1.31-	4.07
8	TP	6.02	0.126	0.294	
8	BP	8.02	0.445	1.21-	4.11
9	TP	6.00	0.115	0.269	
9	BP	8.03	0.464	1.27-	4.72
10	TP	6.02	0.117	0.274	
10	BP	8.02	0.477	1.30-	4.74
11	TP	6.08	0.126	0.295	
11	BP	7.99	0.490	1.33-	4.51
12	TP	6.01	0.164	0.384	
12	BP	8.06	0.478	1.32-	3.44
13	TP	6.02	0.138	0.322	
13	BP	8.04	0.481	1.32-	4.09
14	TP	6.00	0.141	0.329	
14	BP	8.05	0.499	1.37-	4.16
15	TP	6.00	0.164	0.384	
15	BP	8.05	0.496	1.36-	3.55

Average Recovery: 83.7%

RUN NO. 43

Four-Stage pH Parapump

Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_o t_I = Q_o t_{III}$: 70.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	6.02	0.480	0.782
P_2 Buffer	6.02	---	0.417
P_1 Feed	7.99	0.423	0.784
P_1 Buffer	7.99	---	0.421

Hemoglobin Recovery: 89.5%

Albumin Recovery: 89.8%

Protein Recovery: 89.7%

RUN NO. 43 (cont.)

Cycle	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o	$(W)_{Hb}$	$(W)_{Al}$	$(SF)_{Hb}$	$(SF)_{Al}$	α
1 TP	6.00	0.136	0.650	0.282	0.99-	0.220	0.78-			
1 BP	8.11	0.357	0.750	0.864	0.90-	0.490	0.51-	3.06	1.1-	3.4
2 TP	6.02	0.115	0.639	0.239	0.97-	0.198	0.80-			
2 BP	7.98	0.571	0.840	1.35-	0.96-	0.584	0.42-	5.65	1.0-	5.7
3 TP	6.00	0.098	0.624	0.20-	0.93-	0.18-	0.82-			
3 BP	8.10	0.654	0.880	1.58-	0.95-	0.625	0.38-	7.9-	0.98	7.7
4 TP	6.05	0.102	0.640	0.21-	1.01-	0.17-	0.827			
4 BP	8.05	0.666	0.899	1.59-	1.04-	0.605	0.395	7.6-	0.97	7.4
5 TP	6.00	0.097	0.622	0.20-	0.92-	0.18-	0.82-			
5 BP	8.10	0.700	0.907	1.69-	0.99-	0.631	0.37-	8.5-	0.93	7.9
6 TP	6.00	0.087	0.623	0.18-	0.95-	0.16-	0.84-			
6 BP	8.05	0.729	0.882	1.74-	0.78-	0.690	0.31-	9.7-	1.2-	12.-
7 TP	6.00	0.086	0.613	0.18-	0.90-	0.17-	0.83-			
7 BP	8.10	0.728	0.883	1.76-	0.79-	0.690	0.31-	9.8-	1.1-	11.-
8 TP	6.03	0.082	0.617	0.17-	0.93-	0.15-	0.85-			
8 BP	8.10	0.721	0.901	1.75-	0.99-	0.663	0.34-	10.-	0.94	9.4
9 TP	6.00	0.078	0.624	0.16-	0.97-	0.14-	0.86-			
9 BP	7.92	0.727	0.870	1.69-	0.78-	0.684	0.32-	11.-	1.2-	13.-
10 TP	6.08	0.078	0.605	0.16-	0.87-	0.16-	0.84-			
10 BP	7.98	0.672	0.829	1.59-	0.66-	0.707	0.29-	9.9	1.3-	13.-

RUN NO. 43 (cont.)

Cycle	pH	R ₄₀₃	R ₅₉₅	y_{HD}/y_0	y_{Al}/y_0	$(W)_{HD}$	$(W)_{Al}$	$(SF)_{HD}$	$(SF)_{Al}$	α
11 TP	6.00	0.072	0.594	0.15-	0.82-	0.15-	0.85-			
11 BP	8.09	0.666	0.855	1.61-	0.78-	0.674	0.33-	11.-	1.1-	12.-
12 TP	5.98	0.088	0.608	0.18-	0.86-	0.17-	0.83-			
12 BP	8.05	0.671	0.863	1.60-	0.84-	0.656	0.34-	8.9	1.0-	8.9
13 TP	6.00	0.094	0.612	0.20-	0.87-	0.18-	0.82-			
13 BP	7.99	0.678	0.881	1.60-	0.93-	0.632	0.37-	8.0	0.94	7.5
14 TP	6.05	0.087	0.615	0.18-	0.90-	0.17-	0.83-			
14 BP	8.05	0.707	0.899	1.68-	0.95-	0.639	0.36-	9.3	0.95	8.8
15 TP	6.05	0.082	0.615	0.17-	0.91-	0.16-	0.84-			
15 BP	8.05	0.727	0.908	1.73-	0.95-	0.646	0.35	10.-	0.96	9.6
16 TP	5.97	0.084	0.606	0.17-	0.86-	0.17-	0.83-			
16 BP	8.10	0.709	0.888	1.71-	0.86-	0.665	0.33-	10.-	1.0-	10.-
17 TP	6.02	0.083	0.613	0.17-	0.90-	0.16-	0.84-			
17 BP	8.07	0.708	0.895	1.70-	0.91-	0.651	0.35-	10.-	0.99	9.9

RUN NO. 44

Four-Stage pH Parapump

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 6.0$, $P_1 = 8.5$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figures 8 & 14

F_T : 35 cc

F_B : 35 cc

V_T : 105 cc

V_B : 60 cc

$Q_0 t_I = Q_0 t_{III}$: 90.0 cc

			<u>n = 1-6</u>	<u>n = 7-12</u>
	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>R₅₉₅</u>
P_2 Feed	6.05	0.407	0.850	0.850
P_2 Buffer	6.05	---	0.406	0.406
P_1 Feed	8.52	0.343	0.870	0.839
P_1 Buffer	8.52	---	0.407	0.395

Hemoglobin Recovery: 86.6%

Albumin Recovery: 95.2%

Protein Recovery: 90.9%

RUN NO. 44 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	6.20	0.143	0.691	0.351	0.93-	0.274	0.73-	2.96	1.1-	3.3
1 BP	8.43	0.367	0.845	1.04-	0.85-	0.515	0.49-			
2 TP	6.10	0.131	0.699	0.321	1.00-	0.243	0.757	4.27	1.1-	4.7
2 BP	8.30	0.494	0.931	1.37-	0.92-	0.563	0.44-			
3 TP	6.05	0.098	0.658	0.24-	0.90-	0.21-	0.79-	6.1-	0.90	5.5
3 BP	8.42	0.515	0.977	1.46-	1.00-	0.593	0.407			
4 TP	6.10	0.086	0.672	0.21-	0.99-	0.18-	0.82-	7.6-	1.1-	8.4
4 BP	8.52	0.550	0.995	1.60-	0.94-	0.630	0.37-			
5 TP	6.02	0.080	0.668	0.20-	0.99-	0.17-	0.83-	8.2-	1.1-	9.0
5 BP	8.52	0.562	1.003	1.64-	0.94-	0.636	0.36-			
6 TP	6.10	0.058	0.644	0.14-	0.93-	0.13-	0.87-	11.-	0.99	11.-
6 BP	8.52	0.537	0.986	1.57-	0.94-	0.626	0.37-			
7 TP	6.08	0.068	0.677	0.17-	1.05-	0.14-	0.863	9.7	1.2-	12.-
7 BP	8.50	0.568	0.962	1.65-	0.90-	0.647	0.35-			
8 TP	5.90	0.066	0.660	0.16-	0.98-	0.14-	0.86-	10.-	1.1-	11.-
8 BP	8.50	0.550	0.948	1.60-	0.89-	0.642	0.36-			
9 TP	5.90	0.066	0.665	0.16-	1.00-	0.14-	0.862	9.9	1.2-	12.-
9 BP	8.52	0.543	0.929	1.58-	0.86-	0.648	0.35-			
10 TP	5.98	0.070	0.642	0.17-	0.89-	0.16-	0.84-	9.4	0.95	8.9
10 BP	8.50	0.552	0.959	1.60-	0.94-	0.630	0.37-			

RUN NO. 44 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
11 TP	6.02	0.068	0.671	0.17-	1.03-	0.14-	0.860			
11 BP	8.48	0.567	0.972	1.64-	0.96-	0.631	0.37-	9.6	1.1	11.-
12 TP	6.03	0.058	0.678	0.14-	1.09-	0.12-	0.884			
12 BP	8.45	0.555	0.958	1.59-	0.95-	0.626	0.37-	11.-	1.1	12.-

RUN NO. 45

Six-Stage pH Parapump

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 6.0$, $P_1 = 8.5$)

Flow Rate: 2.5 cc/min

Power: None

Procedure: Figure 21

F_T : 35 cc

F_B : 35 cc

F_0 : 0 cc

V_T : 105 cc

V_B : 60 cc

$Q_0 t_I$: 90.0 cc

$Q_0 t_{II}$: 60.0 cc

$Q_0 t_{IV}$: 90.0 cc

$Q_0 t_V$: 120.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.92	0.448	0.763
P_2 Buffer	5.92	---	0.437
P_1 Feed	8.50	0.362	0.778
P_1 Buffer	8.50	---	0.436

Hemoglobin Recovery: 92.1%

Albumin Recovery: 99.1%

Protein Recovery: 95.6%

RUN NO. 45 (cont.)

Cycle	pH	R_{403}	R_{595}	y_{Hb}/y_o	y_{Al}/y_o	$(W)_{Hb}$	$(W)_{Al}$	$(SF)_{Hb}$	$(SF)_{Al}$	α
1 TP	5.93	0.093	0.630	0.21-	0.97-	0.18-	0.82-			
1 BP	8.40	0.658	0.909	1.77-	1.00-	0.639	0.361	8.4	0.97	8.1
2 TP	5.90	0.049	0.626	0.11-	1.05-	0.094	0.906			
2 BP	8.40	0.692	0.908	1.86-	0.91-	0.673	0.33-	17.-	1.2-	20.-
3 TP	5.97	0.042	0.619	0.094	1.02-	0.084	0.916			
3 BP	8.42	0.662	0.906	1.78-	0.97-	0.647	0.35-	19.-	1.1-	21.-
4 TP	6.00	0.041	0.613	0.091	0.99-	0.084	0.92-			
4 BP	8.45	0.651	0.905	1.77-	0.97-	0.645	0.36-	19.-	1.0-	19.-
5 TP	6.05	0.038	0.629	0.085	1.09-	0.072	0.928			
5 BP	8.45	0.642	0.894	1.75-	0.93-	0.653	0.35-	21.-	1.2-	25.-
6 TP	6.10	0.038	0.610	0.086	0.98-	0.080	0.92-			
6 BP	8.45	0.632	0.903	1.72-	1.01-	0.630	0.370	20.-	0.97	19.-
7 TP	6.00	0.040	0.610	0.089	0.97-	0.084	0.92-			
7 BP	8.40	0.634	0.897	1.70-	1.00-	0.630	0.370	19.-	0.97	18.-
8 TP	5.95	0.038	0.617	0.085	1.02-	0.076	0.924			
8 BP	8.35	0.622	0.888	1.65-	1.00-	0.622	0.378	19.-	1.02	19.-
9 TP	6.00	0.037	0.611	0.083	0.98-	0.078	0.92-			
9 BP	8.50	0.592	0.881	1.64-	0.97-	0.630	0.37-	20.-	1.0-	20.-

RUN NO. 46Mode 1Buffer: 0.05M NaH₂PO₄ + 0.05M Na₂HPO₄Feed: 0.01 weight % hemoglobin (P₂ = 6.0, P₁ = 8.0)

Flow Rate: 2.68 cc/min

Power: 5 watts (125 volts)

Procedure: Figure 82

No Power Case: Run 35

F_T: 10 ccF_B: 10 ccV_T: 85 ccV_B: 60 ccQ_ot_I = Q_ot_{III}: 72.4 ccFeed Reading: R₄₀₃ = 0.520 (pH = 6.05)R₄₀₃ = 0.434 (pH = 8.00)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>	<u>δ_{EXP}</u>
1 TP	6.05	0.441	0.848		
1 BP	8.11	0.339	0.801	0.945	82.5
2 TP	6.12	0.324	0.623		
2 BP	8.35	0.360	0.905	1.45-	76.4
3 TP	6.10	0.239	0.460		
3 BP	8.13	0.385	0.914	1.99-	68.7
4 TP	6.10	0.173	0.333		
4 BP	8.17	0.387	0.925	2.78-	62.9
5 TP	6.17	0.227	0.436		
5 BP	8.08	0.384	0.901	2.07-	66.9

RUN NO. 46 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>	<u>δ_{EXP}</u>
6 TP	6.05	0.191	0.368		
6 BP	8.10	0.381	0.899	2.44	64.3
7 TP	6.08	0.168	0.322		
7 BP	8.13	0.362	0.860	2.67	59.1
8 TP	6.05	0.111	0.214		
8 BP	7.95	0.390	0.890	4.16	55.2
9 TP	6.10	0.099	0.191		
9 BP	8.00	0.400	0.921	4.82	55.6
10 TP	6.10	0.073	0.141		
10 BP	8.12	0.402	0.951	6.74	54.6

Average Recovery: 64.6%

(No external buffer cooling)

RUN NO. 47Mode 2Buffer: 0.05M NaH₂PO₄ + 0.05M Na₂HPO₄Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
(P₂ = 6.0, P₁ = 8.0)

Flow Rate: 2.5 cc/min

Power: 240 volts in Stage II

120 volts in Stage III

Procedure: Figure 83

No Power Case: Run 43

F_T: 35 ccF_B: 35 ccV_T: 85 ccV_B: 60 ccQ_ot_I = Q_ot_{III}: 70.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P ₂ Feed	6.00	0.476	0.743
P ₂ Buffer	6.00	---	0.401
P ₁ Feed	7.91	0.424	0.755
P ₁ Buffer	7.91	---	0.395

Hemoglobin Recovery: 71.9%

Albumin Recovery: 89.3%

Protein Recovery: 80.6%

RUN NO. 47 (cont.)

Cycle	pH	R ₄₀₃	R ₅₉₅	y _{Hb} /y _O	y _{Al} /y _O	(W) _{Hb}	(W) _{Al}	(SF) _{Hb}	(SF) _{Al}	α
1 TP	5.92	0.183	0.620	0.384	0.90-	0.299	0.70-			
1 BP	8.12	0.362	0.729	0.894	0.96-	0.482	0.52-	2.33	0.94	2.2-
2 TP	5.94	0.112	0.539	0.235	0.572	0.291	0.709			
2 BP	8.13	0.449	0.775	1.11-	1.00-	0.526	0.474	4.72	0.572	2.71
3 TP	5.98	0.151	0.593	0.317	1.12-	0.221	0.779			
3 BP	8.03	0.489	0.757	1.18-	0.83-	0.587	0.41-	3.72	1.3-	4.8-
4 TP	6.00	0.132	0.567	0.276	0.695	0.284	0.716			
4 BP	8.10	0.498	0.788	1.23-	0.95-	0.564	0.44-	4.46	0.73	3.3-
5 TP	6.00	0.114	0.553	0.239	0.650	0.269	0.731			
5 BP	8.10	0.524	0.787	1.29-	0.89-	0.592	0.41-	5.40	0.73	3.9-
6 TP	5.93	0.113	0.589	0.236	1.09-	0.178	0.822			
6 BP	8.08	0.524	0.778	1.29-	0.84-	0.606	0.39-	5.47	1.3-	7.1-
7 TP	5.95	0.110	0.591	0.231	0.88-	0.208	0.79-			
7 BP	8.08	0.514	0.783	1.26-	0.90-	0.583	0.42-	5.45	0.98	5.3-
8 TP	5.95	0.121	0.605	0.254	0.94-	0.213	0.79-			
8 BP	8.08	0.501	0.780	1.23-	0.91-	0.575	0.43-	4.84	1.0-	4.8-
9 TP	5.95	0.114	0.601	0.239	0.93-	0.204	0.80-			
9 BP	8.10	0.452	0.762	1.11-	0.93-	0.544	0.46-	4.64	1.0-	4.6-
10 TP	6.02	0.132	0.603	0.276	0.90-	0.235	0.77-			
10 BP	8.00	0.461	0.770	1.10-	0.98-	0.529	0.47-	3.99	0.92	3.7-

RUN NO. 48

Mode 4

Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: 9 watts

Procedure: Figure 85

No Power Case: Run 43

F_T : 30 cc

F_B : 30 cc

V_T : 85 cc

V_B : 60 cc

$Q_0 t_I = Q_0 t_{III}$: 60 cc

"Wait" time: 24 min

	<u>pH</u>	<u>R_{403}</u>	<u>R_{595}</u>
P_2 Feed	6.02	0.434	0.725
P_2 Buffer	6.02	---	0.373
P_1 Feed	7.95	0.400	0.720
P_1 Buffer	7.95	---	0.372

Hemoglobin Recovery: 80.1%

Albumin Recovery: 94.1%

Protein Recovery: 87.1%

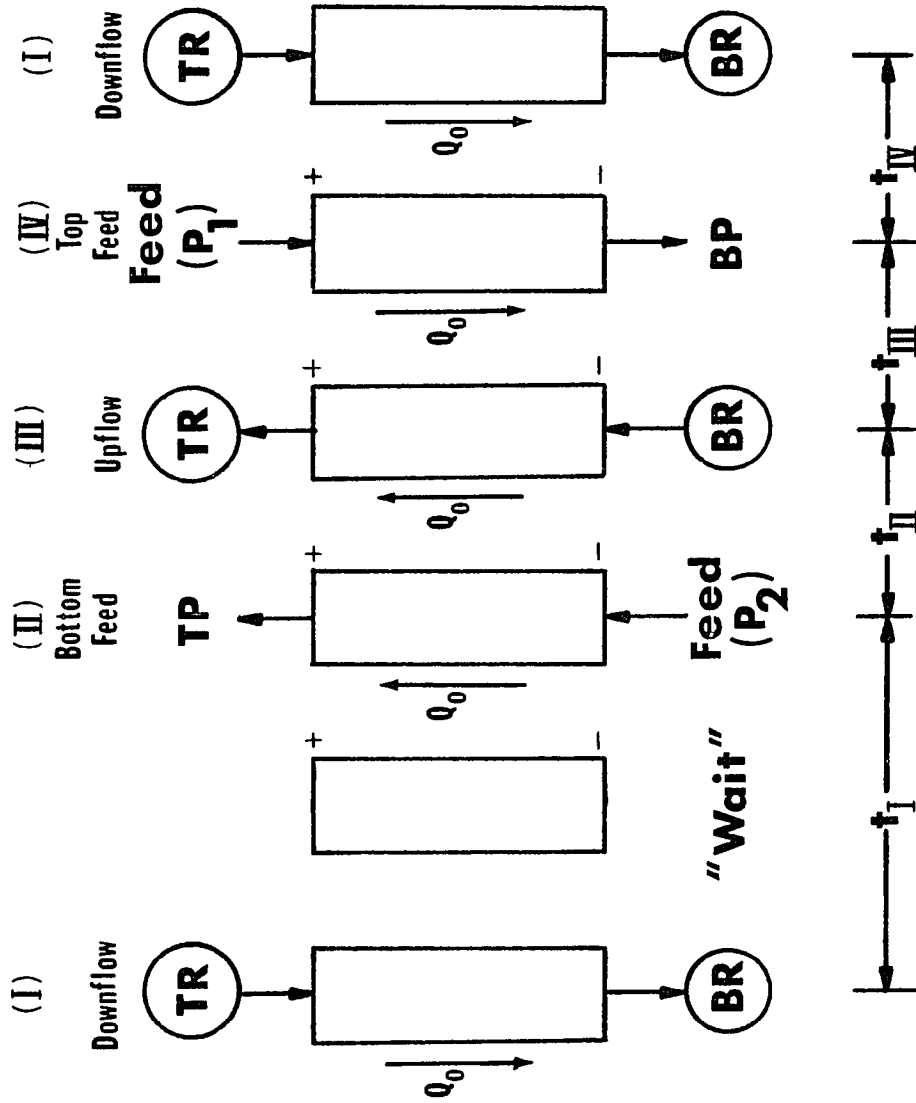


FIGURE 85. Process Diagram for the pH Parapump with Electric Field (Mode 4)

RUN NO. 48 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(M)_{Hb}</u>	<u>(M)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	6.00	0.145	0.597	0.334	0.94-	0.262	0.74-	2.69	0.90	2.4
1 BP	8.00	0.355	0.710	0.897	1.04-	0.463	0.537			
2 TP	5.97	0.129	0.573	0.298	0.84-	0.262	0.74-	3.79	0.87	3.3
2 BP	8.10	0.431	0.728	1.13-	0.97-	0.538	0.46-			
3 TP	5.98	0.132	0.575	0.304	0.85-	0.263	0.74-	4.14	0.83	3.4
3 BP	8.10	0.482	0.771	1.26-	1.03-	0.550	0.450			
4 TP	6.00	0.125	0.574	0.287	0.86-	0.250	0.75-	4.84	0.86	4.2
4 BP	8.09	0.526	0.789	1.39-	1.00-	0.582	0.418			
5 TP	5.92	0.116	0.581	0.268	0.91-	0.228	0.77-	5.30	1.0-	5.3
5 BP	8.13	0.547	0.777	1.42-	0.91-	0.609	0.39-			
6 TP	5.98	0.106	0.581	0.245	0.94-	0.212	0.79-	5.96	1.1-	6.6
6 BP	7.92	0.587	0.776	1.46-	0.87-	0.627	0.37-			
7 TP	6.02	0.117	0.577	0.270	0.89-	0.233	0.77-	5.48	0.86	4.7
7 BP	8.05	0.579	0.809	1.48-	1.04-	0.587	0.413			
8 TP	6.05	0.119	0.585	0.273	0.93-	0.227	0.77-	5.49	0.90	4.9
8 BP	8.05	0.590	0.812	1.50-	1.03-	0.593	0.407			

RUN NO. 49Mode 3

Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4 + 0.05M NaCl

Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: 120 volts (8.5 watts)

Procedure: Figure 79

No Power Case: Run 41

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_0 t_I = Q_0 t_{III}$: 70.0 cc

Feed Reading: $R_{403} = 0.487$ (pH = 5.99)

$R_{403} = 0.444$ (pH = 7.98)

<u>Cycle</u>		<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_0</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>
1	TP	6.12	0.210	0.430	
1	BP	8.48	0.330	0.844	1.96
2	TP	6.10	0.193	0.396	
2	BP	8.10	0.422	0.977	2.47
3	TP	6.10	0.124	0.253	
3	BP	8.25	0.507	1.21-	4.78
4	TP	6.12	0.073	0.15-	
4	BP	8.15	0.573	1.34-	8.9-
5	TP	6.00	0.060	0.12-	
5	BP	8.35	0.584	1.44-	12.-

RUN NO. 49 (cont.)

<u>Cycle</u>		<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>
6	TP	6.05	0.050	0.11-	
6	BP	8.33	0.601	1.46-	14.
7	TP	6.05	0.036	0.075	
7	BP	8.30	0.617	1.50-	20.
8	TP	6.00	0.027	0.055	
8	BP	8.25	0.617	1.48-	27.
9	TP	6.08	0.044	0.089	
9	BP	8.18	0.616	1.45-	16.
10	TP	6.05	0.039	0.080	
10	BP	8.15	0.623	1.46-	18.
11	TP	6.05	0.040	0.081	
11	BP	8.05	0.591	1.35-	17.
12	TP	6.05	0.040	0.082	
12	BP	8.32	0.597	1.45-	18.
13	TP	6.08	0.033	0.068	
13	BP	8.22	0.602	1.43-	21.
14	TP	6.08	0.034	0.070	
14	BP	8.20	0.612	1.45-	21.
15	TP	6.00	0.037	0.076	
15	BP	8.18	0.644	1.52-	20.
16	TP	6.00	0.035	0.072	
16	BP	8.20	0.621	1.47-	20.

Average Recovery: 75.1%

RUN NO. 50Mode 3Buffer: 0.10M NaH_2PO_4 + 0.10M Na_2HPO_4 Feed: 0.01 weight % hemoglobin ($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: 120 volts

Procedure: Figure 79

No Power Case: Run 39

 F_T : 35 cc F_B : 35 cc V_T : 85 cc V_B : 60 cc $Q_o t_I = Q_o t_{III}$: 65 ccFeed Reading: $R_{403} = 0.400$ (pH = 6.05) $R_{403} = 0.379$ (pH = 8.00)

<u>Cycle</u>	<u>pH</u>	<u>R_{403}</u>	<u>y_{Hb}/y_o</u>	<u>$[\text{SF}]_{\text{Hb}}$</u>
1 TP	5.98	0.277	0.693	
1 BP	8.01	0.323	0.852	1.23
2 TP	6.10	0.238	0.594	
2 BP	8.10	0.416	1.13-	1.91
3 TP	6.10	0.172	0.431	
3 BP	8.15	0.461	1.26-	2.93
4 TP	6.04	0.125	0.313	
4 BP	8.08	0.510	1.37-	4.38
5 TP	6.08	0.109	0.272	
5 BP	8.07	0.524	1.41-	5.18

RUN NO. 50 (cont.)

<u>Cycle</u>		<u>pH</u>	<u>R₄₀₃</u>	<u>y_{Hb}/y_o</u>	<u>[SF]_{Hb}</u>
6	TP	6.02	0.087	0.22-	
6	BP	8.01	0.565	1.49-	6.9
7	TP	6.07	0.089	0.22-	
7	BP	8.10	0.584	1.58-	7.1
8	TP	6.10	0.077	0.19-	
8	BP	8.09	0.566	1.53-	8.0
9	TP	6.03	0.051	0.13-	
9	BP	8.12	0.595	1.61-	13.-
10	TP	6.05	0.051	0.13-	
10	BP	8.12	0.605	1.64-	13.-
11	TP	6.05	0.055	0.14-	
11	BP	8.15	0.607	1.66-	12.-
12	TP	6.10	0.058	0.14-	
12	BP	8.08	0.619	1.67-	12.-
13	TP	6.00	0.056	0.14-	
13	BP	8.10	0.626	1.69-	12.-
14	TP	6.08	0.073	0.18-	
14	BP	8.10	0.560	1.52-	8.4

Average Recovery: 86.4%

RUN NO. 51Mode 3Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4 Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: 5 watts (130 volts)

Procedure: Figure 79

No Power Case: Run 43

 F_T : 35 cc F_B : 35 cc V_T : 85 cc V_B : 60 cc $Q_o t_I = Q_o t_{III}$: 70.0 cc

	<u>pH</u>	<u>R_{403}</u>	<u>R_{595}</u>
P_2 Feed	6.00	0.478	0.735
P_2 Buffer	6.00	---	0.384
P_1 Feed	8.00	0.434	0.746
P_1 Buffer	8.00	---	0.385

Hemoglobin Recovery: 85.0%

Albumin Recovery: 88.5%

Protein Recovery: 86.8%

RUN NO. 51 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	5.99	0.145	0.539	0.303	0.577	0.343	0.657	2.80	1.0-	2.8
1 BP	8.14	0.357	0.638	0.848	0.56-	0.602	0.40-			
2 TP	6.00	0.129	0.574	0.270	0.81-	0.250	0.75-	4.41	0.84	3.7
2 BP	8.04	0.501	0.775	1.19-	0.97-	0.551	0.45-			
3 TP	5.98	0.125	0.574	0.262	0.82-	0.242	0.76-	5.57	0.95	5.3
3 BP	8.12	0.618	0.804	1.46-	0.86-	0.629	0.37-			
4 TP	6.02	0.115	0.569	0.240	0.81-	0.229	0.77-	6.42	0.88	5.6
4 BP	8.08	0.654	0.829	1.54-	0.92-	0.626	0.37-			
5 TP	6.00	0.095	0.564	0.20-	0.83-	0.19-	0.81-	8.0-	0.79	6.3
5 BP	8.10	0.673	0.861	1.59-	1.05-	0.602	0.40-			
6 TP	6.00	0.095	0.557	0.20-	0.79-	0.20-	0.80-	7.8-	0.83	6.5
6 BP	8.00	0.673	0.835	1.55-	0.95-	0.620	0.38-			
7 TP	6.00	0.091	0.566	0.19-	0.84-	0.18-	0.82-	8.3-	0.94	7.8
7 BP	8.10	0.664	0.829	1.57-	0.89-	0.638	0.36-			
8 TP	6.05	0.087	0.571	0.18-	0.88-	0.17-	0.83-	8.6-	0.90	7.7
8 BP	8.02	0.665	0.827	1.54-	0.98-	0.611	0.39-			
9 TP	6.03	0.082	0.575	0.17-	0.88-	0.16-	0.84-	8.8-	1.0-	8.8
9 BP	8.00	0.651	0.814	1.50-	0.88-	0.630	0.37-			
10 TP	6.00	0.080	0.566	0.17-	0.87-	0.16-	0.84-	9.1-	0.97	8.8
10 BP	8.10	0.653	0.826	1.55-	0.90-	0.632	0.37-			

RUN NO. 51 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₂₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(M)_{Hb}</u>	<u>(M)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
11 TP	6.00	0.070	0.569	0.15-	0.91-	0.14-	0.86-	10.-	1.0-	10.-
11 BP	8.08	0.661	0.825	1.56-	0.89-	0.637	0.36-			
12 TP	6.00	0.076	0.576	0.16-	0.94-	0.14-	0.86-			
12 BP	8.15	0.660	0.853	1.57-	1.03-	0.604	0.396	9.8	0.91	8.9
13 TP	6.00	0.081	0.552	0.17-	0.79-	0.18-	0.82-			
13 BP	8.08	0.685	0.854	1.61-	0.99-	0.619	0.38-	9.5	0.80	7.6
14 TP	6.00	0.068	0.553	0.14-	0.82-	0.15-	0.85-			
14 BP	8.10	0.683	0.862	1.63-	1.02-	0.615	0.385	12.-	0.80	9.6
15 TP	5.95	0.069	0.559	0.14-	0.85-	0.14-	0.86-			
15 BP	8.18	0.680	0.863	1.64-	1.01-	0.619	0.381	12.-	0.84	10.-
16 TP	6.00	0.057	0.551	0.12-	0.83-	0.12-	0.88-			
16 BP	8.05	0.707	0.889	1.65-	1.15-	0.589	0.411	14.-	0.72	10.-
17 TP	5.90	0.069	0.559	0.14-	0.85-	0.14-	0.86-			
17 BP	7.92	0.712	0.853	1.61-	0.99-	0.620	0.38-	12.-	0.86	10.-
18 TP	5.87	0.075	0.554	0.16-	0.81-	0.16-	0.84-			
18 BP	8.10	0.690	0.843	1.63-	0.91-	0.642	0.36-	10.-	0.89	8.9

RUN NO. 52Mode 3

Buffer: 0.05M NaH_2PO_4 + 0.05M Na_2HPO_4

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 6.0$, $P_1 = 8.0$)

Flow Rate: 2.5 cc/min

Power: 10 watts

Procedure: Figure 79

No Power Case: Run 43

F_T : 35 cc

F_B : 35 cc

V_T : 85 cc

V_B : 60 cc

$Q_o t_I = Q_o t_{III}$: 70.0 cc

	<u>pH</u>	<u>R_{403}</u>	<u>R_{595}</u>
P_2 Feed	6.05	0.480	0.753
P_2 Buffer	6.05	---	0.396
P_1 Feed	7.94	0.421	0.757
P_1 Buffer	7.94	---	0.391

Hemoglobin Recovery: 74.1%

Albumin Recovery: 85.2%

Protein Recovery: 79.7%

RUN NO. 52 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	6.02	0.192	0.643	0.400	0.98-	0.290	0.71-	2.03	1.1-	2.2
1 BP	8.05	0.334	0.707	0.813	0.91-	0.472	0.53-			
2 TP	6.05	0.184	0.616	0.382	0.85-	0.231	0.77-	2.91	1.1-	3.2
2 BP	8.20	0.439	0.733	1.11-	0.76-	0.594	0.41-			
3 TP	6.00	0.151	0.581	0.314	0.78-	0.287	0.71-	3.88	0.84	3.3
3 BP	8.40	0.458	0.785	1.22-	0.93-	0.567	0.43-			
4 TP	6.08	0.122	0.593	0.254	0.83-	0.234	0.77-	4.84	0.91	4.4
4 BP	8.20	0.488	0.779	1.23-	0.91-	0.575	0.43-			
5 TP	6.05	0.117	0.589	0.244	0.84-	0.225	0.77-	5.08	0.91	4.6
5 BP	7.90	0.527	0.786	1.24-	0.92-	0.574	0.43-			
6 TP	6.00	0.111	0.582	0.230	0.81-	0.213	0.79-	5.65	1.0-	5.7
6 BP	8.30	0.502	0.775	1.30-	0.80-	0.619	0.38-			
7 TP	6.00	0.111	0.588	0.230	0.85-	0.213	0.79-	5.17	1.2-	6.2
7 BP	8.03	0.491	0.741	1.19-	0.72-	0.623	0.38-			
8 TP	6.29	0.126	0.611	0.263	0.94-	0.219	0.78-	4.56	1.0-	4.6
8 BP	8.13	0.485	0.779	1.20-	0.92-	0.566	0.43-			
9 TP	6.05	0.111	0.583	0.230	0.82-	0.219	0.78-	5.65	0.93	5.3
9 BP	8.25	0.509	0.790	1.30-	0.88-	0.596	0.40-			
10 TP	6.03	0.106	0.593	0.220	0.88-	0.200	0.80-	5.77	1.1-	6.3
10 BP	7.90	0.540	0.771	1.27-	0.80-	0.614	0.39-			

RUN NO. 52 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_o</u>	<u>y_{Al}/y_o</u>	<u>(M)_{Hb}</u>	<u>(M)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
11 TP	6.05	0.107	0.564	0.222	0.719	0.236	0.764	5.99	0.80	4.8
11 BP	8.15	0.536	0.799	1.33-	0.90-	0.596	0.40-			
12 TP	6.00	0.104	0.586	0.217	0.85-	0.203	0.80-	6.32	0.99	6.3
12 BP	8.10	0.564	0.799	1.37-	0.86-	0.614	0.39-			

RUN NO. 53Mode 5

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 86

No Power Case: Run 45

F_T : 35 cc

F_B : 35 cc

F_o : 45 cc

V_T : 82.5 cc

V_B : 60.0 cc

$Q_o t_I$: 67.5 cc

$Q_o t_{II}$: 67.5 cc

$Q_p t_{IV}$: 112.5 cc

$Q_o t_V$: 0 cc

		<u>Cycles 1-6</u>		<u>Cycles 7-9</u>	
	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.82	0.411	0.728	0.411	0.771
P_2 Buffer	5.82	---	0.373	---	0.397
P_1 Feed	8.52	0.367	0.773	0.367	0.822
P_1 Buffer	8.52	---	0.378	---	0.405

Hemoglobin Recovery: 86.1%

Albumin Recovery: 107%

Protein Recovery: 96%

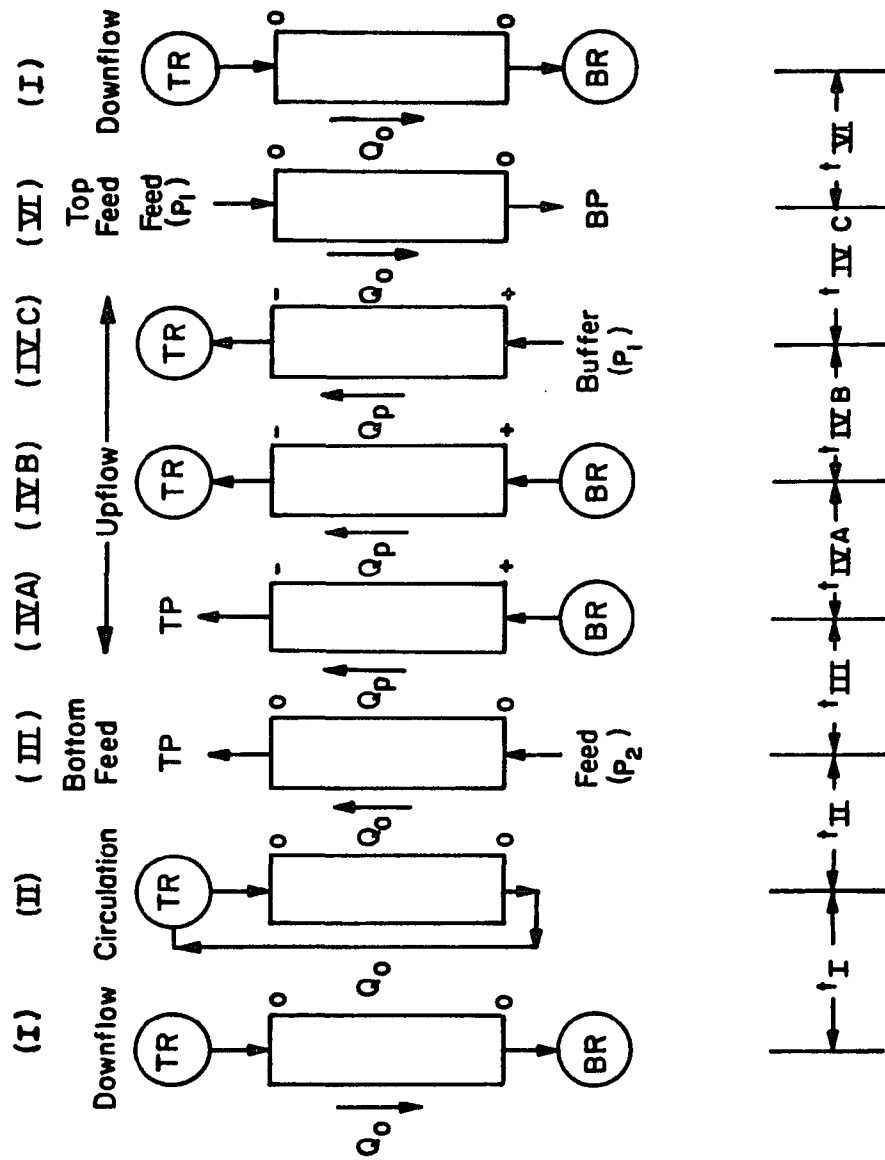


FIGURE 86. Process Diagram for the pH Parapump with Electric Field (Mode 5)

RUN NO. 53 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	6.10	0.171	0.694	0.416	0.95-	0.304	0.70-			
1 BP	8.52	0.324	0.686	0.883	0.68-	0.567	0.43-	2.12	1.4-	3.0-
2 TP	6.00	0.045	0.546	0.11-	0.87-	0.11-	0.89-			
2 BP	8.50	0.568	0.778	1.54-	0.49-	0.759	0.24-	14.-	1.8-	25.-
3 TP	6.05	0.041	0.530	0.098	0.785	0.11-	0.889			
3 BP	8.43	0.681	0.842	1.80-	0.55-	0.766	0.23-	18.-	1.4-	26.-
4 TP	5.97	0.053	0.545	0.13-	0.84-	0.13-	0.87-			
4 BP	8.45	0.490	0.712	1.31-	0.38-	0.775	0.22-	10.-	2.2-	22.-
5 TP	6.13	0.041	0.518	0.098	0.716	0.12-	0.88-			
5 BP	8.40	0.569	0.738	1.50-	0.32-	0.824	0.18-	15.-	2.2-	34.-
6 TP	6.02	0.042	0.505	0.102	0.639	0.138	0.862			
6 BP	8.52	0.479	0.715	1.30-	0.36-	0.783	0.22-	13.-	1.8-	23.-
7 TP	6.02	0.037	0.548	0.090	0.717	0.112	0.888			
7 BP	8.43	0.654	0.813	1.73-	0.23-	0.884	0.12-	19.-	3.1-	60.-
8 TP	6.10	0.037	0.543	0.090	0.688	0.116	0.884			
8 BP	8.43	0.419	0.700	1.11-	0.30-	0.787	0.21-	12.-	2.3-	28.-
9 TP	6.00	0.040	0.541	0.096	0.671	0.13-	0.875			
9 BP	8.33	0.593	0.762	1.52-	0.19-	0.889	0.11-	16.-	3.5-	56.-

RUN NO. 54Mode 6

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 36

No Power Case: Run 45

 F_T : 35 cc F_B : 35 cc F_o : 45 cc V_T : 82.5 cc V_B : 60.0 cc $Q_o t_I$: 67.5 cc $Q_o t_{II}$: 67.5 cc $Q_p t_{IV}$: 112.5 cc $Q_o t_V$: 135.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.82	0.423	0.765
P_2 Buffer	5.82	---	0.394
P_1 Feed	8.58	0.369	0.807
P_1 Buffer	8.58	---	0.394

Hemoglobin Recovery: 83.3%

Albumin Recovery: 107%

Protein Recovery: 100%

RUN NO. 54 (cont.)

Cycle	pH	R ₄₀₃	R ₅₉₅	y _{Hb} /y _O	y _{Al} /y _O	(W) _{Hb}	(W) _{Al}	(SF) _{Hb}	(SF) _{Al}	α
1 TP	6.10	0.179	0.676	0.422	1.07-	0.283	0.717			
1 BP	8.40	0.569	0.840	1.48-	0.68-	0.685	0.31-	3.51	1.6	5.5
2 TP	6.05	0.064	0.589	0.15-	0.90-	0.14-	0.86-			
2 BP	8.50	0.569	0.841	1.52-	0.64-	0.704	0.30-	10.-	1.4	14.-
3 TP	6.00	0.045	0.588	0.11-	0.94-	0.10-	0.90-			
3 BP	8.45	0.564	0.828	1.49-	0.61-	0.710	0.29-	14.-	1.5	21.-
4 TP	6.10	0.039	0.548	0.092	0.819	0.10-	0.90-			
4 BP	8.45	0.537	0.790	1.41-	0.51-	0.734	0.27-	15.-	1.6	25.-
5 TP	6.12	0.042	0.556	0.10-	0.77-	0.12-	0.88-			
5 BP	8.45	0.509	0.760	1.34-	0.43-	0.757	0.24-	13.-	1.8	24.-
6 TP	5.91	0.042	0.541	0.098	0.692	0.12-	0.876			
6 BP	8.57	0.486	0.757	1.33-	0.43-	0.756	0.24-	14.-	1.6	22.-
7 TP	6.15	0.046	0.537	0.11-	0.66-	0.14	0.86-			
7 BP	8.45	0.469	0.747	1.24-	0.47-	0.725	0.27-	11.-	1.4	16.-
8 TP	6.20	0.050	0.544	0.12-	0.69-	0.15-	0.85-			
8 BP	8.48	0.477	0.736	1.27-	0.39-	0.765	0.23-	11.-	1.8	20.-
9 TP	6.08	0.040	0.550	0.095	0.746	0.11-	0.887			
9 BP	8.49	0.462	0.731	1.23-	0.40-	0.755	0.25-	13.-	1.9	24.-
10 TP	6.00	0.036	0.554	0.084	0.779	0.10-	0.903			
10 BP	8.48	0.458	0.750	1.22-	0.50-	0.709	0.29-	15.-	1.6	23.-

RUN NO. 55Mode 7

Buffer: 0.05M Tris-Maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 31

No Power Case: not run

F_T : 17.5 cc

F_B : 52.5 cc

F_O : 40.0 cc

V_T : 112.5 cc

V_B : 90.0 cc

$Q_o t_I$: 67.5 cc

$Q_o t_{II}$: 67.5 cc

$Q_p t_{IV}$: 107.5 cc

$Q_o t_V$: 60.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.80	0.452	0.700
P_2 Buffer	5.80	---	0.402
P_1 Feed	8.52	0.372	0.718
P_1 Buffer	8.52	---	0.407

Hemoglobin Recovery: 71.6%

Albumin Recovery: 128%

Protein Recovery: 100%

RUN NO. 55 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	5.90	0.218	0.613	0.480	0.93-	0.340	0.66-	3.33	1.7	5.6
1 BP	8.40	0.614	0.742	1.60-	0.55-	0.744	0.26-			
2 TP	5.88	0.142	0.589	0.314	0.95-	0.248	0.75-			
2 BP	8.35	0.636	0.771	1.64-	0.70-	0.702	0.30-	5.22	1.4	7.1
3 TP	5.92	0.077	0.561	0.17-	0.90-	0.16-	0.84-			
3 BP	8.42	0.610	0.745	1.60-	0.57-	0.738	0.26-	9.4-	1.6	15.-
4 TP	5.82	0.046	0.561	0.10-	0.95-	0.096	0.90-			
4 BP	8.42	0.812	0.788	2.14-	0.31-	0.873	0.13-	21.-	3.1	66.-
5 TP	5.90	0.045	0.555	0.099	0.92-	0.096	0.90-			
5 BP	8.60	0.715	0.760	1.99-	0.27-	0.881	0.12-	20.-	3.4	68.-
6 TP	5.80	0.034	0.548	0.075	0.902	0.077	0.923			
6 BP	8.37	0.888	0.809	2.30-	0.28-	0.891	0.11-	31.-	3.2	99.-
7 TP	5.90	0.040	0.546	0.088	0.878	0.091	0.909			
7 BP	8.58	0.706	0.759	1.95-	0.31-	0.863	0.14-	22.-	2.8	63.-
8 TP	5.85	0.031	0.535	0.068	0.822	0.076	0.924			
8 BP	8.52	0.900	0.828	2.43-	0.28-	0.897	0.10-	36.-	2.9	105.-
9 TP	5.88	0.030	0.559	0.066	0.98-	0.063	0.94-			
9 BP	8.68	0.889	0.851	2.54-	0.31-	0.891	0.11-	38.-	3.2	122.-
10 TP	5.88	0.029	0.538	0.065	0.846	0.071	0.929			
10 BP	8.58	0.875	0.836	2.41-	0.35-	0.873	0.13-	37.-	2.4	90.-

RUN NO. 55 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
11 TP	6.00	0.048	0.551	0.11-	0.89-	0.11-	0.89-	21.-	2.6-	56.-
11 BP	8.43	0.897	0.828	2.36-	0.34-	0.873	0.13-			
12 TP	5.83	0.030	0.538	0.067	0.844	0.074	0.926	36.-	3.2	118.-
12 BP	8.60	0.875	0.825	2.43-	0.26-	0.905	0.095			

RUN NO. 56Mode 7

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 31

No Power Case: Run 45

F_T : 35 cc

F_B : 35 cc

F_O : 40 cc

V_T : 112.5 cc

V_V : 90.0 cc

$Q_o t_I$: 67.5 cc

$Q_o t_{II}$: 67.5 cc

$Q_p t_{IV}$: 107.5 cc

$Q_o t_V$: 60.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.85	0.446	0.712
P_2 Buffer	5.85	---	0.434
P_1 Feed	8.50	0.371	0.727
P_1 Buffer	8.50	---	0.434

Hemoglobin Recovery: 89.3%

Albumin Recovery: 119%

Protein Recovery: 104%

RUN NO. 56 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(N)_{Hb}</u>	<u>(N)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	6.03	0.289	0.637	0.649	0.81-	0.394	0.61-			
1 BP	8.48	0.356	0.629	0.956	0.37-	0.719	0.28-	1.47	2.2	3.2
2 TP	5.80	0.115	0.598	0.257	0.93-	0.209	0.79-			
2 BP	8.48	0.663	0.742	1.78-	0.32-	0.848	0.15-	6.93	2.9	20.-
3 TP	5.90	0.087	0.597	0.20-	0.98-	0.17-	0.83-			
3 BP	8.43	0.439	0.659	1.17-	0.36-	0.766	0.23-	5.9-	2.7	16.-
4 TP	5.87	0.046	0.587	0.10-	1.00-	0.091	0.909			
4 BP	8.48	0.598	0.728	1.61-	0.40-	0.801	0.20-	16.-	2.5	40.-
5 TP	6.00	0.043	0.578	0.097	0.94-	0.093	0.91-			
5 BP	8.52	0.479	0.673	1.30-	0.33-	0.796	0.20-	13.-	2.8	38.-
6 TP	5.85	0.034	0.574	0.075	0.93-	0.075	0.93-			
6 BP	8.38	0.495	0.682	1.29-	0.40-	0.763	0.24-	17.-	2.3	40.-
7 TP	6.00	0.030	0.570	0.067	0.913	0.068	0.932			
7 BP	8.48	0.647	0.752	1.74-	0.43-	0.802	0.20-	26.-	2.1	55.-
8 TP	5.82	0.026	0.568	0.057	0.916	0.059	0.941			
8 BP	8.48	0.601	0.725	1.62-	0.37-	0.814	0.19-	28.-	2.5	70.-
9 TP	6.00	0.036	0.573	0.081	0.92-	0.081	0.92-			
9 BP	8.50	0.547	0.705	1.48-	0.37-	0.800	0.20-	18.-	2.5	45.-
10 TP	5.85	0.029	0.578	0.064	0.97-	0.062	0.94-			
10 BP	8.50	0.524	0.706	1.41-	0.44-	0.763	0.24-	22.-	2.2	49.-

RUN NO. 57Mode 7

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 31

No Power Case: not run

F_T : 17.5 cc

F_B : 17.5 cc

F_O : 40.0 cc

V_T : 112.5 cc

V_B : 90.0 cc

$Q_o t_I$: 67.5 cc

$Q_o t_{II}$: 67.5 cc

$Q_p t_{IV}$: 107.5 cc

$Q_o t_V$: 60.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.88	0.436	0.792
P_2 Buffer	5.88	---	0.405
P_1 Feed	8.50	0.376	0.812
P_1 Buffer	8.50	---	0.405

Hemoglobin Recovery: 49.1%

Albumin Recovery: 61.3%

Protein Recovery: 59.2%

RUN NO. 57 (cont.)

Cycle	pH	R ₄₀₃	R ₅₂₅	y_{Hb}/y_o	y_{Al}/y_o	(W) _{Hb}	(W) _{Al}	(SF) _{Hb}	(SF) _{Al}	α
1 TP	6.15	0.274	0.705	0.629	0.92-	0.406	0.59-			
1 BP	8.50	0.259	0.661	0.688	0.57-	0.547	0.45-	1.09	1.6	1.8
2 TP	6.10	0.108	0.631	0.248	0.92-	0.212	0.79-			
2 BP	8.52	0.324	0.693	0.867	0.55-	0.613	0.39-	3.50	1.7	5.8
3 TP	6.10	0.057	0.613	0.13-	0.95-	0.12-	0.88-			
3 BP	8.45	0.350	0.700	0.919	0.53-	0.635	0.37-	7.1-	1.8	13.-
4 TP	6.10	0.042	0.584	0.095	0.828	0.10-	0.898			
4 BP	8.48	0.369	0.706	0.975	0.50-	0.661	0.34-	10.-	1.7	17.-
5 TP	6.10	0.033	0.556	0.075	0.705	0.096	0.904			
5 BP	8.50	0.228	0.592	0.607	0.307	0.664	0.336	8.1	2.3	19.-
6 TP	6.08	0.029	0.567	0.065	0.770	0.078	0.922			
6 BP	8.50	0.290	0.656	0.771	0.46-	0.626	0.37-	12.-	1.7	20.-
7 TP	6.02	0.027	0.554	0.062	0.705	0.081	0.909			
7 BP	8.60	0.323	0.656	0.885	0.35-	0.717	0.28-	14.-	2.0	29.-
8 TP	6.08	0.027	0.546	0.062	0.667	0.085	0.915			
8 BP	8.50	0.328	0.667	0.772	0.51-	0.602	0.40-	13.-	1.3	16.-
9 TP	6.05	0.025	0.546	0.056	0.673	0.077	0.923			
9 BP	8.50	0.386	0.709	1.03-	0.46-	0.691	0.31-	18.-	1.5	27.-
10 TP	6.15	0.026	0.531	0.060	0.590	0.092	0.908			
10 BP	8.42	0.321	0.636	0.835	0.31-	0.729	0.27-	14.-	1.9	26.-

RUN NO. 58Mode 7

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 31

No Power Case: not run

F_T : 52.5 cc

F_B : 17.5 cc

F_O : 40.0 cc

V_T : 112.5 cc

V_B : 90.0 cc

$Q_o t_I$: 67.5 cc

$Q_o t_{II}$: 67.5 cc

$Q_p t_{IV}$: 107.5 cc

$Q_o t_V$: 60.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.82	0.418	0.776
P_2 Buffer	5.82	---	0.369
P_1 Feed	8.68	0.373	0.807
P_1 Buffer	8.68	---	0.369

Hemoglobin Recovery: 89.8%

Albumin Recovery: 107%

Protein Recovery: 98%

RUN NO. 58 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	6.15	0.286	0.687	0.684	0.88-	0.428	0.56-			
1 BP	8.48	0.269	0.663	0.707	0.63-	0.527	0.47-	1.03	1.4	1.4
2 TP	6.09	0.117	0.615	0.280	0.93-	0.232	0.77-			
2 BP	8.68	0.505	0.807	1.42-	0.58-	0.711	0.29-	5.07	1.6	8.1
3 TP	6.05	0.092	0.603	0.22-	0.93-	0.19-	0.81-			
3 BP	8.58	0.369	0.705	1.00-	0.53-	0.653	0.35-	4.5-	1.8	8.0
4 TP	6.05	0.050	0.576	0.12-	0.89-	0.12-	0.88-			
4 BP	8.55	0.346	0.700	0.925	0.58-	0.613	0.39-	7.7-	1.5	12.-
5 TP	6.08	0.038	0.568	0.090	0.888	0.092	0.908			
5 BP	8.55	0.494	0.773	1.33-	0.52-	0.718	0.28-	15.-	1.7	25.-
6 TP	6.05	0.054	0.573	0.13-	0.87-	0.13-	0.87-			
6 BP	8.50	0.397	0.708	1.06-	0.48-	0.687	0.31-	8.2	1.8	15.-
7 TP	6.05	0.074	0.569	0.18-	0.73-	0.19-	0.81-			
7 BP	8.52	0.311	0.653	0.825	0.47-	0.638	0.36-	4.6	1.6	7.2
8 TP	6.09	0.041	0.559	0.10-	0.83-	0.11-	0.89-			
8 BP	8.48	0.389	0.675	1.02-	0.38-	0.731	0.27-	10.-	2.2	22.-
9 TP	6.08	0.036	0.561	0.085	0.859	0.090	0.910			
9 BP	8.50	0.327	0.656	0.864	0.45-	0.658	0.34-	10.-	1.9	19.-
10 TP	6.20	0.032	0.532	0.077	0.722	0.10-	0.904			
10 BP	8.62	0.247	0.588	0.679	0.32-	0.678	0.32-	8.8	2.3	20.-

RUN NO. 59Mode 7

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 31

No Power Case: not run

F_T : 52.5 cc

F_B : 52.5 cc

F_O : 40.0 cc

V_T : 112.5 cc

V_B : 90.0 cc

$Q_o t_I$: 67.5 cc

$Q_o t_{II}$: 67.5 cc

$Q_p t_{IV}$: 107.5 cc

$Q_o t_V$: 60.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.80	0.409	0.689
P_2 Buffer	5.80	---	0.326
P_1 Feed	8.45	0.379	0.712
P_1 Buffer	8.45	---	0.326

Hemoglobin Recovery: 57.6%

Albumin Recovery: 87.4%

Protein Recovery: 72.5%

RUN NO. 59 (cont.)

<u>Cycle</u>	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>	<u>y_{Hb}/y_O</u>	<u>y_{Al}/y_O</u>	<u>(W)_{Hb}</u>	<u>(W)_{Al}</u>	<u>(SF)_{Hb}</u>	<u>(SF)_{Al}</u>	<u>α</u>
1 TP	5.98	0.132	0.552	0.323	0.92-	0.260	0.74-			
1 BP	8.52	0.291	0.580	0.781	0.54-	0.593	0.41-	2.42	1.7	4.1
2 TP	6.05	0.105	0.515	0.257	0.79-	0.246	0.75-			
2 BP	8.58	0.243	0.544	0.668	0.46-	0.591	0.41-	2.60	1.7	4.5
3 TP	6.05	0.056	0.479	0.13-	0.71-	0.16-	0.84-			
3 BP	8.45	0.298	0.579	0.787	0.52-	0.602	0.40-	6.1-	1.4	8.3
4 TP	6.00	0.040	0.469	0.098	0.690	0.12-	0.876			
4 BP	8.55	0.432	0.663	1.18-	0.57-	0.674	0.33-	12.-	1.2	15.-
5 TP	6.05	0.033	0.468	0.081	0.702	0.10-	0.897			
5 BP	8.55	0.272	0.573	0.735	0.54-	0.576	0.42-	9.1	1.3	12.-
6 TP	6.00	0.039	0.460	0.095	0.642	0.13-	0.871			
6 BP	8.53	0.365	0.605	0.992	0.46-	0.683	0.32-	10.-	1.4	15.-
7 TP	6.00	0.039	0.459	0.095	0.639	0.13-	0.871			
7 BP	8.53	0.364	0.593	0.989	0.39-	0.717	0.28-	10.-	1.6	17.-
8 TP	6.12	0.031	0.459	0.076	0.655	0.10-	0.896			
8 BP	8.50	0.407	0.617	1.09-	0.42-	0.722	0.28-	14.-	1.6	22.-
9 TP	6.08	0.033	0.461	0.079	0.666	0.11-	0.894			
9 BP	8.58	0.351	0.605	0.966	0.48-	0.669	0.33-	12.-	1.4	17.-

RUN NO. 60Mode 8

Buffer: 0.05M Tris-maleate + 0.05M NaOH

Feed: 0.01 weight % hemoglobin + 0.01 weight % albumin
($P_2 = 5.8$, $P_1 = 8.5$)

Flow Rate: $Q_o = 2.5$ cc/min, $Q_p = 0.5$ cc/min

Power: 210 volts

Procedure: Figure 40

No Power Case: Run 45

F_T : 35 cc

F_B : 35 cc

F_o : 40 cc

V_T : 112.5 cc

V_B : 90.0 cc

$Q_o t_I$: 67.5 cc

$Q_o t_{II}$: 67.5 cc

$Q_p t_{IV}$: 107.5 cc

$Q_o t_V$: 45.0 cc

	<u>pH</u>	<u>R₄₀₃</u>	<u>R₅₉₅</u>
P_2 Feed	5.80	0.663	0.868
P_2 Buffer	5.80	---	0.411
P_1 Feed	8.52	0.487	0.879
P_1 Buffer	8.52	---	0.415

Hemoglobin Recovery: 112%

Albumin Recovery: 99.5%

Protein Recovery: 106%

RUN NO. 60 (cont.)

Cycle	pH	R ₄₀₃	R ₅₉₅	y_{Hb}/y_o	y_{Al}/y_o	$(M)_{Hb}$	$(M)_{Al}$	$(SF)_{Hb}$	$(SF)_{Al}$	α
1 TP	6.00	0.617	0.850	0.931	0.99-	0.484	0.52-			
1 BP	8.40	1.321	1.083	2.614	0.265	0.908	0.092	2.81	3.7	11.
2 TP	6.08	0.301	0.700	0.455	0.81-	0.329	0.67-			
2 BP	8.52	1.042	0.969	2.131	0.26-	0.892	0.11-	4.68	4.3	20.
3 TP	6.02	0.200	0.678	0.302	0.87-	0.259	0.74-			
3 BP	8.50	0.981	0.936	2.00-	0.25-	0.890	0.11-	6.62	3.5	23.
4 TP	6.10	0.111	0.625	0.168	0.767	0.180	0.820			
4 BP	8.52	0.702	0.795	1.44-	0.20-	0.878	0.12-	8.57	3.8	33.
5 TP	6.12	0.048	0.594	0.072	0.727	0.090	0.910			
5 BP	8.58	0.619	0.776	1.29-	0.27-	0.828	0.17-	18.-	2.7	49.
6 TP	6.09	0.041	0.593	0.062	0.734	0.078	0.922			
6 BP	8.45	0.703	0.789	1.41-	0.20-	0.876	0.12-	23.-	3.7	83.
7 TP	6.09	0.073	0.632	0.11-	0.86-	0.11-	0.89-			
7 BP	8.52	0.731	0.813	1.49-	0.22-	0.869	0.13-	14.-	3.9	53.
8 TP	6.03	0.055	0.601	0.082	0.750	0.099	0.901			
8 BP	8.60	0.651	0.779	1.36-	0.21-	0.866	0.13-	17.-	3.6	59.
9 TP	6.11	0.044	0.626	0.066	0.877	0.070	0.930			
9 BP	8.58	0.752	0.829	1.56-	0.23-	0.873	0.13-	24.-	3.9	92.

APPENDIX C
COMPUTER CALCULATIONS

```

1 C
2 C   PROGRAM #1 - HEMOGLOBIN CONCENTRATION WAVES
3 C
4 C
5 C   PROGRAM WRITTEN BY H. CONNIE HOLLFIN
6 C   DOCTOR OF ENGINEERING SCIENCE IN CHEMICAL ENGINEERING
7 C   NEW JERSEY INSTITUTE OF TECHNOLOGY, NEWARK, N.J.07102
8 C
9 C
10 C  HEMOGLOBIN BREAKTHROUGH CURVES WITH STEP OR PULSE INPUT
11 C
12 C  CALCULATE PH AND CONCENTRATION WAVES
13 C
14 C  FINITE PASS TRANSFER MODEL
15 C
16 C
17 C      DIMENSION PH(200), PHJM(200), PHOUT(750),
18 C          VOUT(750), VAVG(750), B(200), BJM(200),
19 C          ZK(200), ZKJM(200), YHB(200), YHBJM(200),
20 C          HBOUT(750), XHB(200), XHBJM(200)
21 C
22 C  FIX OPERATING CONDITIONS FOR THE RUN
23 C      V = 45.0
24 C      EPS = 0.565
25 C      E1 = (1.-EPS)/EPS
26 C      ZKL = 0.10
27 C      QP = 0.5
28 C      VO = QP/3.0
29 C      READ(5,895) RECHB
30 C 895  FORMAT(F6.1)
31 C      READ(5,900) M,PHOLD,BOLD,HBOLD,PHNEW,BNEW,HBNFW,PULSE
32 C 900  FORMAT(I3,F6.3,F6.3,F6.3,F6.3,F6.3,F6.3,F6.3,F6.1)
33 C      S = M
34 C      VDEL = V/S
35 C      TDEL = VDEL/QP
36 C      K = M*4
37 C      READ(5,907) ZKOLD,ZKNEW
38 C 907  FORMAT(F6.3,F6.3)
39 C      READ(5,1205) NDATA
40 C 1205 FORMAT(I2)
41 C 105  READ(5,905) VHR,RPOLD,RPNEW
42 C 905  FORMAT(F8.4,F6.3,F6.3)
43 C      DO 980 NREAD=1,NDATA
44 C      READ(5,1215) FLAM
45 C 1215 FORMAT(F10.6)
46 C      VEA = VHB
47 C      RA = VO / (VO+VEA)
48 C      VINI = 0.
49 C
50 C  SET INITIAL CONDITIONS IN THE COLUMN
51 C      DO 110 I = 1,M
52 C      IF (VEA .EQ. 0.0) B(I) = BOLD
53 C      IF (VEA .NE. 0.0) B(I) = BPOLD
54 C      ZK(I) = ZKOLD
55 C      YHB(I) = HBOLD
56 C      IF (YHB(I) .LT. 7KL) XHB(I)=0.0

```

```

57      IF (YHB(I) .GT. ZKL) XHB(I)=ZK(I)+(YHB(I)-ZKL)
58 110  PH(I) = PHOLD
59  C
60  C  THIS IS THE GO STEP
61      DO 130 J = 1,K
62      VINI = VINI + VDFL
63      VOUT(J) = VINI
64      VAVG(J) = VINI - VDEL/2.
65      PHOUT(J) = PH(M)
66      HBOUT(J) = YHB(M)*(1./RA)+RECHB/100.
67      IF (VOUT(J) .GT. PULSE) GO TO 112
68      IF (VEA .EQ. 0.0) BJM(1) = BNEW
69      IF (VEA .NE. 0.0) BJM(1) = BPNEW
70      ZKJM(1) = ZKNFW
71      YHBJM(1) = HBNEW + (1.-1./RA)*YHB(1)
72      XHBJM(1) = XHB(1)
73      PHJM(1) = PHNEW
74      GO TO 122
75 112  VEA = 0.0
76      RA = VO / (VO+VEA)
77      BJM(1) = POLD
78      ZKJM(1) = ZKOLD
79      YHBJM(1) = HBOLD
80      XHBJM(1) = XHB(1)
81      PHJM(1) = PHOLD
82  C
83 122  DO 123 I = 2,M
84      IM = I-1
85      BJM(I) = B(IM)
86      ZKJM(I) = ZK(IM)
87      YHBJM(I) = YHB(IM)*(1./RA) + YHB(I)*(1.-1./RA)
88      XHBJM(I) = XHB(I)
89 123  PHJM(I) = PH(IM)
90  C
91  C  THIS IS THE STOP STEP
92      DO 130 I = 1,M
93      B(I) = BJM(I)
94      PH(I) = B(I)*PHJM(I)+(1.-F(I))*PH(I)
95      IF (PHNEW .EQ. PHOLD) GO TO 124
96      ZK(I) = ZKOLD+((ZKNFW-ZKOLD)/(PHNEW-PHOLD))*(PH(I)-PHOLD)
97      GO TO 125
98 124  ZK(I) = ZKOLD
99 125  E2 = EXP((-E1-1./ZK(I))*FLAM*TDEL)
100     XHB(I) = ((YHBJM(I)+E1*XHBJM(I)-ZKL)/(F1+1./ZK(I)))*
101     C      (1.-E2)+XHBJM(I)*E2
102     IF (XHB(I) .LT. 0.0) XHB(I) = 0.0
103     YHB(I) = YHBJM(I)+E1*(XHBJM(I)-XHB(I))
104 130  CONTINUE
105  C
106  C  PRINTOUT OF RESULTS
107     WRITE(6,998)
108 998  FORMAT('1'. 'SIMULATION OF PH AND CONCENTRATION WAVES')
109     WRITE(6,1000) RECHB
110 1000 FORMAT(' '. 'HEMOGLOBIN RECOVERY = '.F6.1.'%')
111     WRITE(6,995) VHB
112 995  FORMAT(' '. 'HEMOGLOBIN MIGRATION VELOCITY = '.F8.3.

```



```

113      C      'CM/MIN')
114      WRITE(6,1008) M
115 1008  FORMAT(' ',NUMBER OF STAGES = ',I5)
116      WRITE (6,1050) FLAM
117 1050  FORMAT(' ',LAMBDA - LOW FLOW RATE = ',F10.6)
118      WRITE(6,1002) PHOLD
119 1002  FORMAT(' ',INITIAL PH = ',F5.1)
120      WRITE(6,1003) HBOLD
121 1003  FORMAT(' ',THE INITIAL CONCENTRATION IS ',F6.3)
122      WRITE(6,1001) ZKOLD
123 1001  FORMAT(' ',THE INITIAL VALUE FOR K* IS ',F6.3)
124      WRITE(6,1010) BOLD
125 1010  FORMAT(' ',THE INITIAL VALUE FOR B WITH ZERO ',
126      C      'ELECTRIC FIELD IS ',F6.3)
127      IF (RA .EQ. 1.0) GO TO 131
128      WRITE(6,1025) BPOLD
129 1025  FORMAT(' ',THE INITIAL VALUE FOR B WITH FINITE ',
130      C      'ELECTRIC FIELD IS ',F6.3)
131 131   WRITE(6,1004) PHNEW
132 1004  FORMAT(' ',FEED PH = ',F5.1)
133      WRITE(6,1005) HBNEW
134 1005  FORMAT(' ',THE FEED CONCENTRATION IS ',F6.3)
135      WRITE(6,1007) ZKNEW
136 1007  FORMAT(' ',THE FEED VALUE FOR K* IS ',F6.3)
137      WRITE(6,990) BNEW
138 990   FORMAT(' ',THE FEED VALUE FOR B WITH ZERO ',
139      C      'ELECTRIC FIELD IS ',F6.3)
140      IF (RA .EQ. 1.0) GO TO 132
141      WRITE(6,1012) BPNEW
142 1012  FORMAT(' ',THE FEED VALUE FOR B WITH FINITE ',
143      C      'ELECTRIC FIELD IS ',F6.3)
144 132   WRITE(6,1006) PULSE
145 1006  FORMAT(' ',VOLUME OF PULSE = ',F6.1,' CC')
146      WRITE(6,1014)
147 1014  FORMAT('0',TIME',3X,'V OUT,CC',5X,'V AVG,CC',
148      C      '7X,'PH OUT',7X,'Y(HR)')
149      C
150      DO 135 J = 1,K
151      IF (M .EQ. 9) GO TO 134
152      IF (VOUT(J) .LT. (V-VDEL)) GO TO 135
153      IF (J .NE. 50) GO TO 134
154      WRITE(6,1020)
155 1020  FORMAT('1')
156      WRITE(6,1019)
157 1019  FORMAT(' ',TIME',3X,'V OUT,CC',5X,'V AVG,CC',
158      C      '7X,'PH OUT',7X,'Y(HR)')
159 134   WRITE(6,1016) J,VOUT(J),VAVG(J),PHOUT(J),HBOUT(J)
160 1016  FORMAT(2X,I4,F13.6,F13.6,F13.6,F13.6)
161 135   CONTINUE
162      C
163 980   CONTINUE
164      C
165      STOP
166      END

```

```

1 C
2 C          PROGRAM #2 - PARAMETRIC PUMPING
3 C
4 C
5 C PROGRAM WRITTEN BY Mr CONNIE HOLLEIN
6 C DOCTOR OF ENGINEERING SCIENCE IN CHEMICAL ENGINEERING
7 C NEW JERSEY INSTITUTE OF TECHNOLOGY, NEWARK, N.J. 07102
8 C
9 C
10 C PARAMETRIC PUMPING CALCULATIONS FOR MODES 6-8 AND NO POWER
11 C
12 C EASE CASE FOR NO POWER IS MODE 6 WITH VHB=0, VAL=0 & FO=0
13 C
14 C THE BATCH PARAPUMP IS MODE 6 WITH FB=0, FT=0 & FO=0
15 C
16 C
17 C   DIMENSION YTAL(100), YTRAL(100), YBAL(100), YBRAL(100),
18 C           WTAL(100), WBAL(100), SFAL(100), RECAL(100)
19 C   DIMENSION YTHB(100), YBHB(100), WTHB(100), WBHB(100),
20 C           SFHB(100), ALPHA(100), RECHB(100)
21 C   DIMENSION PH(200), PHJM(200), PHLM(200), ZK(200),
22 C           ZKJM(200), ZKLM(200), YHB(200), YHBJM(200),
23 C           YHBLM(200), XHB(200), XHBJM(200), XHBLM(200),
24 C           ZKX(200), YHBX(200), XHBX(200), PHX(200)
25 C
26 C SET OPERATING CONDITIONS FOR THE RUN
27 C   V = 45.0
28 C   EPS = 0.565
29 C   E1 = (1.-EPS)/EPS
30 C   ZKL = 0.10
31 C   QP = 0.5
32 C   QO = 2.5
33 C   VO = QP/3.0
34 C   YTRAL(1) = 1.0
35 C   YBRAL(1) = 1.0
36 C   BZERO = 0.436
37 C   BPOW = 0.371
38 C   READ (5,1200) MODE, VAL, VHB, PH1, ZK1, PH2, ZK2
39 120C   FORMAT(I1,2F7.3,4F5.2)
40 C   IF (MODE .LT. 6) GO TO 985
41 C   IF (MODE .GT. 8) GO TO 985
42 C   READ (5,1210) VB, VT, QOTI, QOTII, QOTV
43 121C   FORMAT(5F6.1)
44 C   READ (5,1215) FLAMO, FLAMP
45 1215   FORMAT(2F10.6)
46 C   READ(5,1205) NDATA, NSTOP
47 1205   FORMAT(2I3)
48 C   DO 980 NREAD=1,NDATA
49 C   READ (5,1220) MO, MP, FB, FT, FO
50 122C   FORMAT(2I3,3F6.1)
51 C   CHAN = MO/MP
52 C   IF (CHAN .LT. 1.) GO TO 985
53 C   YTRHB = 1.0
54 C   YBRHB = 1.0
55 C
56 C

```

```

57 C ALBUMIN CALCULATIONS - MODES 6 TO 8
58 C
59 C
60 C CALCULATE RETARDATION COEFFICIENT - ALBUMIN
61     VEB = VAL
62     RB = VO / (VO + VEB)
63 C
64 C REPEAT CALCULATIONS CYCLE-BY-CYCLE TO STEADY STATE
65     DO 60 N = 1, NSTOP
66 C
67 C CALCULATE TOP CONCENTRATIONS - ALBUMIN
68     NP = N+1
69     IF (FO .GT. RB*(V - FB)) GO TO 20
70     YTRAL(NP) = ((2.*V+VT-QOTI-FB-FO/RB)*YTRAL(N)+FB+
71     C           (QOTI-RB*V+FO)*YBRAL(N))/(V+VT)
72     IF (FB .NE. 0.0) GO TO 15
73     YTAL(N) = YTRAL(NP)
74     GO TO 30
75 15  YTAL(N) = ((FB+FO/RB)*YTRAL(N))/(FB+FO)
76     GO TO 30
77 20  YTRAL(NP) = ((V+VT-QOTI)*YTRAL(N)+V-FO/RB+
78     C           (QOTI-RB*V+FO)*YBRAL(N))/(V+VT)
79     IF (FT .NE. 0.0) GO TO 25
80     YTAL(N) = YTRAL(NP)
81     GO TO 30
82 25  YTAL(N) = (V*YTRAL(N)+FO/RB-V+FB)/(FB+FO)
83 30  CONTINUE
84 C
85 C CALCULATE BOTTOM CONCENTRATIONS - ALBUMIN
86     IF (MODE-7) 35,45,45
87 35  BRNEW6 = (RB*V-FO+VB)*YBRAL(N)/(V+VB)
88     IF (FT .GT. V) GO TO 40
89     YBAL(N) = BRNEW6
90     YBRAL(NP) = ((V+VB-FT)*BRNEW6+FT+(QOTI-V)*YTRAL(NP))/
91     C           (QOTI+VB)
92     GO TO 60
93 40  YBAL(N) = (V*BRNEW6+FT-V)/FT
94     YBRAL(NP) = (VB*BRNEW6+V+(QOTI-V)*YTRAL(NP))/(QOTI+VB)
95     GO TO 60
96 45  YBAL(N) = ((RB*V-FO+QOTV-V)*YBRAL(N))/QOTV
97     YBRAL(NP) = ((V+VB-QOTV)*YBRAL(N)+(QOTV-FT)*YBAL(N)+
98     C           FT+(QOTI-V)*YTRAL(NP))/(QOTI+VB)
99 60  CONTINUE
100 C
101 C
102 C HEMOGLOBIN CALCULATIONS - MODES 6 TO 8
103 C
104 C
105 C SET INITIAL CONDITIONS IN THE COLUMN FOR THE RUN
106     M = MO
107     FLAM = FLAM0
108     S = M
109     VDEL = V/S
110     TDEL = VDEL/QO
111     DO 80 I=1,M
112     ZK(I) = ZK1

```

FORTRAN IV (VER 55) SOURCE LISTING:

```

113      YHB(I) = 1.0
114      XHB(I) = ZK(I)*(YHB(I)-ZKL)
115 80    PH(I) = PH1
116 C
117 C REPEAT CALCULATIONS CYCLE-BY-CYCLE TO STEADY STATE
118      DO 540 N = 1,NSTOP
119 C
120 C FOLLOW CONCENTRATIONS FROM STAGE-TO-STAGE
121      DO 520 L = 1,8
122 C
123 C STATE THE INLET AND OUTLET CONDITIONS FOR EACH STAGE
124 C
125 C STAGE I--DOWN (TOP RESERVOIR TO BOTTOM RESERVOIR)
126      IF (L .GT. 1) GO TO 100
127      VEA = 0.0
128      K = QOTI/VDEL
129      PHNEW = PH2
130      ZKNEW = ZK2
131      HBNEW = YTRHB
132      VOL = VB
133      VMASS = VB*YBPHB
134      GO TO 240
135 C
136 C STAGE II--DOWN (RECYCLE TO TOP RESERVOIR)
137 100    IF (L .GT. 2) GO TO 120
138      IF (QOTII .EQ. 0.0) GO TO 520
139      VEA = 0.0
140      K = QOTII/VDEL
141      PHNEW = PH2
142      ZKNEW = ZK2
143      HBNEW = YTRHB
144      VOL = VT+V-QOTI
145      VMASS = VOL*YTRHB
146      GO TO 240
147 C
148 C STAGE III--UP (BOTTOM FEED TO TOP PRODUCT)
149 120    IF (L .GT. 3) GO TO 140
150      IF (FB .EQ. 0.0) GO TO 520
151      VEA = 0.0
152      K = FB/VDEL
153      PHNEW = PH2
154      ZKNEW = ZK2
155      HBNEW = 1.0
156      VOL = 0.0
157      VMASS = 0.0
158      GO TO 240
159 C
160 C STAGE IVA--UP (BOTTOM RESERVOIR TO TOP PRODUCT)
161 140    IF (L .GT. 4) GO TO 160
162      IF (FO .EQ. 0.0) GO TO 520
163      VEA = VHB
164      M = MP
165      FLAM = FLAMP
166      S = M
167      VDEL = V/S
168      TDEL = VDEL/QP

```

FORTRAN IV (VER 55) SOURCE LISTING:

```

169      K = FO/VDEL
170      IF (MO .EQ. MP) GO TO 158
171      C1 = 0.0
172      C2 = 0.0
173      C3 = 0.0
174      C4 = 0.0
175      CHEK = 0.0
176      DO 145 I=1,MO
177      ZKX(I) = C1+ZK(I)/CHAN
178      C1 = ZKX(I)
179      YHBX(I) = C2+YHB(I)/CHAN
180      C2 = YHBX(I)
181      XHBX(I) = C3+XHB(I)/CHAN
182      C3 = XHBX(I)
183      PHX(I) = C4+PH(I)/CHAN
184      C4 = PHX(I)
185      CHEK = CHEK+1.
186      IF (CHEK .LT. CHAN) GO TO 145
187      C1 = 0.0
188      C2 = 0.0
189      C3 = 0.0
190      C4 = 0.0
191      CHEK = 0.0
192 145  CONTINUE
193      DO 150 I=1,MP
194      ICHAN = I*CHAN
195      ZK(I) = ZKX(ICHAN)
196      YHB(I) = YHBX(ICHAN)
197      XHB(I) = XHBX(ICHAN)
198      PH(I) = PHX(ICHAN)
199 150  CONTINUE
200 158  PHNEW = PH1
201      ZKNEW = ZK1
202      HBNEW = YBRHB
203      VOL = FB
204      VMASS = FB*YT3HB
205      GO TO 240
206 C
207 C  STAGE IVB--UP (BOTTOM RESERVOIR TO TOP RESERVOIR)
208 160  IF (L .GT. 5) GO TO 180
209      IF (FO .EQ. QOTI) GO TO 520
210      IF (FO .NE. 0.0) GO TO 162
211      VEA = 0.0
212      GO TO 164
213 162  VEA = VHB
214 164  K = (QOTI-FO)/VDEL
215      PHNEW = PH1
216      ZKNEW = ZK1
217      HBNEW = YBRHB
218      VOL = VT+V-QOTI
219      VMASS = VOL*YTRHB
220      GO TO 240
221 C
222 C  STAGE IVC--UP (BUFFER FEED TO TOP RESERVOIR)
223 180  IF (L .GT. 6) GO TO 200
224      IF (FO .EQ. 0.0) GO TO 520

```

```

225      VEA = VHB
226      K = FO/VDEL
227      PHNEW = PH1
228      ZKNEW = ZK1
229      HBNEW = 0.0
230      VOL = (VT+V-QOTI)+(QOTI-FO)
231      VMASS = VOL*YTRHB
232      GO TO 240
233 C
234 C   FLOW DIRECTION IN STAGE V DEPENDS ON MODE OF OPERATION
235 C   NO POWER--UP (RECYCLE TO BOTTOM RESERVOIR)
236 C   MODE 6--UP (RECYCLE TO BOTTOM RESERVOIR)
237 C   MODE 7--DOWN (BOTTOM RESERVOIR TO HOLDING TANK)
238 C   MODE 8--UP (BOTTOM RESERVOIR TO HOLDING TANK)
239 200  IF (L .GT. 7) GO TO 220
240      IF (FO .EQ. 0.0) GO TO 214
241      IF (MO .EQ. MP) GO TO 214
242      M = MO
243      FLAM = FLAM0
244      S = M
245      VDEL = V/S
246      TDEL = VDEL/QO
247      INI = 1
248      C1 = ZK(INI)
249      C2 = YHB(INI)
250      C3 = XHB(INI)
251      C4 = PH(INI)
252      CHEK = 0.0
253      DO 205 I=1,MO
254      ZKX(I) = C1
255      YHBX(I) = C2
256      XHBX(I) = C3
257      PHX(I) = C4
258      CHEK = CHEK+1.
259      IF (CHEK .LT. CHAN) GO TO 205
260      INI = INI+1
261      C1 = ZK(INI)
262      C2 = YHB(INI)
263      C3 = XHB(INI)
264      C4 = PH(INI)
265      CHEK = 0.0
266 205  CONTINUE
267      DO 210 I=1,MO
268      ZK(I) = ZKX(I)
269      YHB(I) = YHBX(I)
270      XHB(I) = XHBX(I)
271      PH(I) = PHX(I)
272 210  CONTINUE
273 214  VEA = 0.0
274      K = QOTV/VDEL
275      PHNEW = PH1
276      ZKNEW = ZK1
277      HBNEW = YBRHB
278      IF (MODE-7) 216,218,218
279 216  IF (QOTV .EQ. 0.0) GO TO 520
280      VOL = VB

```

```

281      VMASS = VB*YBRHB
282      GO TO 240
283 218  VOL = 0.0
284      VMASS = 0.0
285      GO TO 240
286 C
287 C  STAGE VI, L=8 FOR LAST STAGE IN CYCLE
288 C  MODE 6 & NO POWER--DOWN (TOP FEED TO BOTTOM PRODUCT)
289 C  MODES 7 & 8--DOWN (TOP FEED TO BOTTOM RESERVOIR)
290 220  CONTINUE
291      IF (FT .EQ. 0.0) GO TO 510
292      VEA = 0.0
293      K = FT/VDEL
294      PHNEW = PH1
295      ZKNEW = ZK1
296      HBNEW = 1.0
297      IF (MODE-7) 222,224,224
298 222  VOL = 0.0
299      VMASS = 0.0
300      GO TO 240
301 224  VOL = (VB-QOTV)+(QOTV-FT)
302      VMASS = VOL*YBRHB
303 240  CONTINUE
304      RA = VO / (VO+VEA)
305      IF (VEA .EQ. 0.0) B = BZERO
306      IF (VEA .NE. 0.0) B = BPOW
307 C
308 C  SET INITIAL CONDITIONS IN THE COLUMN FOR EACH STAGE
309 C
310      IF (L .LT. 7 .AND. L .NE. 3) GO TO 340
311      IF (L .EQ. 7 .AND. MODE .NE. 7) GO TO 340
312      IF (L .EQ. 8 .AND. MODE .EQ. 7) GO TO 340
313 C
314 280  DO 300 I = 1,M
315      ZKLM(I) = ZK(I)
316      YHBLM(I) = YHB(I)
317      XHBLM(I) = XHB(I)
318 300  PHLM(I) = PH(I)
319      DO 320 I = 1,M
320      IREV = M+1 - I
321      ZK(I) = ZKLM(IREV)
322      YHB(I) = YHBLM(IREV)
323      XHB(I) = XHBLM(IREV)
324 320  PH(I) = PHLM(IREV)
325 340  CONTINUE
326 C
327 C  THIS IS THE GO STEP
328      DO 500 J = 1,K
329 C
330      IF (L .EQ. 2) HBNEW = YTRHB
331      IF (L .EQ. 7 .AND. MODE .EQ. 6) HBNEW = YBRHB
332 C
333 C  MUST CALCULATE THE RECYCLE STAGES
334      IF (L .NE. 2) GO TO 360
335      VMASS = VMASS+VDEL*YHB(M)*(1./RA)-VDEL*YTRHB
336      HBOUT = VMASS/VOL

```

```

337      YTRHB = HBOUT
338      GO TO 400
339 360    IF (MODE .NE. 6) GO TO 380
340      IF (L .NE. 7) GO TO 380
341      VMASS = VMASS+VDEL*YHB(M)*(1./RA)-VDEL*YBRHB
342      HBOUT = VMASS/VOL
343      YBRHB = HBOUT
344      GO TO 400
345 C
346 C   NOW CALCULATE THE STAGES WITHOUT RECYCLE
347 380    VOL = VOL+VDEL
348      VMASS = VMASS+VDEL*YHB(M)*(1./RA)
349      HBOUT = VMASS/VOL
350 C
351      IF (J .LT. K) GO TO 400
352 C
353      IF (L .EQ. 1) YBRHB=HBOUT
354      IF (L .EQ. 3 .AND. FO .EQ. 0.0) YTHB(N)=HBOUT
355      IF (L .EQ. 3 .AND. FO .NE. 0.0) YT3HB=HBOUT
356      IF (L .EQ. 4) YTHB(N)=HBOUT
357      IF (L .EQ. 5) YTRHB=HBOUT
358      IF (L .EQ. 6) YTRHB=HBOUT
359      IF (L .LT. 7) GO TO 400
360      IF (L .GT. 7) GO TO 390
361      YBHB(N) = HBOUT
362      YBRHB = ((VB-QOTV)*YBRHB+(QOTV-FT)*HBOUT)/(VB-FT)
363      GO TO 400
364 C   STAGE VI, L=8 FOR LAST STAGE IN CYCLE
365 390    IF (MODE .EQ. 6) YBHB(N)=HBOUT
366      IF (MODE .GE. 7) YBRHB=HBOUT
367 400    ZKJM(1) = ZKNEW
368      YHBJM(1) = HBNEW + (1.-1./RA)*YHB(1)
369      XHBJM(1) = XHB(1)
370      PHJM(1) = PHNEW
371 C
372      DO 420 I = 2,M
373      IM = I-1
374      ZKJM(I) = ZK(IM)
375      YHBJM(I) = YHB(IM)*(1./RA) + YHB(I)*(1.-1./RA)
376      XHBJM(I) = XHB(I)
377 420    PHJM(I) = PH(IM)
378 C
379 C   THIS IS THE STOP STEP
380      DO 480 I = 1,M
381      PH(I) = B*PHJM(I)+(1.-B)*PH(I)
382      ZK(I) = ZK1+((ZK2-ZK1)/(PH2-PH1))*(PH(I)-PH1)
383      E2 = EXP((-E1-1./ZK(I))*FLAM*TDEL)
384      XHB(I) = ((YHBJM(I)+E1*XHBJM(I)-ZKL)/(E1+1./ZK(I)))*
385      C      (1.-E2)+XHBJM(I)*E2
386      IF (XHB(I) .LT. 0.0) XHB(I) = 0.0
387      YHB(I) = YHBJM(I)+E1*(XHBJM(I)-XHB(I))
388 480    CONTINUE
389 500    CONTINUE
390      GO TO 520
391 510    YTHB(N) = YTRHB
392      YBHB(N) = YBRHB

```



```

393 520   CONTINUE
394 540   CONTINUE
395 C
396 C   CALCULATE SEPARATION FACTORS AND WEIGHT PERCENTS
397 C   CALCULATE ALBUMIN RECOVERY
398       DO 850 N = 1,NSTOP
399       SFAL(N) = YTAL(N)/YBAL(N)
400       SFHB(N) = YBHB(N)/YTHB(N)
401       ALPHA(N) = SFAL(N)*SFHB(N)
402       WTAL(N) = 100.*YTAL(N)/(YTAL(N)+YTHB(N))
403       WTHB(N) = 100.*YTHB(N)/(YTAL(N)+YTHB(N))
404       WBAL(N) = 100.*YBAL(N)/(YBAL(N)+YBHB(N))
405       WBHB(N) = 100.*YBHB(N)/(YBAL(N)+YBHB(N))
406       IF (FB .NE. 0.0) GO TO 820
407       IF (FT .NE. 0.0) GO TO 820
408       RECAL(N) = 1.0000
409       RECHB(N) = 1.0000
410       GO TO 850
411 820   RECAL(N) = ((FB+FO)*YTAL(N)+FT*YBAL(N))/(FB+FT)
412       RECHB(N) = ((FB+FO)*YTHB(N)+FT*YBHB(N))/(FB+FT)
413 850   CONTINUE
414 C
415 C   PRINTOUT OF PARAMETRIC PUMPING CONCENTRATIONS
416       WRITE (6,1000) MODE
417 100C  FORMAT('1', 'THIS IS MODE', I3)
418       WRITE (6,1005) FB
419 1005  FORMAT(' ', 'VOLUME OF BOTTOM FEED =', F7.1, ' CC')
420       WRITE (6,1010) FT
421 101C  FORMAT(' ', 'VOLUME OF TOP FEED =', F7.1, ' CC')
422       WRITE (6,1015) FO
423 1015  FORMAT(' ', 'VOLUME OF BUFFER TO STAGE IV =', F7.1, ' CC')
424       WRITE (6,1020) VT
425 102C  FORMAT(' ', 'TOP RESERVOIR DEAD VOLUME =', F7.1, ' CC')
426       WRITE (6,1025) VB
427 1025  FORMAT(' ', 'BOTTOM RESERVOIR DEAD VOLUME =', F7.1, ' CC')
428       WRITE (6,1030) QOTI
429 103C  FORMAT(' ', 'STAGE I DISPLACEMENT =', F7.1, ' CC')
430       WRITE (6,1035) QOTII
431 1035  FORMAT(' ', 'STAGE II DISPLACEMENT =', F7.1, ' CC')
432       WRITE (6,1040) QOTV
433 104C  FORMAT(' ', 'STAGE V DISPLACEMENT =', F7.1, ' CC')
434       WRITE (6,1045) VHB
435 1045  FORMAT(' ', 'HEMOGLOBIN MIGRATION VELOCITY =', F8.3, 2X,
436 C      ' CM/MIN')
437       WRITE (6,1050) VAL
438 105C  FORMAT(' ', 'ALBUMIN MIGRATION VELOCITY =', F8.3, 2X,
439 C      ' CM/MIN')
440       WRITE (6,1055) MO
441 1055  FORMAT(' ', 'NUMBER OF CELLS - NO POWER = ', I5)
442       WRITE (6,1057) FLAMO
443 1057  FORMAT(' ', 'LAMBDA - HIGH FLOW RATE = ', F8.4)
444       IF (FO .EQ. 0.0) GO TO 860
445       WRITE (6,1060) MP
446 106C  FORMAT(' ', 'NUMBER OF CELLS - POWER = ', I5)
447       WRITE (6,1062) FLAMP
448 1062  FORMAT(' ', 'LAMBDA - LOW FLOW RATE = ', F8.4)

```

FORTRAN IV (VER 55) SOURCE LISTING:

```

449 860 WRITE (6,1065) PH1
450 1065 FORMAT(' ',HIGH PH = ',F5.1)
451 WRITE (6,1070) ZK1
452 1070 FORMAT(' ',THE HIGH PH VALUE FOR K* IS ',F6.3)
453 WRITE (6,1075) PH2
454 1075 FORMAT(' ',LOW PH = ',F5.1)
455 WRITE (6,1080) ZK2
456 1080 FORMAT(' ',THE LOW PH VALUE FOR K* IS ',F6.3)
457 WRITE (6,1085) BZERO
458 1085 FORMAT(' ',THE VALUE FOR B WITH ZERO ',
459 C 'ELECTRIC FIELD IS ',F6.3)
460 WRITE (6,1090) BPOW
461 1090 FORMAT(' ',THE VALUE FOR B WITH FINITE ',
462 C 'ELECTRIC FIELD IS ',F6.3)
463 C
464 WRITE (6,1105)
465 1105 FORMAT('0', ' N',6X,'YT(HB)',7X,'YB(HB)',7X,'SF(HB)',
466 C 6X,'REC(HB)')
467 C
468 DO 900 N = 1,NSTOP
469 WRITE (6,1110) N, YTHB(N), YBHB(N), SFHB(N), RECHB(N)
470 1110 FORMAT(2X,I2,4F13.6)
471 900 CONTINUE
472 C
473 WRITE (6,1095)
474 1095 FORMAT('1', ' N',6X,'YT(AL)',7X,'YB(AL)',7X,'SF(AL)',7X,
475 C 'REC(AL)')
476 C
477 DO 910 N = 1,NSTOP
478 WRITE (6,1100) N, YTAL(N), YBAL(N), SFAL(N), RECAL(N)
479 1100 FORMAT(2X,I2,4F13.6)
480 910 CONTINUE
481 C
482 WRITE (6,1115)
483 1115 FORMAT('0', ' N',4X,'WT(AL)',4X,'WT(HB)',4X,'WB(AL)',
484 C 4X,'WB(HB)',4X,'ALPHA')
485 C
486 DO 920 N = 1,NSTOP
487 WRITE (6,1120) N, WTAL(N), WTHB(N), WBAL(N), WBHB(N),
488 C ALPHA(N)
489 1120 FORMAT(2X,I2,5F10.3)
490 920 CONTINUE
491 C
492 980 CONTINUE
493 C
494 985 STOP
495 END

```

```

1 C
2 C          PROGRAM #3 - ALBUMIN SEPARATION
3 C
4 C
5 C PROGRAM WRITTEN BY H. CONNIE HOLLEIN
6 C DOCTOR OF ENGINEERING SCIENCE IN CHEMICAL ENGINEERING
7 C NEW JERSEY INSTITUTE OF TECHNOLOGY, NEWARK, N.J. D7102
8 C
9 C
10 C ALBUMIN CALCULATIONS FOR MODES 6 TO 10
11 C
12 C
13 C     DIMENSION YTAL(60), YTRAL(60), YBAL(60), YBRAL(60),
14 C           SFAL(60), REC(60)
15 C
16 C SET INITIAL CONDITIONS FOR THE RUN
17 C     V = 45.0
18 C     QP = 0.5
19 C     YTRAL(1) = 1.0
20 C     YBRAL(1) = 1.0
21 C     READ (5,2) FO, QOT1, VEB
22 C     FORMAT(F7.1,F7.1,F7.3)
23 C     READ (5,1000) NDATA
24 C     1000 FORMAT(I2)
25 C
26 C     DO 200 NREAD=1,NDATA
27 C     READ (5,10) MODE, FB, FT, VT, VP
28 C     10 FORMAT(I2,F7.1,F7.1,F7.1,F7.1)
29 C     IF (MODE .EQ. 8 .AND. FT .LE. V) QOTV = V
30 C     IF (MODE .EQ. 8 .AND. FT .GT. V) QOTV = FT
31 C     IF (MODE .NE. 8 .AND. FT .LE. 60.0) QOTV = 60.0
32 C     IF (MODE .NE. 8 .AND. FT .GT. 60.0) QOTV = FT
33 C
34 C CALCULATE RETARDATION COEFFICIENT
35 C     VO = QP/3.0
36 C     RB = VO / (VO + VEB)
37 C
38 C REPEAT CALCULATIONS CYCLE-BY-CYCLE TO STEADY STATE
39 C     DO 60 N = 1,25
40 C
41 C CALCULATE TOP CONCENTRATIONS
42 C     NP = N+1
43 C     IF (MODE .GT. 8) GO TO 11
44 C     A = YBRAL(N)
45 C     B = A
46 C     GO TO 15
47 C 11 IF (MODE .GT. 9) GO TO 12
48 C     BRNEW9 = (QOTI+VB)*YBRAL(N)/(QOTI+VB+FO)
49 C     A = BRNEW9
50 C     B = A
51 C     GO TO 15
52 C 12 IF (MODE .GT. 10) GO TO 200
53 C     A = 0.0
54 C     E = YBRAL(N)
55 C 15 IF (FO .GT. RB*(V-FB)) GO TO 20
56 C     YTAL(N) = ((FB+FO/RP)*YTRAL(N))/(FB+FO)

```

```

57      YTRAL(NP) = ((2.*V+VT-QOTI-FB-FO/RB)*YTRAL(N)+FB+
58      C          FO*A+(QOTI-RB*V)*B)/(V+VT)
59      GO TO 30
60 20    YTRAL(N) = (V*YTRAL(N)+FO/RB-V+FB)/(FB+FO)
61      YTRAL(NP) = ((V+VT-QOTI)*YTRAL(N)+V-FO/RB+
62      C          FO*A+(QOTI-RB*V)*B)/(V+VT)
63 30    CONTINUE
64      C
65      C CALCULATE BOTTOM CONCENTRATIONS - ALBUMIN
66      M = MODE - 5
67      GO TO (35, 45, 45, 50, 55), M
68 35    BRNEW6 = (RB*V-FO+VB)*YBRAL(N)/(V+VB)
69      IF (FT .GT. V) GO TO 40
70      YBAL(N) = BRNEW6
71      YBRAL(NP) = ((V+VB-FT)*BRNEW6+FT+(QOTI-V)*YTRAL(NP))/
72      C          (QOTI+VB)
73      GO TO 58
74 40    YBAL(N) = (V*BRNEW6+FT-V)/FT
75      YBRAL(NP) = (VB*BRNEW6+V+(QOTI-V)*YTRAL(NP))/(QCTI+VB)
76      GO TO 58
77 45    YBAL(N) = ((RB*V-FO+QOTV-V)*YBRAL(N))/QOTV
78      YBRAL(NP) = ((V+VB-QOTV)*YBRAL(N)+(QOTV-FT)*YBAL(N)+
79      C          FT+(QOTI-V)*YTRAL(NP))/(QOTI+VB)
80      GO TO 58
81 50    YBAL(N) = (RB*V+QOTV-V)*BRNEW9/QOTV
82      YBRAL(NP) = ((V+VB-QOTV)*BRNEW9+(QOTV-FT)*YBAL(N)+
83      C          FT+(QOTI-V)*YTRAL(NP))/(QOTI+VB)
84      GO TO 58
85 55    YBAL(N) = ((RB*V+QOTV-V)*YBRAL(N))/QOTV
86      YBRAL(NP) = ((V+VB-QOTV)*YBRAL(N)+(QOTV-FT)*YBAL(N)+
87      C          FT+(QOTI-V)*YTRAL(NP))/(QOTI+VB)
88 58    CONTINUE
89      C
90      C CALCULATE SEPARATION FACTORS
91      SFAL(N) = YTRAL(N)/YBAL(N)
92      C
93      C HAS STEADY STATE BEEN REACHED
94      REC(N) = ((FB+FO)*YTRAL(N)+FT*YBAL(N))/(FB+FT)
95 60    CONTINUE
96      C
97      C PRINTOUT OF RESULTS
98      WRITE (6,70) MODE
99 70    FORMAT('1', 'THIS IS MODE', I3)
100     WRITE (6,72) FB
101 72    FORMAT(' ', 'VOLUME OF BOTTOM FEED =', F7.1, ' CC')
102     WRITE (6,74) FT
103 74    FORMAT(' ', 'VOLUME OF TOP FEED =', F7.1, ' CC')
104     WRITE (6,75) FO
105 75    FORMAT(' ', 'VOLUME OF BUFFER TO STAGE IV =', F7.1, ' CC')
106     WRITE (6,76) VT
107 76    FORMAT(' ', 'TOP RESERVOIR DEAD VOLUME =', F7.1, ' CC')
108     WRITE (6,78) VB
109 78    FORMAT(' ', 'BOTTOM RESERVOIR DEAD VOLUME =', F7.1, ' CC')
110     WRITE (6,79) QOTI
111 79    FORMAT(' ', 'STAGE I DISPLACEMENT =', F7.1, ' CC')
112     WRITE (6,80) QOTV

```

```
113 80  FORMAT(' ', 'STAGE V DISPLACEMENT =', F7.1, ' CC')
114      WRITE (6,81) RB
115 81  FORMAT(' ', 'RETARDATION COEFFICIENT =', F10.6)
116      WRITE (6,82)
117 82  FORMAT('0', '  N', 7X, 'YTAL', 9X, 'YBAL', 9X, 'SFAL', 9X, 'REC')
118 C
119      DO 90 N=1,25
120      WRITE (6,85) N, YTAL(N), YBAL(N), SFAL(N), REC(N)
121 85  FORMAT(2X, I2, F13.6, F13.6, F13.6, F13.6)
122 90  CONTINUE
123 C
124 200  CONTINUE
125 C
126      STOP
127      END
```

```

1 C
2 C
3 C      PROGRAM #4 - ALBUMIN OPTIMIZATION
4 C
5 C      PROGRAM WRITTEN BY H. CONNIE HOLLEIN
6 C      DOCTOR OF ENGINEERING SCIENCE IN CHEMICAL ENGINEERING
7 C      NEW JERSEY INSTITUTE OF TECHNOLOGY, NEWARK, N.J. 07102
8 C
9 C      OPTIMIZATION OF MODES 7 AND 8
10 C
11      DIMENSION YTAL(60), YTRAL(60), YBAL(60), YBRAL(60),
12 C          SFAL(60), REC(60)
13 C
14 C      SET INITIAL CONDITIONS
15      V = 45.0
16      QP = 0.5
17      YTRAL(1) = 1.0
18      YBRAL(1) = 1.0
19      COUNT = 0.
20      READ(5,2) MODE, FO, VT, VB, QOTI, VEB, CONST
21 2      FORMAT(I1,F7.1,F7.1,F7.1,F7.1,F7.3,F10.6)
22      READ(5,300) DATA
23 300    FORMAT(F4.0)
24 5      READ(5,10) FEED
25 10     FORMAT(F7.1)
26      FT = FEED/(1.+CONST)
27      IF (FT .GT. VB) GO TO 200
28      FB = CONST*FT
29      COUNT = COUNT + 1.
30      IF (MODE .EQ. 8 .AND. FT .LE. V) QOTV = V
31      IF (MODE .EQ. 8 .AND. FT .GT. V) QOTV = FT
32      IF (MODE .NE. 8 .AND. FT .LE. 60.0) QOTV = 60.0
33      IF (MODE .NE. 8 .AND. FT .GT. 60.0) QOTV = FT
34 C
35 C      CALCULATE RETARDATION COEFFICIENT
36      VO = QP/3.0
37      RB = VO / (VO + VEB)
38 C
39 C      REPEAT CALCULATIONS CYCLE-BY-CYCLE TO STEADY STATE
40 C      CALCULATE TOP CONCENTRATIONS
41 C
42      DO 60 N = 1,50
43      NP = N+1
44      IF (FO .GT. RB*(V - FB)) GO TO 20
45      YTAL(N) = ((FB+FO/RB)*YTRAL(N))/(FB+FO)
46      YTRAL(NP) = ((2.*V+VT-QOTI-FB-FO/RB)*YTRAL(N)+FB+
47 C          (QOTI-RB*V+FO)*YBRAL(N))/(V+VT)
48      GO TO 30
49 20     YTAL(N) = (V*YTRAL(N)+FO/RB-V+FB)/(FB+FO)
50      YTRAL(NP) = ((V+VT-QOTI)*YTRAL(N)+V-FO/RB+
51 C          (QOTI-RB*V+FO)*YBRAL(N))/(V+VT)
52 30     CONTINUE
53 C
54 C      CALCULATE BOTTOM CONCENTRATIONS
55      YBAL(N) = ((RB*V-FO+QOTV-V)*YBRAL(N))/QOTV
56      YBRAL(NP) = ((V+VB-QOTV)*YBRAL(N)+(QOTV-FT)*YBAL(N)+

```

```

57      C          FT+(QOTI-V)*YTRAL(NP))/(QOTI+VB)
58 C
59 C  CALCULATE SEPARAION FACTORS
60      SFAL(N) = YTAL(N)/YBAL(N)
61 C
62 C  HAS STEADY STATE BEEN REACHED
63      REC(N) = ((FB+FO)*YTAL(N)+FT*YBAL(N))/Y(FB+FT)
64 60  CONTINUE
65 C
66 C  PRINTOUT OF RESULTS
67      IF (COUNT .GT. 1.) GO TO 85
68      WRITE (6,70) MODE
69 70  FORMAT('1', 'OPTIMIZATION OF MODE', I3)
70      WRITE (6,74) CONST
71 71  FORMAT(' ', 'FB =', F10.6, ' TIMES FT')
72      WRITE (6,75) FO
73 75  FORMAT(' ', 'VOLUME OF BUFFER TO STAGE IV =', F7.1, ' CC')
74      WRITE (6,76) VT
75 76  FORMAT(' ', 'TOP RESERVOIR DEAD VOLUME =', F7.1, ' CC')
76      WRITE (6,78) VB
77 78  FORMAT(' ', 'BOTTOM RESERVOIR DEAD VOLUME =', F7.1, ' CC')
78      WRITE (6,79) QOTI
79 79  FORMAT(' ', 'STAGE I DISPLACEMENT =', F7.1, ' CC')
80      WRITE (6,81) RB
81 81  FORMAT(' ', 'RETARDATION COEFFICIENT =', F10.6)
82      WRITE (6,83)
83 83  FORMAT('0', ' FT', 8X, 'FB', 9X, 'FEED', 7X, 'YTAL', 9X, 'YBAL',
84      C      9X, 'SFAL', 9X, 'REC')
85 85  WRITE (6,87) FT, FB, FEED, YTAL(50), YBAL(50), SFAL(50)
86      C      ,REC(50)
87 87  FORMAT(F5.1, F10.1, F11.2, F13.6, F13.6, F13.6, F13.6)
88 C
89      IF (COUNT .LT. DATA) GO TO 5
90 C
91 200 CONTINUE
92 C
93      STOP
94      END

```

COMPUTER NOTATION

ALPHA	:	α
B(I)	:	B(i,j)
BJM(I)	:	B(i-1,j-1)
BNEW	:	feed value of B with zero field
BOLD	:	initial value of B with zero field
BPNEW	:	feed value of B with power
BPOLD	:	initial value of B with power
BPOW	:	value of B with power
BRNEW6	:	$[\langle y_{BR}^* \rangle_{n+1}]_{A1}$
BZERO	:	value of B with zero field
CHAN	:	ratio of MO to MP -- must be whole number
C1	:	dummy variable used to switch number of cells from MO to MP and vice-versa
C2	:	ditto
C3	:	ditto
C4	:	ditto
EPS	:	ϵ
E1	:	$(1 - \epsilon) / \epsilon$
E2	:	$\text{EXP} \left(- \lambda \Delta t \left(\frac{1 - \epsilon}{\epsilon} + \frac{1}{k} \right) \right)$
FB	:	F_B , cc
FT	:	F_T , cc
FLAM	:	λ , min^{-1}
FLAMO	:	λ at Q_0 , min^{-1}
FLAMP	:	λ at Q_p , min^{-1}
FO	:	F_0 , cc

HBNEW : feed hemoglobin concentration, dimensionless
HBOLD : initial hemoglobin concentration, dimensionless
HBOUT : outlet hemoglobin concentration, dimensionless
I : i -- cell number
J : j -- transfer-step number
K : Γ -- total number of transfer-steps
L : l -- stage number
M : M -- total number of cells-in-series
MO : M at Q_0
MODE : mode of operation
MP : M at Q_p
N : n -- cycle number
NDATA : number of data points in "DO loop" of "READ" command
NSTOP : N -- total number of cycles
PH(I) : pH(i,j)
PHJM(I) : pH(i-1,j-1)
PHLM(I) : dummy variable used to switch flow direction
PHX(I) : dummy variable used to switch number of cells from
MO to MP and vice-versa
PHNEW : feed value of pH
PHOLD : initial value of pH
PHOUT : outlet value of pH
PH1 : P_1 or high pH
PH2 : P_2 or low pH
PULSE : total volume of feed with a given pH, I.S., and
solute concentration, cc
Q0 : Q_0 , cc/min

QOTI : $Q_o t_I$, cc
 QOTII : $Q_o t_{II}$, cc
 QOTV : $Q_o t_V$, cc
 QP : Q_p , cc/min
 RA : R_{Hb}
 RB : R_{Al}
 RECHB : δ_{Hb} , %
 RECHB(N) : $[\delta_n]_{Hb}$, weight fraction
 RECAL(N) : $[\delta_n]_{Al}$, weight fraction
 SFAL(N) : $[\langle SF \rangle_n]_{Al}$
 SFHB(N) : $[\langle SF \rangle_n]_{Hb}$
 TDEL : Δt , min
 V : V , cc
 VAL : value of $\nu_{E,Al}$ in Stage IV, cm/min
 VAVG(J) : $\frac{1}{2} (VOUT(J) + VOUT(J-1))$
 VB : V_B , cc
 VDEL : ΔV , cc
 VEA : value of $\nu_{E,Hb}$ in any stage, cm/min
 VEB : value of $\nu_{E,Al}$ in any stage, cm/min
 VHB : value of $\nu_{E,Hb}$ in Stage IV, cm/min
 VINI : initial volume of outlet fluid for transfer-step "j"
 VMASS : volume times concentration of outlet fluid
 VO : ν_o , cm/min

VOL : total outlet fluid volume, cc
 VOUT(J) : ditto
 VT : V_T , cc
 WBAL(N) : $[\langle w_B \rangle_n]_{A1}$
 WTAL(N) : $[\langle w_T \rangle_n]_{A1}$
 WBHB(N) : $[\langle w_B \rangle_n]_{Hb}$
 WTHB(N) : $[\langle w_T \rangle_n]_{Hb}$
 XHB(I) : $x(i, j)$
 XHBJM(I) : $x(i, j-1)$
 XHBLM(I) : dummy variable used to switch flow direction
 XHBX(I) : dummy variable used to switch number of cells from
 MO to MP and vice-versa.
 YHB(I) : $y(i, j)$
 YHBJM(I) : $y^*(i-1, j-1)$
 YHBLM(I) : dummy variable used to switch flow direction
 YHBX(I) : dummy variable used to switch number of cells from
 MO to MP and vice-versa
 YBAL(N) : $[\langle y_B \rangle_n]_{A1}$
 YBRAL(N) : $[\langle y_{BR} \rangle_n]_{A1}$
 YTAL(N) : $[\langle y_T \rangle_n]_{A1}$
 YTRAL(N) : $[\langle y_{TR} \rangle_n]_{A1}$
 YBHB(N) : $[\langle y_B \rangle_n]_{Hb}$
 YBRHB(N) : $[\langle y_{BR} \rangle_n]_{Hb}$
 YTHB(N) : $[\langle y_T \rangle_n]_{Hb}$
 YTRHB(N) : $[\langle y_{TR} \rangle_n]_{Hb}$

ZK(I) : $k(i,j)$
ZKJM(I) : $k(i-1,j-1)$
ZKLM(I) : dummy variable used to switch flow direction
ZKX(I) : dummy variable used to switch number of cells from
MO to MP and vice-versa
ZKL : b -- constant in Equation 6-3
ZKNEW : k_{pH} at feed pH
ZKOLD : k_{pH} at initial pH
ZK1 : k_{pH} at $pH = P_1$
ZK2 : k_{pH} at $pH = P_2$

RUN NO. 5-A

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HR)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.100000
23	49.285644	48.214202	5.999980	0.100000
24	51.428497	50.357055	5.999984	0.100000
25	53.571350	52.499908	6.000005	0.100000
26	55.714202	54.642761	6.000076	0.100000
27	57.857055	56.785614	6.000278	0.100000
28	59.999908	58.928466	6.000774	0.100000
29	62.142761	61.071319	6.001857	0.100000
30	64.285614	63.214172	6.003994	0.100001
31	66.428466	65.357025	6.007877	0.100004
32	68.571319	67.499877	6.014448	0.100013
33	70.714172	69.642730	6.024893	0.100040
34	72.857025	71.785583	6.040604	0.100114
35	74.999877	73.928436	6.063097	0.100312
36	77.142730	76.071289	6.093906	0.100824
37	79.285583	78.214141	6.134452	0.102094
38	81.428436	80.356994	6.185905	0.105117
39	83.571289	82.499847	6.249063	0.112007
40	85.714141	84.642700	6.324265	0.126975
41	87.856994	86.785552	6.411325	0.157812
42	89.999847	88.928405	6.509530	0.217719
43	92.142700	91.071258	6.617667	0.326730
44	94.285552	93.214111	6.734101	0.511106
45	96.428405	95.356964	6.856874	0.798371
46	98.571258	97.499816	6.983820	1.206261
47	100.714110	99.642669	7.112697	1.726763
48	102.856960	101.785520	7.241296	2.311867
49	104.999810	103.928370	7.367552	2.872038

RUN NO. 5-A (cont.)

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HR)
50	107.142660	106.071220	7.519141	3.375237
51	109.285520	108.214080	7.660729	3.571247
52	111.428370	110.356930	7.790316	3.304240
53	113.571220	112.499780	7.906612	2.600473
54	115.714080	114.642630	8.008879	1.699033
55	117.856930	116.785490	8.096702	0.898039
56	119.999780	118.928340	8.169740	0.369452
57	122.142630	121.071190	8.227497	0.104482
58	124.285490	123.214050	8.269176	0.001460
59	126.428340	125.356900	8.293654	0.000000
60	128.571190	127.499750	8.299586	0.000000
61	130.714050	129.642600	8.285625	0.000000
62	132.856900	131.785460	8.250703	0.000000
63	134.999750	133.928310	8.194316	0.000000
64	137.142600	136.071160	8.116756	0.000000
65	139.285460	138.214010	8.019253	0.000000
66	141.428310	140.356870	7.903972	0.000000
67	143.571160	142.499720	7.773919	0.000000
68	145.714010	144.642570	7.632734	0.000000
69	147.856870	146.785430	7.484426	0.000000
70	149.999720	148.928280	7.333085	0.000000
71	152.142570	151.071130	7.182621	0.000000
72	154.285430	153.213980	7.036534	0.000000
73	156.428280	155.356840	6.897757	0.000000
74	158.571130	157.499690	6.768552	0.000000
75	160.713980	159.642540	6.650498	0.000000
76	162.856840	161.785400	6.544508	0.000000
77	164.999690	163.928250	6.450903	0.000000
78	167.142540	166.071100	6.369503	0.000000
79	169.285400	168.213950	6.299742	0.000000
80	171.428250	170.356810	6.240775	0.000000
81	173.571100	172.499660	6.191579	0.000000
82	175.713950	174.642510	6.151039	0.000000
83	177.856810	176.785360	6.118024	0.000000
84	179.999660	178.928220	6.091434	0.000000

2TN 14 7/8 X 11

22"

MOORE BUSINESS FORMS, INC. HO

RUN NO. 5-B

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 1.000

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HR)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.100008
23	49.285644	48.214202	5.999980	0.100086
24	51.428497	50.357055	5.999984	0.100486
25	53.571350	52.499908	6.000005	0.101913
26	55.714202	54.642761	6.000076	0.105899
27	57.857055	56.785614	6.000278	0.115171
28	59.999908	58.928466	6.000774	0.133838
29	62.142761	61.071319	6.001857	0.167229
30	64.285614	63.214172	6.003994	0.221278
31	66.428466	65.357025	6.007877	0.301515
32	68.571319	67.499877	6.014448	0.411810
33	70.714172	69.642730	6.024893	0.553134
34	72.857025	71.785583	6.040604	0.722616
35	74.999877	73.928436	6.063097	0.913138
36	77.142730	76.071289	6.093906	1.113667
37	79.285583	78.214141	6.134452	1.310392
38	81.428436	80.356994	6.185905	1.488575
39	83.571289	82.499847	6.249063	1.634842
40	85.714141	84.642700	6.324265	1.739447
41	87.856994	86.785552	6.411325	1.797916
42	89.999847	88.928405	6.509530	1.811626
43	92.142700	91.071258	6.617667	1.787079
44	94.285552	93.214111	6.734101	1.734142
45	96.428405	95.356964	6.856874	1.663746
46	98.571258	97.499816	6.983820	1.585838
47	100.714110	99.642669	7.112697	1.508034
48	102.856960	101.785520	7.241296	1.435197
49	104.999810	103.928370	7.367552	1.369778

RUN NO. 5-B (cont.)

TIME	V OUT.CC	V AVG.CC	PH OUT	V(HR)
50	107.142660	106.071220	7.519141	1.086437
51	109.285520	108.214080	7.660729	0.617875
52	111.428370	110.356930	7.790316	0.232617
53	113.571220	112.499780	7.906612	0.034454
54	115.714080	114.642630	8.008879	0.000000
55	117.856930	116.785490	8.096702	0.000000
56	119.999780	118.928340	8.169740	0.000000
57	122.142630	121.071190	8.227497	0.000000
58	124.285490	123.214050	8.269176	0.000000
59	126.428340	125.356900	8.293654	0.000000
60	128.571190	127.499750	8.299586	0.000000
61	130.714050	129.642600	8.285625	0.000000
62	132.856900	131.785460	8.250703	0.000000
63	134.999750	133.928310	8.194316	0.000000
64	137.142600	136.071160	8.116756	0.000000
65	139.285460	138.214010	8.019253	0.000000
66	141.428310	140.356870	7.903972	0.000000
67	143.571160	142.499720	7.773919	0.000000
68	145.714010	144.642570	7.632734	0.000000
69	147.856870	146.785430	7.484426	0.000000
70	149.999720	148.928280	7.333085	0.000000
71	152.142570	151.071130	7.182621	0.000000
72	154.285430	153.213980	7.036534	0.000000
73	156.428280	155.356840	6.897757	0.000000
74	158.571130	157.499690	6.768552	0.000000
75	160.713980	159.642540	6.650498	0.000000
76	162.856840	161.785400	6.544508	0.000000
77	164.999690	163.928250	6.450903	0.000000
78	167.142540	166.071100	6.369503	0.000000
79	169.285400	168.213950	6.299742	0.000000
80	171.428250	170.356810	6.240775	0.000000
81	173.571100	172.499660	6.191579	0.000000
82	175.713950	174.642510	6.151039	0.000000
83	177.856810	176.785360	6.118024	0.000000
84	179.999660	178.928220	6.091434	0.000000

27 IN 14 /IR X 11

27"

MOORE BUSINESS FORMS, INC. 110

RUN NO. 5-C

SIMULATION OF PH AND CONCENTRATION WAVES
 HEMOGLOBIN RECOVERY = 100.0%
 HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN
 NUMBER OF STAGES = 21
 LAMBDA - LOW FLOW RATE = 2.500000
 INITIAL PH = 6.0
 THE INITIAL CONCENTRATION IS 0.000
 THE INITIAL VALUE FOR K* IS 10.000
 THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493
 FEED PH = 8.5
 THE FEED CONCENTRATION IS 1.000
 THE FEED VALUE FOR K* IS 0.010
 THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 VOLUME OF PULSE = 60.0 CC

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HR)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.100000
23	49.285644	48.214202	5.999980	0.100000
24	51.428497	50.357055	5.999984	0.100000
25	53.571350	52.499908	6.000005	0.100000
26	55.714202	54.642761	6.000076	0.100000
27	57.857055	56.785614	6.000278	0.100000
28	59.999908	58.928466	6.000774	0.100000
29	62.142761	61.071319	6.001857	0.100000
30	64.285614	63.214172	6.003994	0.100000
31	66.428466	65.357025	6.007877	0.100000
32	68.571319	67.499877	6.014448	0.100000
33	70.714172	69.642730	6.024893	0.100000
34	72.857025	71.785583	6.040604	0.100000
35	74.999877	73.928436	6.063097	0.100000
36	77.142730	76.071289	6.093906	0.100000
37	79.285583	78.214141	6.134452	0.100000
38	81.428436	80.356994	6.185905	0.100000
39	83.571289	82.499847	6.249063	0.100001
40	85.714141	84.642700	6.324265	0.100004
41	87.856994	86.785552	6.411325	0.100017
42	89.999847	88.928405	6.509530	0.100066
43	92.142700	91.071258	6.617667	0.100242
44	94.285552	93.214111	6.734101	0.100835
45	96.428405	95.356964	6.856874	0.102719
46	98.571258	97.499816	6.983820	0.108287
47	100.714110	99.642669	7.112697	0.123514
48	102.856960	101.785520	7.241296	0.161732
49	104.999810	103.928370	7.367552	0.249095

212 14

RUN NO. 5-C (cont.)

<u>TIME</u>	<u>V OUT. CC</u>	<u>V AVG. CC</u>	<u>PH OUT</u>	<u>Y (HR)</u>
50	107.142660	106.071220	7.519141	0.447419
51	109.285520	108.214080	7.660729	0.847387
52	111.428370	110.356930	7.790316	1.518178
53	113.571220	112.499780	7.906612	2.465874
54	115.714080	114.642630	8.008879	3.477253
55	117.856930	116.785490	8.096702	4.153942
56	119.999780	118.928340	8.169740	4.131882
57	122.142630	121.071190	8.227497	3.384584
58	124.285490	123.214050	8.269176	2.278894
59	126.428340	125.356900	8.293654	1.278023
60	128.571190	127.499750	8.299586	0.611249
61	130.714050	129.642600	8.285625	0.258145
62	132.856900	131.785460	8.250703	0.103111
63	134.999750	133.928310	8.194316	0.002410
64	137.142600	136.071160	8.116756	0.000000
65	139.285460	138.214010	8.019253	0.000000
66	141.428310	140.356870	7.903972	0.000000
67	143.571160	142.499720	7.773919	0.000000
68	145.714010	144.642570	7.632734	0.000000
69	147.856870	146.785430	7.484426	0.000000
70	149.999720	148.928280	7.333085	0.000000
71	152.142570	151.071130	7.182621	0.000000
72	154.285430	153.213980	7.036534	0.000000
73	156.428280	155.356840	6.897757	0.000000
74	158.571130	157.499690	6.768552	0.000000
75	160.713980	159.642540	6.650498	0.000000
76	162.856840	161.785400	6.544508	0.000000
77	164.999690	163.928250	6.450903	0.000000
78	167.142540	166.071100	6.369503	0.000000
79	169.285400	168.213950	6.299742	0.000000
80	171.428250	170.356810	6.240775	0.000000
81	173.571100	172.499660	6.191579	0.000000
82	175.713950	174.642510	6.151039	0.000000
83	177.856810	176.785360	6.118024	0.000000
84	179.999660	178.928220	6.091434	0.000000

RUN NO. 5-D

SIMULATION OF PH AND CONCENTRATION WAVES
 HEMOGLOBIN RECOVERY = 100.0%
 HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN
 NUMBER OF STAGES = 180
 LAMBDA - LOW FLOW RATE = 2.500000
 INITIAL PH = 6.0
 THE INITIAL CONCENTRATION IS 0.000
 THE INITIAL VALUE FOR K* IS 3.800
 THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493
 FEED PH = 8.5
 THE FEED CONCENTRATION IS 1.000
 THE FEED VALUE FOR K* IS 0.010
 THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 VOLUME OF PULSE = 60.0 CC

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HR)
181	45.250000	45.125000	5.999828	0.100000
191	47.750000	47.625000	5.999819	0.100000
201	50.250000	50.125000	5.999809	0.100000
211	52.750000	52.625000	5.999800	0.100000
221	55.250000	55.125000	5.999790	0.100000
231	57.750000	57.625000	5.999781	0.100000
241	60.250000	60.125000	5.999771	0.100000
251	62.750000	62.625000	5.999762	0.100000
261	65.250000	65.125000	5.999752	0.100000
271	67.750000	67.625000	5.999743	0.100000
281	70.250000	70.125000	5.999733	0.100000
291	72.750000	72.625000	5.999723	0.100000
301	75.250000	75.125000	5.999714	0.100000
311	77.750000	77.625000	5.999704	0.100000
321	80.250000	80.125000	5.999703	0.100000
331	82.750000	82.625000	5.999789	0.100000
341	85.250000	85.125000	6.000509	0.100000
351	87.750000	87.625000	6.004516	0.100000
361	90.250000	90.125000	6.020855	0.100000
371	92.750000	92.625000	6.071238	0.100033
381	95.250000	95.125000	6.191358	0.101531
391	97.750000	97.625000	6.417100	0.153020
401	100.250000	100.125000	6.757172	1.152287
411	102.750000	102.625000	7.174090	7.848581
421	105.250000	105.125000	7.605859	11.197748
431	107.750000	107.625000	8.032809	1.532074
441	110.250000	110.125000	8.297375	0.000000
451	112.750000	112.625000	8.426774	0.000000
461	115.250000	115.125000	8.477677	0.000000
471	117.750000	117.625000	8.494052	0.000000
481	120.250000	120.125000	8.498423	0.000000
491	122.750000	122.625000	8.499404	0.000000
501	125.250000	125.125000	8.499594	0.000000
511	127.750000	127.625000	8.499626	0.000000
521	130.250000	130.125000	8.499626	0.000000

RUN NO. 5-D (cont.)

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HB)
388	97.000000	96.875000	6.336948	0.119126
389	97.250000	97.125000	6.362466	0.126999
390	97.500000	97.375000	6.389185	0.137932
391	97.750000	97.625000	6.417100	0.153020
392	98.000000	97.875000	6.446199	0.173710
393	98.250000	98.125000	6.476469	0.201883
394	98.500000	98.375000	6.507888	0.239964
395	98.750000	98.625000	6.540430	0.291034
396	99.000000	98.875000	6.574064	0.358945
397	99.250000	99.125000	6.608756	0.448442
398	99.500000	99.375000	6.644461	0.565252
399	99.750000	99.625000	6.681133	0.716143
400	100.000000	99.875000	6.718722	0.908914
401	100.250000	100.125000	6.757172	1.152287
402	100.500000	100.375000	6.796422	1.455651
403	100.750000	100.625000	6.836408	1.828641
404	101.000000	100.875000	6.877063	2.280487
405	101.250000	101.125000	6.918314	2.819145
406	101.500000	101.375000	6.960090	3.450180
407	101.750000	101.625000	7.002313	4.175461
408	102.000000	101.875000	7.044904	4.991704
409	102.250000	102.125000	7.087786	5.889093
410	102.500000	102.375000	7.130875	6.850056
411	102.750000	102.625000	7.174090	7.848581
412	103.000000	102.875000	7.217351	8.850120
413	103.250000	103.125000	7.260575	9.812622
414	103.500000	103.375000	7.303683	10.688615
415	103.750000	103.625000	7.346594	11.428417
416	104.000000	103.875000	7.389230	11.984411
417	104.250000	104.125000	7.431515	12.316067
418	104.500000	104.375000	7.473376	12.394672
419	104.750000	104.625000	7.514743	12.207714
420	105.000000	104.875000	7.555548	11.761518
421	105.250000	105.125000	7.605859	11.197748
422	105.500000	105.375000	7.655038	10.414418
423	105.750000	105.625000	7.702980	9.458054
424	106.000000	105.875000	7.749589	8.387153
425	106.250000	106.125000	7.794779	7.262467
426	106.500000	106.375000	7.838478	6.137043
427	106.750000	106.625000	7.880623	5.049821
428	107.000000	106.875000	7.921163	4.026464
429	107.250000	107.125000	7.960058	3.085817
430	107.500000	107.375000	7.997279	2.247036
431	107.750000	107.625000	8.032809	1.532074
432	108.000000	107.875000	8.066638	0.958281
433	108.250000	108.125000	8.098768	0.534088
434	108.500000	108.375000	8.129209	0.251149
435	108.750000	108.625000	8.157978	0.081155
436	109.000000	108.875000	8.185102	0.000000
437	109.250000	109.125000	8.210612	0.000000
438	109.500000	109.375000	8.234548	0.000000

RUN NO. 5-E

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 9

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HB)
1	5.000000	2.500000	6.000000	0.000000
2	10.000000	7.500000	5.999999	0.000000
3	15.000000	12.500000	5.999998	0.000000
4	20.000000	17.500000	5.999997	0.000000
5	25.000000	22.500000	5.999996	0.000000
6	30.000000	27.500000	5.999995	0.000000
7	35.000000	32.500000	5.999994	0.000000
8	40.000000	37.500000	5.999993	0.000000
9	45.000000	42.500000	5.999992	0.000000
10	50.000000	47.500000	6.001414	0.100008
11	55.000000	52.500000	6.008638	0.100080
12	60.000000	57.500000	6.029012	0.100466
13	65.000000	62.500000	6.071146	0.102035
14	70.000000	67.500000	6.142437	0.107344
15	75.000000	72.500000	6.246979	0.122865
16	80.000000	77.500000	6.384556	0.162576
17	85.000000	82.500000	6.550828	0.251529
18	90.000000	87.500000	6.738382	0.424775
19	95.000000	92.500000	6.938191	0.714469
20	100.000000	97.500000	7.141036	1.122568
21	105.000000	102.500000	7.338645	1.592506
22	110.000000	107.500000	7.562443	1.985257
23	115.000000	112.500000	7.743052	1.954533
24	120.000000	117.500000	7.862567	1.484304
25	125.000000	122.500000	7.908580	0.894961
26	130.000000	127.500000	7.878054	0.460738
27	135.000000	132.500000	7.778046	0.218332
28	140.000000	137.500000	7.623468	0.100307
29	145.000000	142.500000	7.433306	0.000317
30	150.000000	147.500000	7.226808	0.000000
31	155.000000	152.500000	7.020632	0.000000
32	160.000000	157.500000	6.827299	0.000000
33	165.000000	162.500000	6.654816	0.000000
34	170.000000	167.500000	6.507125	0.000000
35	175.000000	172.500000	6.384982	0.000000
36	180.000000	177.500000	6.286950	0.000000

RUN NO. 5-F

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 1.000

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 1.000

VOLUME OF PULSE = 60.0 CC

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HR)
21	44.999938	43.928497	6.000000	0.000000
22	47.142791	46.071350	8.500000	0.866100
23	49.285644	48.214202	8.500000	0.989032
24	51.428497	50.357055	8.500000	0.999365
25	53.571350	52.499908	8.500000	0.999971
26	55.714202	54.642761	8.500000	0.999999
27	57.857055	56.785614	8.500000	1.000000
28	59.999908	58.928466	8.500000	1.000000
29	62.142761	61.071319	8.500000	1.000000
30	64.285614	63.214172	8.500000	1.000000
31	66.428466	65.357025	8.500000	1.000000
32	68.571319	67.499877	8.500000	1.000000
33	70.714172	69.642730	8.500000	1.000000
34	72.857025	71.785583	8.500000	1.000000
35	74.999877	73.928436	8.500000	1.000000
36	77.142730	76.071289	8.500000	1.000000
37	79.285583	78.214141	8.500000	1.000000
38	81.428436	80.356994	8.500000	1.000000
39	83.571289	82.499847	8.500000	1.000000
40	85.714141	84.642700	8.500000	1.000000
41	87.856994	86.785552	8.500000	1.000000
42	89.999847	88.928405	8.500000	1.000000
43	92.142700	91.071258	8.500000	1.000000
44	94.285552	93.214111	8.500000	1.000000
45	96.428405	95.356964	8.500000	1.000000
46	98.571258	97.499816	8.500000	1.000000
47	100.714110	99.642669	8.500000	1.000000
48	102.856960	101.785520	8.500000	1.000000
49	104.999810	103.928370	8.500000	1.000000

NOTE: This run shows that the pH velocity must be included!

RUN NO. 5-F (cont.)

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HB)
50	107.142660	106.071220	6.000000	0.102368
51	109.285520	108.214080	6.000000	0.043162
52	111.428370	110.356930	6.000000	0.000000
53	113.571220	112.499780	6.000000	0.000000
54	115.714080	114.642630	6.000000	0.000000
55	117.856930	116.785490	6.000000	0.000000
56	119.999780	118.928340	6.000000	0.000000
57	122.142630	121.071190	6.000000	0.000000
58	124.285490	123.214050	6.000000	0.000000
59	126.428340	125.356900	6.000000	0.000000
60	128.571190	127.499750	6.000000	0.000000
61	130.714050	129.642600	6.000000	0.000000
62	132.856900	131.785460	6.000000	0.000000
63	134.999750	133.928310	6.000000	0.000000
64	137.142600	136.071160	6.000000	0.000000
65	139.285460	138.214010	6.000000	0.000000
66	141.428310	140.356870	6.000000	0.000000
67	143.571160	142.499720	6.000000	0.000000
68	145.714010	144.642570	6.000000	0.000000
69	147.856870	146.785430	6.000000	0.000000
70	149.999720	148.928280	6.000000	0.000000
71	152.142570	151.071130	6.000000	0.000000
72	154.285430	153.213980	6.000000	0.000000
73	156.428280	155.356840	6.000000	0.000000
74	158.571130	157.499690	6.000000	0.000000
75	160.713980	159.642540	6.000000	0.000000
76	162.856840	161.785400	6.000000	0.000000
77	164.999690	163.928250	6.000000	0.000000
78	167.142540	166.071100	6.000000	0.000000
79	169.285400	168.213950	6.000000	0.000000
80	171.428250	170.356810	6.000000	0.000000
81	173.571100	172.499660	6.000000	0.000000
82	175.713950	174.642510	6.000000	0.000000
83	177.856810	176.785360	6.000000	0.000000
84	179.999660	178.928220	6.000000	0.000000

RUN NO. 5-G

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 1.300000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TIME	V OUT, CC	V AVG, CC	PH OUT	Y(HB)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.100000
23	49.285644	48.214202	5.999980	0.100000
24	51.428497	50.357055	5.999984	0.100000
25	53.571350	52.499908	6.000005	0.100000
26	55.714202	54.642761	6.000076	0.100000
27	57.857055	56.785614	6.000278	0.100000
28	59.999908	58.928466	6.000774	0.100000
29	62.142761	61.071319	6.001857	0.100000
30	64.285614	63.214172	6.003994	0.100001
31	66.428466	65.357025	6.007877	0.100005
32	68.571319	67.499877	6.014448	0.100014
33	70.714172	69.642730	6.024893	0.100042
34	72.857025	71.785583	6.040604	0.100120
35	74.999877	73.928436	6.063097	0.100327
36	77.142730	76.071289	6.093906	0.100857
37	79.285583	78.214141	6.134452	0.102164
38	81.428436	80.356994	6.185905	0.105258
39	83.571289	82.499847	6.249063	0.112272
40	85.714141	84.642700	6.324265	0.127437
41	87.856994	86.785552	6.411325	0.158555
42	89.999847	88.928405	6.509530	0.218806
43	92.142700	91.071258	6.617667	0.328149
44	94.285552	93.214111	6.734101	0.512713
45	96.428405	95.356964	6.856874	0.799866
46	98.571258	97.499816	6.983820	1.207253
47	100.714110	99.642669	7.112697	1.726921
48	102.856960	101.785520	7.241296	2.311089
49	104.999810	103.928370	7.367552	2.870546
50	107.142660	106.071220	7.519141	3.373413
51	109.285520	108.214080	7.660729	3.569565
52	111.428370	110.356930	7.790316	3.303002
53	113.571220	112.499780	7.906612	2.599711
54	115.714080	114.642630	8.008879	1.698624
55	117.856930	116.785490	8.096702	0.897843
56	119.999780	118.928340	8.169740	0.369369
57	122.142630	121.071190	8.227497	0.104457
58	124.285490	123.214050	8.269176	0.001452
59	126.428340	125.356900	8.293654	0.000000

RUN NO. 5-G (cont.)

TIME	V OUT, CC	V AVG, CC	PH OUT	Y (HB)
60	128.571190	127.499750	8.299586	0.000000
61	130.714050	129.642600	8.285625	0.000000
62	132.856900	131.785460	8.250703	0.000000
63	134.999750	133.928310	8.194316	0.000000
64	137.142600	136.071160	8.116756	0.000000
65	139.285460	138.214010	8.019253	0.000000
66	141.428310	140.356870	7.903972	0.000000
67	143.571160	142.499720	7.773919	0.000000
68	145.714010	144.642570	7.632734	0.000000
69	147.856870	146.785430	7.484426	0.000000
70	149.999720	148.928280	7.333085	0.000000
71	152.142570	151.071130	7.182621	0.000000
72	154.285430	153.213980	7.036534	0.000000
73	156.428280	155.356840	6.897757	0.000000
74	158.571130	157.499690	6.768552	0.000000
75	160.713980	159.642540	6.650498	0.000000
76	162.856840	161.785400	6.544508	0.000000
77	164.999690	163.928250	6.450903	0.000000
78	167.142540	166.071100	6.369503	0.000000
79	169.285400	168.213950	6.299747	0.000000
80	171.428250	170.356810	6.240775	0.000000
81	173.571100	172.499660	6.191579	0.000000
82	175.713950	174.642510	6.151039	0.000000
83	177.856810	176.785360	6.118024	0.000000
84	179.999660	178.928220	6.091434	0.000000

RUN NO. 5-H

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 0.100000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TYPE	V OUT, CC	V AVG, CC	PH OUT	Y (HB)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.101367
23	49.285644	48.214232	5.999980	0.102497
24	51.428497	50.357055	5.999984	0.104238
25	53.571350	52.499908	6.000005	0.106714
26	55.714202	54.642761	6.000076	0.110195
27	57.857055	56.785614	6.000278	0.114875
28	59.999908	58.928466	6.000774	0.121101
29	62.142761	61.071319	6.001857	0.129259
30	64.285614	63.214172	6.003994	0.139820
31	66.428466	65.357025	6.007877	0.153356
32	68.571319	67.499877	6.014448	0.170552
33	70.714172	69.642730	6.024893	0.192220
34	72.857025	71.785583	6.040604	0.219320
35	74.999877	73.928436	6.063097	0.252966
36	77.142730	76.071289	6.093906	0.294435
37	79.285583	78.214141	6.134452	0.345159
38	81.428436	80.356994	6.185905	0.406702
39	83.571289	82.499847	6.249063	0.480712
40	85.714141	84.642700	6.324265	0.568839
41	87.856994	86.785552	6.411325	0.672614
42	89.999347	88.928405	6.509530	0.793256
43	92.142700	91.071258	6.617667	0.931436
44	94.285552	93.214111	6.734101	1.086962
45	96.428405	95.356964	6.856874	1.258394
46	98.571258	97.499816	6.983820	1.442627
47	100.714110	99.642669	7.112697	1.634480
48	102.856960	101.785520	7.241296	1.826382
49	104.999810	103.928370	7.367552	2.008271
50	107.142660	106.071220	7.519141	2.127068
51	109.285520	108.214080	7.660729	2.139447
52	111.428370	110.356930	7.790316	2.038517
53	113.571220	112.499780	7.906612	1.822526
54	115.714080	114.642630	8.008879	1.506570
55	117.856930	116.785490	8.096702	1.129875
56	119.999780	118.928340	8.169740	0.750704
57	122.142630	121.071190	8.227497	0.429641
58	124.285490	123.214050	8.269176	0.202213
59	126.428340	125.356900	8.293654	0.071738

RUN NO. 5-H (cont.)

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HB)
60	128.571190	127.499750	8.299586	0.012808
61	130.714050	129.642600	8.285625	0.000000
62	132.856900	131.785460	8.250703	0.000000
63	134.999750	133.928310	8.194316	0.000000
64	137.142600	136.071160	8.116756	0.000000
65	139.285460	138.214010	8.019253	0.000000
66	141.428310	140.356870	7.903972	0.000000
67	143.571160	142.499720	7.773919	0.000000
68	145.714010	144.642570	7.632734	0.000000
69	147.856870	146.785430	7.484426	0.000000
70	149.999720	148.928280	7.333085	0.000000
71	152.142570	151.071130	7.182621	0.000000
72	154.285430	153.213980	7.036534	0.000000
73	156.428280	155.356840	6.897757	0.000000
74	158.571130	157.499690	6.768552	0.000000
75	160.713980	159.642540	6.650498	0.000000
76	162.856840	161.785400	6.544508	0.000000
77	164.999690	163.928250	6.450903	0.000000
78	167.142540	166.071100	6.369503	0.000000
79	169.285400	168.213950	6.299742	0.000000
80	171.428250	170.356810	6.240775	0.000000
81	173.571100	172.499660	6.191579	0.000000
82	175.713950	174.642510	6.151039	0.000000
83	177.856810	176.785360	6.118024	0.000000
84	179.999660	178.928220	6.091434	0.000000

RUN NO. 5-I

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 0.010000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TIME	V OUT, CC	V AVG, CC	PH OUT	Y(HB)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.551974
23	49.285644	48.214212	5.999980	0.556195
24	51.428497	50.357055	5.999984	0.561710
25	53.571350	52.499908	6.000005	0.569606
26	55.714202	54.642761	6.000076	0.581325
27	57.857055	56.785614	6.000278	0.598021
28	59.999908	58.928466	6.000774	0.619031
29	62.142761	61.071319	6.001857	0.641046
30	64.285614	63.214172	6.003994	0.661474
31	66.428466	65.357025	6.007877	0.682255
32	68.571319	67.499877	6.014448	0.705112
33	70.714172	69.642730	6.024893	0.728472
34	72.857025	71.785583	6.040604	0.752170
35	74.999877	73.928436	6.063097	0.776823
36	77.142730	76.071289	6.093906	0.802040
37	79.285583	78.214141	6.134452	0.827843
38	81.428436	80.356994	6.185905	0.854297
39	83.571289	82.499847	6.249063	0.881273
40	85.714141	84.642700	6.324265	0.908794
41	87.856994	86.785552	6.411325	0.936795
42	89.999847	88.928405	6.509530	0.965225
43	92.142700	91.071258	6.617667	0.994018
44	94.285552	93.214111	6.734101	1.023091
45	96.428405	95.356964	6.856874	1.052354
46	98.571258	97.499816	6.983820	1.081677
47	100.714110	99.642669	7.112697	1.110921
48	102.856960	101.785520	7.241296	1.139908
49	104.999810	103.928370	7.367552	1.168427
50	107.142660	106.071220	7.519141	0.551484
51	109.285520	108.214080	7.660729	0.508648
52	111.428370	110.356930	7.790316	0.468543
53	113.571220	112.499780	7.906612	0.440392
54	115.714080	114.642630	8.008879	0.409768
55	117.856930	116.785490	8.096702	0.376648
56	119.999780	118.928340	8.169740	0.340225
57	122.142630	121.071190	8.227497	0.298406
58	124.285490	123.214050	8.269176	0.254741
59	126.428340	125.356900	8.293654	0.210749

RUN NO. 5-I (cont.)

TIME	V OUT, CC	V AVG, CC	PH OUT	Y (HB)
60	128.571190	127.499750	8.299586	0.168912
61	130.714050	129.642600	8.285625	0.131677
62	132.856900	131.785460	8.250703	0.101800
63	134.999750	133.928310	8.194316	0.081071
64	137.142600	136.071160	8.116756	0.066608
65	139.285460	138.214010	8.019253	0.056643
66	141.428310	140.356870	7.903972	0.049424
67	143.571160	142.499720	7.773919	0.042352
68	145.714010	144.642570	7.632734	0.038836
69	147.856870	146.785430	7.484426	0.036240
70	149.999720	148.928280	7.333085	0.034277
71	152.142570	151.071130	7.182621	0.032761
72	154.285430	153.213980	7.036534	0.030841
73	156.428280	155.356840	6.897757	0.028040
74	158.571130	157.499690	6.768552	0.027269
75	160.713980	159.642540	6.650498	0.026625
76	162.856840	161.785400	6.544508	0.026078
77	164.999690	163.928250	6.450903	0.025604
78	167.142540	166.071100	6.369503	0.024379
79	169.285400	168.213950	6.299742	0.022169
80	171.428250	170.356810	6.240775	0.021853
81	173.571100	172.499660	6.191579	0.021562
82	175.713950	174.642510	6.151039	0.021292
83	177.856810	176.785360	6.118024	0.021037
84	179.999660	178.928220	6.091434	0.019504

RUN NO. 5-J

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 0.001000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TIME	V OUT, CC	V AVG, CC	PH OUT	Y (HR)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.939783
23	49.285644	48.214202	5.999980	0.939862
24	51.428497	50.357055	5.999984	0.939967
25	53.571350	52.499908	6.000005	0.940125
26	55.714202	54.642761	6.000076	0.940379
27	57.857055	56.785614	6.000278	0.940805
28	59.999908	58.928466	6.000774	0.941512
29	62.142761	61.071319	6.001857	0.942626
30	64.285614	63.214172	6.003994	0.944225
31	66.428466	65.357025	6.007877	0.946259
32	68.571319	67.499877	6.014448	0.948555
33	70.714172	69.642730	6.024893	0.950973
34	72.857025	71.785583	6.040604	0.953485
35	74.999877	73.928436	6.063097	0.956113
36	77.142730	76.071289	6.093906	0.958839
37	79.285583	78.214141	6.134452	0.961644
38	81.428436	80.356994	6.185905	0.964522
39	83.571289	82.499847	6.249063	0.967466
40	85.714141	84.642700	6.324265	0.970466
41	87.856994	86.785552	6.411325	0.973519
42	89.999847	88.928405	6.509530	0.976618
43	92.142700	91.071258	6.617667	0.979759
44	94.285552	93.214111	6.734101	0.982934
45	96.428405	95.356964	6.856874	0.986144
46	98.571258	97.499816	6.983820	0.989379
47	100.714110	99.642669	7.112697	0.992639
48	102.856960	101.785520	7.241296	0.995921
49	104.999810	103.928370	7.367552	0.999217
50	107.142660	106.071220	7.519141	0.040552
51	109.285520	108.214080	7.660729	0.032832
52	111.428370	110.356930	7.790316	0.027987
53	113.571220	112.499780	7.906612	0.024583
54	115.714080	114.642630	8.008879	0.022010
55	117.856930	116.785490	8.096702	0.019944
56	119.999780	118.928340	8.169740	0.018193
57	122.142630	121.071190	8.227497	0.016635
58	124.285490	123.214050	8.269176	0.015193
59	126.428340	125.356900	8.293654	0.013834

RUN NO. 5-J (cont.)

<u>TIME</u>	<u>V OUT,CC</u>	<u>V AVG,CC</u>	<u>PH OUT</u>	<u>Y(HB)</u>
60	128.571190	127.499750	8.299586	0.012565
61	130.714050	129.642600	8.285625	0.011430
62	132.856900	131.785460	8.250703	0.010480
63	134.999750	133.928310	8.194316	0.009732
64	137.142600	136.071160	8.116756	0.009169
65	139.285460	138.214010	8.019253	0.008753
66	141.428310	140.356870	7.903972	0.008447
67	143.571160	142.499720	7.773919	0.008220
68	145.714010	144.642570	7.632734	0.008050
69	147.856870	146.785430	7.484426	0.007921
70	149.999720	148.928280	7.333085	0.007764
71	152.142570	151.071130	7.182621	0.007105
72	154.285430	153.213980	7.036534	0.006755
73	156.428280	155.356840	6.897757	0.006707
74	158.571130	157.499690	6.768552	0.006466
75	160.713980	159.642540	6.650498	0.006325
76	162.856840	161.785400	6.544508	0.006298
77	164.999690	163.928250	6.450903	0.006277
78	167.142540	166.071100	6.369503	0.006258
79	169.285400	168.213950	6.299742	0.006242
80	171.428250	170.356810	6.240775	0.006154
81	173.571100	172.499660	6.191579	0.005904
82	175.713950	174.642510	6.151039	0.005893
83	177.856810	176.785360	6.118024	0.005884
84	179.999660	178.928220	6.091434	0.005875

RUN NO. 8-A

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = -0.048CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 6.0

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 3.800

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

THE INITIAL VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.391

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

THE FEED VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

VOLUME OF PULSE = 225.0 CC

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HB)
21	44.999938	43.928497	5.999981	0.000000
22	47.142791	46.071350	5.999980	0.000162
23	49.285644	48.214202	5.999979	0.001030
24	51.428497	50.357055	5.999978	0.003467
25	53.571350	52.499908	5.999978	0.008224
26	55.714202	54.642761	5.999981	0.015465
27	57.857055	56.785614	5.999992	0.024605
28	59.999908	58.928466	6.000023	0.034543
29	62.142761	61.071319	6.000100	0.044095
30	64.285614	63.214172	6.000272	0.052361
31	66.428466	65.357025	6.000621	0.058886
32	68.571319	67.499877	6.001279	0.063632
33	70.714172	69.642730	6.002447	0.066833
34	72.857025	71.785583	6.004406	0.068844
35	74.999877	73.928436	6.007535	0.070023
36	77.142730	76.071289	6.012316	0.070666
37	79.285583	78.214141	6.019331	0.070990
38	81.428436	80.356994	6.029261	0.071138
39	83.571289	82.499847	6.042855	0.071195
40	85.714141	84.642700	6.060908	0.071211
41	87.856994	86.785552	6.084216	0.071241
42	89.999847	88.928405	6.113538	0.071311
43	92.142700	91.071258	6.149547	0.071470
44	94.285552	93.214111	6.192786	0.071820
45	96.428405	95.356964	6.243634	0.072568
46	98.571258	97.499816	6.302270	0.074119
47	100.714110	99.642669	6.368659	0.077238
48	102.856960	101.785520	6.442540	0.083312
49	104.999810	103.928370	6.523435	0.094736

RUN NO. 8-A (cont.)

<u>TIME</u>	<u>V OUT,CC</u>	<u>V AVG,CC</u>	<u>PH OUT</u>	<u>Y(HR)</u>
50	107.142660	106.071220	6.610662	0.115429
51	109.285520	108.214080	6.703365	0.151413
52	111.428370	110.356930	6.800550	0.211288
53	113.571220	112.499780	6.901118	0.306264
54	115.714080	114.642630	7.003911	0.449317
55	117.856930	116.785490	7.107753	0.653001
56	119.999780	118.928340	7.211493	0.925778
57	122.142630	121.071190	7.314031	1.267300
58	124.285490	123.214050	7.414358	1.663976
59	126.428340	125.356900	7.511576	2.086817
60	128.571190	127.499750	7.604910	2.493542
61	130.714050	129.642600	7.693725	2.835742
62	132.856900	131.785460	7.777520	3.069795
63	134.999750	133.928310	7.855938	3.168159
64	137.142600	136.071160	7.928751	3.126733
65	139.285460	138.214010	7.995852	2.965334
66	141.428310	140.356870	8.057244	2.721096
67	143.571160	142.499720	8.113023	2.437643
68	145.714010	144.642570	8.163362	2.154308
69	147.856870	146.785430	8.208498	1.899026
70	149.999720	148.928280	8.248719	1.686219
71	152.142570	151.071130	8.284344	1.518814
72	154.285430	153.213980	8.315715	1.392335
73	156.428280	155.356840	8.343185	1.298974
74	158.571130	157.499690	8.367109	1.230564
75	160.713980	159.642540	8.387837	1.180143
76	162.856840	161.785400	8.405704	1.142415
77	164.999690	163.928250	8.421028	1.113644
78	167.142540	166.071100	8.434110	1.091294
79	169.285400	168.213950	8.445226	1.073662
80	171.428250	170.356810	8.454629	1.059583
81	173.571100	172.499660	8.462547	1.048248
82	175.713950	174.642510	8.469189	1.039078
83	177.856810	176.785360	8.474735	1.031631
84	179.999660	178.928220	8.479350	1.025578

21IN. 14 7/16 X 11

22"

MOORE BUSINESS FORMS, INC. HO

RUN NO. 13-A

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 8.5

THE INITIAL CONCENTRATION IS 1.000

THE INITIAL VALUE FOR K* IS 0.010

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

FEED PH = 6.0

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 3.800

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

VOLUME OF PULSE = 180.0 CC

TIME	V OUT, CC	V AVG, CC	PH OUT	Y (HB)
21	44.999938	43.928497	8.499981	0.999980
22	47.142791	46.071350	8.499979	0.318438
23	49.285644	48.214202	8.499969	0.193897
24	51.428497	50.357055	8.499914	0.162437
25	53.571350	52.499908	8.499709	0.146402
26	55.714202	54.642761	8.499085	0.135867
27	57.857055	56.785614	8.497507	0.128395
28	59.999908	58.928466	8.494040	0.122895
29	62.142761	61.071319	8.487259	0.118753
30	64.285614	63.214172	8.475227	0.115590
31	66.428466	65.357025	8.455571	0.113155
32	68.571319	67.499877	8.425676	0.111274
33	70.714172	69.642730	8.382963	0.109822
34	72.857025	71.785583	8.325215	0.108704
35	74.999877	73.928436	8.250895	0.107852
36	77.142730	76.071289	8.159385	0.107214
37	79.285583	78.214141	8.051127	0.106750
38	81.428436	80.356994	7.927633	0.106433
39	83.571289	82.499847	7.791362	0.106242
40	85.714141	84.642700	7.645505	0.106164
41	87.856994	86.785552	7.493713	0.106193
42	89.999847	88.928405	7.339799	0.106325
43	92.142700	91.071258	7.187445	0.106563
44	94.285552	93.214111	7.039980	0.106913
45	96.428405	95.356964	6.900203	0.107386
46	98.571258	97.499816	6.770279	0.107996
47	100.714110	99.642669	6.651711	0.108764
48	102.856960	101.785520	6.545356	0.109715
49	104.999810	103.928370	6.451491	0.110879

RUN NO. 13-A (cont.)

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HB)
50	107.142660	106.071220	6.369909	0.112293
51	109.285520	108.214080	6.303322	0.113997
52	111.428370	110.356930	6.240968	0.116042
53	113.571220	112.499780	6.191710	0.118479
54	115.714080	114.642630	6.151129	0.121369
55	117.856930	116.785490	6.118084	0.124775
56	119.999780	118.928340	6.091475	0.128763
57	122.142630	121.071190	6.070275	0.133402
58	124.285490	123.214050	6.053555	0.138762
59	126.428340	125.356900	6.040496	0.144910
60	128.571190	127.499750	6.030391	0.151910
61	130.714050	129.642600	6.022639	0.159822
62	132.856900	131.785460	6.016746	0.168700
63	134.999750	133.928310	6.012299	0.178587
64	137.142600	136.071160	6.008971	0.189519
65	139.285460	138.214010	6.006499	0.201518
66	141.428310	140.356870	6.004677	0.214599
67	143.571160	142.499720	6.003342	0.228759
68	145.714010	144.642570	6.002371	0.243988
69	147.856870	146.785430	6.001668	0.260259
70	149.999720	148.928280	6.001164	0.277536
71	152.142570	151.071130	6.000804	0.295769
72	154.285430	153.213980	6.000548	0.314899
73	156.428280	155.356840	6.000368	0.334855
74	158.571130	157.499690	6.000242	0.355557
75	160.713980	159.642540	6.000154	0.376919
76	162.856840	161.785400	6.000093	0.398847
77	164.999690	163.928250	6.000050	0.421243
78	167.142540	166.071100	6.000019	0.444007
79	169.285400	168.213950	5.999999	0.467034
80	171.428250	170.356810	5.999986	0.490224
81	173.571100	172.499660	5.999976	0.513466
82	175.713950	174.642510	5.999970	0.536668
83	177.856810	176.785360	5.999966	0.559727
84	179.999660	178.928220	5.999964	0.582552

RUN NO. 13-B

SIMULATION OF PH AND CONCENTRATION WAVES
 HEMOGLOBIN RECOVERY = 100.0%
 HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN
 NUMBER OF STAGES = 180
 LAMBDA - LOW FLOW RATE = 2.500000
 INITIAL PH = 8.5
 THE INITIAL CONCENTRATION IS 1.000
 THE INITIAL VALUE FOR K* IS 0.010
 THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 FEED PH = 6.0
 THE FEED CONCENTRATION IS 1.000
 THE FEED VALUE FOR K* IS 3.800
 THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493
 VOLUME OF PULSE = 180.0 CC

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HB)
181	45.250000	45.125000	8.499828	0.813204
191	47.750000	47.625000	8.499819	0.125594
201	50.250000	50.125000	8.499809	0.106516
211	52.750000	52.625000	8.499800	0.102123
221	55.250000	55.125000	8.499790	0.100784
231	57.750000	57.625000	8.499781	0.100313
241	60.250000	60.125000	8.499771	0.100132
251	62.750000	62.625000	8.499762	0.100057
261	65.250000	65.125000	8.499752	0.100027
271	67.750000	67.625000	8.499743	0.100012
281	70.250000	70.125000	8.499733	0.100006
291	72.750000	72.625000	8.499702	0.100002
301	75.250000	75.125000	8.499396	0.100001
311	77.750000	77.625000	8.496691	0.100000
321	80.250000	80.125000	8.480960	0.100000
331	82.750000	82.625000	8.419548	0.100000
341	85.250000	85.125000	8.252814	0.100000
351	87.750000	87.625000	7.927886	0.100000
361	90.250000	90.125000	7.460636	0.100000
371	92.750000	92.625000	6.952674	0.100000
381	95.250000	95.125000	6.526186	0.100000
391	97.750000	97.625000	6.244347	0.100000
401	100.250000	100.125000	6.095256	0.100000
411	102.750000	102.625000	6.031160	0.100000
421	105.250000	105.125000	6.008458	0.100000
431	107.750000	107.625000	6.001751	0.100000
441	110.250000	110.125000	6.000080	0.100000
451	112.750000	112.625000	5.999724	0.100001
461	115.250000	115.125000	5.999660	0.100003

27N 14 7/8 X 11

77

MOORE BUSINESS FORMS, INC. HQ

RUN NO. 13-B (cont.)

<u>TIME</u>	<u>V OUT,CC</u>	<u>V AVG,CC</u>	<u>PH OUT</u>	<u>Y(HB)</u>
471	117.750000	117.625000	5.999652	0.100006
481	120.250000	120.125000	5.999652	0.100015
491	122.750000	122.625000	5.999652	0.100033
501	125.250000	125.125000	5.999652	0.100072
511	127.750000	127.625000	5.999652	0.100154
521	130.250000	130.125000	5.999652	0.100320
531	132.750000	132.625000	5.999652	0.100644
541	135.250000	135.125000	5.999652	0.101249
551	137.750000	137.625000	5.999652	0.102326
561	140.250000	140.125000	5.999652	0.104165
571	142.750000	142.625000	5.999652	0.107173
581	145.250000	145.125000	5.999652	0.111888
591	147.750000	147.625000	5.999652	0.118984
601	150.250000	150.125000	5.999652	0.129244
611	152.750000	152.625000	5.999652	0.143519
621	155.250000	155.125000	5.999652	0.162651
631	157.750000	157.625000	5.999652	0.187379
641	160.250000	160.125000	5.999652	0.218240
651	162.750000	162.625000	5.999652	0.255467
661	165.250000	165.125000	5.999652	0.298923
671	167.750000	167.625000	5.999652	0.348057
681	170.250000	170.125000	5.999652	0.401920
691	172.750000	172.625000	5.999652	0.459228
701	175.250000	175.125000	5.999652	0.518455
711	177.750000	177.625000	5.999652	0.577970

RUN NO. 13-C

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 9

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 8.5

THE INITIAL CONCENTRATION IS 1.000

THE INITIAL VALUE FOR K* IS 0.010

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

FEED PH = 6.0

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 3.800

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

VOLUME OF PULSE = 180.0 CC

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HR)
1	5.000000	2.500000	8.500000	1.000000
2	10.000000	7.500000	8.499999	0.999999
3	15.000000	12.500000	8.499998	0.999998
4	20.000000	17.500000	8.499997	0.999997
5	25.000000	22.500000	8.499996	0.999996
6	30.000000	27.500000	8.499995	0.999995
7	35.000000	32.500000	8.499994	0.999994
8	40.000000	37.500000	8.499993	0.999993
9	45.000000	42.500000	8.499992	0.999992
10	50.000000	47.500000	8.495690	0.253402
11	55.000000	52.500000	8.476064	0.186670
12	60.000000	57.500000	8.426313	0.164251
13	65.000000	62.500000	8.333828	0.152492
14	70.000000	67.500000	8.193158	0.146058
15	75.000000	72.500000	8.007727	0.142925
16	80.000000	77.500000	7.788363	0.142136
17	85.000000	82.500000	7.550038	0.143234
18	90.000000	87.500000	7.308378	0.146045
19	95.000000	92.500000	7.076948	0.150564
20	100.000000	97.500000	6.865745	0.156899
21	105.000000	102.500000	6.680788	0.165229
22	110.000000	107.500000	6.524500	0.175769
23	115.000000	112.500000	6.396501	0.188746
24	120.000000	117.500000	6.294521	0.204359
25	125.000000	122.500000	6.215242	0.222755
26	130.000000	127.500000	6.154951	0.244005
27	135.000000	132.500000	6.109999	0.268083
28	140.000000	137.500000	6.077078	0.294857
29	145.000000	142.500000	6.053360	0.324099
30	150.000000	147.500000	6.036525	0.355492
31	155.000000	152.500000	6.024737	0.388646
32	160.000000	157.500000	6.016588	0.423133
33	165.000000	162.500000	6.011020	0.458499
34	170.000000	167.500000	6.007256	0.494292
35	175.000000	172.500000	6.004736	0.530080
36	180.000000	177.500000	6.003066	0.565459

RUN NO. 15-A

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = -0.048CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 8.5

THE INITIAL CONCENTRATION IS 1.000

THE INITIAL VALUE FOR K* IS 0.010

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

THE INITIAL VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

FEED PH = 6.0

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 3.800

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.493

THE FEED VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.391

VOLUME OF PULSE = 180.0 CC

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HB)
21	44.999938	43.928497	8.499981	0.711985
22	47.142791	46.071350	8.499980	0.711242
23	49.285644	48.214202	8.499979	0.706997
24	51.428497	50.357055	8.499977	0.694280
25	53.571350	52.499908	8.499973	0.667737
26	55.714202	54.642761	8.499962	0.624375
27	57.857055	56.785614	8.499931	0.565324
28	59.999908	58.928466	8.499851	0.495554
29	62.142761	61.071319	8.499662	0.422035
30	64.285614	63.214172	8.499260	0.351514
31	66.428466	65.357025	8.498472	0.288924
32	68.571319	67.499877	8.497034	0.236811
33	70.714172	69.642730	8.494565	0.195602
34	72.857025	71.785583	8.490558	0.164294
35	74.999877	73.928436	8.484364	0.141181
36	77.142730	76.071289	8.475202	0.124419
37	79.285583	78.214141	8.462184	0.112358
38	81.428436	80.356994	8.444346	0.103669
39	83.571289	82.499847	8.420703	0.097363
40	85.714141	84.642700	8.390306	0.092728
41	87.856994	86.785552	8.352308	0.089275
42	89.999847	88.928405	8.306027	0.086664
43	92.142700	91.071258	8.250999	0.084663
44	94.285552	93.214111	8.187022	0.083114
45	96.428405	95.356964	8.114180	0.081904
46	98.571258	97.499816	8.032851	0.080955
47	100.714110	99.642669	7.943699	0.080209
48	102.856960	101.785520	7.847642	0.079627
49	104.999810	103.928370	7.745811	0.079177

RUN NO. 15-A (cont.)

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HR)
50	107.142660	106.071220	7.639500	0.078837
51	109.285520	108.214080	7.530106	0.078591
52	111.428370	110.356930	7.419070	0.078427
53	113.571220	112.499780	7.307822	0.078337
54	115.714080	114.642630	7.197729	0.078314
55	117.856930	116.785490	7.090049	0.078355
56	119.999780	118.928340	6.985896	0.078457
57	122.142630	121.071190	6.886222	0.078622
58	124.285490	123.214050	6.791797	0.078849
59	126.428340	125.356900	6.703209	0.079141
60	128.571190	127.499750	6.620864	0.079502
61	130.714050	129.642600	6.544998	0.079937
62	132.856900	131.785460	6.475696	0.080452
63	134.999750	133.928310	6.412902	0.081055
64	137.142600	136.071160	6.356451	0.081754
65	139.285460	138.214010	6.306083	0.082560
66	141.428310	140.356870	6.261466	0.083483
67	143.571160	142.499720	6.222217	0.084537
68	145.714010	144.642570	6.187922	0.085735
69	147.856870	146.785430	6.158149	0.087092
70	149.999720	148.928280	6.132462	0.088626
71	152.142570	151.071130	6.110434	0.090353
72	154.285430	153.213980	6.091652	0.092291
73	156.428280	155.356840	6.075729	0.094462
74	158.571130	157.499690	6.062303	0.096884
75	160.713980	159.642540	6.051041	0.099578
76	162.856840	161.785400	6.041641	0.102566
77	164.999690	163.928250	6.033835	0.105869
78	167.142540	166.071100	6.027384	0.109508
79	169.285400	168.213950	6.022076	0.113503
80	171.428250	170.356810	6.017729	0.117877
81	173.571100	172.499660	6.014185	0.122647
82	175.713950	174.642510	6.011307	0.127833
83	177.856810	176.785360	6.008979	0.133452
84	179.999660	178.928220	6.007104	0.139520

RUN NO. 20-A

SIMULATION OF PH AND CONCENTRATION WAVES

HEMOGLOBIN RECOVERY = 100.0%

HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN

NUMBER OF STAGES = 21

LAMBDA - LOW FLOW RATE = 2.500000

INITIAL PH = 8.5

THE INITIAL CONCENTRATION IS 0.000

THE INITIAL VALUE FOR K* IS 0.010

THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

FEED PH = 8.5

THE FEED CONCENTRATION IS 1.000

THE FEED VALUE FOR K* IS 0.010

THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

VOLUME OF PULSE = 60.0 CC

TIME	V OUT,CC	V AVG,CC	PH OUT	Y(HB)
21	44.999938	43.928497	8.499981	0.000000
22	47.142791	46.071350	8.499980	0.866115
23	49.285644	48.214202	8.499979	0.989035
24	51.428497	50.357055	8.499978	0.999366
25	53.571350	52.499908	8.499977	0.999970
26	55.714202	54.642761	8.499976	0.999999
27	57.857055	56.785614	8.499975	1.000000
28	59.999908	58.928466	8.499974	1.000000
29	62.142761	61.071319	8.499973	1.000000
30	64.285614	63.214172	8.499972	1.000000
31	66.428466	65.357025	8.499971	1.000000
32	68.571319	67.499877	8.499970	1.000000
33	70.714172	69.642730	8.499969	1.000000
34	72.857025	71.785583	8.499969	1.000000
35	74.999877	73.928436	8.499968	1.000000
36	77.142730	76.071289	8.499967	1.000000
37	79.285583	78.214141	8.499966	1.000000
38	81.428436	80.356994	8.499965	1.000000
39	83.571289	82.499847	8.499964	1.000000
40	85.714141	84.642700	8.499963	1.000000
41	87.856994	86.785552	8.499962	1.000000
42	89.999847	88.928405	8.499961	1.000000
43	92.142700	91.071258	8.499960	1.000000
44	94.285552	93.214111	8.499959	1.000000
45	96.428405	95.356964	8.499958	1.000000
46	98.571258	97.499816	8.499957	1.000000
47	100.714110	99.642669	8.499956	1.000000
48	102.856960	101.785520	8.499955	1.000000
49	104.999810	103.928370	8.499954	1.000000

RUN NO. 20-A (cont.)

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HB)
50	107.142660	106.071220	8.499954	0.144209
51	109.285520	108.214080	8.499954	0.001304
52	111.428370	110.356930	8.499954	0.000000
53	113.571220	112.499780	8.499954	0.000000
54	115.714080	114.642630	8.499954	0.000000
55	117.856930	116.785490	8.499954	0.000000
56	119.999780	118.928340	8.499954	0.000000
57	122.142630	121.071190	8.499954	0.000000
58	124.285490	123.214050	8.499954	0.000000
59	126.428340	125.356900	8.499954	0.000000
60	128.571190	127.499750	8.499954	0.000000
61	130.714050	129.642600	8.499954	0.000000
62	132.856900	131.785460	8.499954	0.000000
63	134.999750	133.928310	8.499954	0.000000
64	137.142600	136.071160	8.499954	0.000000
65	139.285460	138.214010	8.499954	0.000000
66	141.428310	140.356870	8.499954	0.000000
67	143.571160	142.499720	8.499954	0.000000
68	145.714010	144.642570	8.499954	0.000000
69	147.856870	146.785430	8.499954	0.000000
70	149.999720	148.928280	8.499954	0.000000
71	152.142570	151.071130	8.499954	0.000000
72	154.285430	153.213980	8.499954	0.000000
73	156.428280	155.356840	8.499954	0.000000
74	158.571130	157.499690	8.499954	0.000000
75	160.713980	159.642540	8.499954	0.000000
76	162.856840	161.785400	8.499954	0.000000
77	164.999690	163.928250	8.499954	0.000000
78	167.142540	166.071100	8.499954	0.000000
79	169.285400	168.213950	8.499954	0.000000
80	171.428250	170.356810	8.499954	0.000000
81	173.571100	172.499660	8.499954	0.000000
82	175.713950	174.642510	8.499954	0.000000
83	177.856810	176.785360	8.499954	0.000000
84	179.999660	178.928220	8.499954	0.000000

RUN NO. 20-B

SIMULATION OF PH AND CONCENTRATION WAVES
 HEMOGLOBIN RECOVERY = 100.0%
 HEMOGLOBIN MIGRATION VELOCITY = 0.000CM/MIN
 NUMBER OF STAGES = 21
 LAMBDA - LOW FLOW RATE = 2.500000
 INITIAL PH = 8.5
 THE INITIAL CONCENTRATION IS 0.000
 THE INITIAL VALUE FOR K* IS 1.000
 THE INITIAL VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 FEED PH = 8.5
 THE FEED CONCENTRATION IS 1.000
 THE FEED VALUE FOR K* IS 1.000
 THE FEED VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 VOLUME OF PULSE = 60.0 CC

TIME	V OUT.CC	V AVG.CC	PH OUT	Y(HB)
21	44.999938	43.928497	8.499981	0.000000
22	47.142791	46.071350	8.499980	0.100006
23	49.285644	48.214202	8.499979	0.100057
24	51.428497	50.357055	8.499978	0.100301
25	53.571350	52.499908	8.499977	0.101115
26	55.714202	54.642761	8.499976	0.103241
27	57.857055	56.785614	8.499975	0.107866
28	59.999908	58.928466	8.499974	0.116582
29	62.142761	61.071319	8.499973	0.131208
30	64.285614	63.214172	8.499972	0.153475
31	66.428466	65.357025	8.499971	0.184687
32	68.571319	67.499877	8.499970	0.225418
33	70.714172	69.642730	8.499969	0.275350
34	72.857025	71.785583	8.499969	0.333272
35	74.999877	73.928436	8.499968	0.397231
36	77.142730	76.071289	8.499967	0.464799
37	79.285583	78.214141	8.499966	0.533381
38	81.428436	80.356994	8.499965	0.600505
39	83.571289	82.499847	8.499964	0.664057
40	85.714141	84.642700	8.499963	0.722417
41	87.856994	86.785552	8.499962	0.774527
42	89.999847	88.928405	8.499961	0.819862
43	92.142700	91.071258	8.499960	0.858365
44	94.285552	93.214111	8.499959	0.890340
45	96.428405	95.356964	8.499958	0.916344
46	98.571258	97.499816	8.499957	0.937082
47	100.714110	99.642669	8.499956	0.953320
48	102.856960	101.785520	8.499955	0.965817
49	104.999810	103.928370	8.499954	0.975280

27N 14 7/8 X 11

27"

AOCRT BUSINESS FORMS, INC. HO

RUN NO. 20-B (cont.)

TIME	V OUT, CC	V AVG, CC	PH OUT	Y(HB)
50	107.142660	106.071220	8.499954	0.982331
51	109.285520	108.214080	8.499954	0.987461
52	111.428370	110.356930	8.499954	0.990950
53	113.571220	112.499780	8.499954	0.992733
54	115.714080	114.642630	8.499954	0.992264
55	117.856930	116.785490	8.499954	0.988416
56	119.999780	118.928340	8.499954	0.979526
57	122.142630	121.071190	8.499954	0.963578
58	124.285490	123.214050	8.499954	0.938522
59	126.428340	125.356900	8.499954	0.902640
60	128.571190	127.499750	8.499954	0.854859
61	130.714050	129.642600	8.499954	0.794919
62	132.856900	131.785460	8.499954	0.723395
63	134.999750	133.928310	8.499954	0.641623
64	137.142600	136.071160	8.499954	0.551186
65	139.285460	138.214010	8.499954	0.453790
66	141.428310	140.356870	8.499954	0.351690
67	143.571160	142.499720	8.499954	0.244731
68	145.714010	144.642570	8.499954	0.133594
69	147.856870	146.785430	8.499954	0.025864
70	149.999720	148.928280	8.499954	0.000000
71	152.142570	151.071130	8.499954	0.000000
72	154.285430	153.213980	8.499954	0.000000
73	156.428280	155.356840	8.499954	0.000000
74	158.571130	157.499690	8.499954	0.000000
75	160.713980	159.642540	8.499954	0.000000
76	162.856840	161.785400	8.499954	0.000000
77	164.999690	163.928250	8.499954	0.000000
78	167.142540	166.071100	8.499954	0.000000
79	169.285400	168.213950	8.499954	0.000000
80	171.428250	170.356810	8.499954	0.000000
81	173.571100	172.499660	8.499954	0.000000
82	175.713950	174.642510	8.499954	0.000000
83	177.856810	176.785360	8.499954	0.000000
84	179.999660	178.928220	8.499954	0.000000

RUN NO. 54-B

THIS IS MODE 6
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 45.0 CC
 TOP RESERVOIR DEAD VOLUME = 82.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 60.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 135.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 18
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

<u>N</u>	<u>YT(HB)</u>	<u>YP(HB)</u>	<u>SF(HB)</u>	<u>REC(HB)</u>
1	0.571402	1.450956	2.539291	1.378509
2	0.322943	1.651464	5.113791	1.194809
3	0.204887	1.736876	8.477230	1.112593
4	0.148743	1.770920	11.905890	1.055451
5	0.122010	1.782750	14.611487	1.030814
6	0.109260	1.785480	16.341522	1.017609
7	0.103165	1.784851	17.300872	1.010328
8	0.100242	1.783266	17.789557	1.006195
9	0.098834	1.781656	18.026718	1.003780
10	0.098152	1.780321	18.138442	1.002334
11	0.097818	1.779303	18.189880	1.001443
12	0.097654	1.778564	18.212997	1.000886
13	0.097571	1.778044	18.223052	1.000531
14	0.097529	1.777682	18.227233	1.000302
15	0.097507	1.777433	18.228759	1.000153
16	0.097495	1.777267	18.229232	1.000055
17	0.097489	1.777153	18.229263	0.999992
18	0.097485	1.777077	18.229171	0.999950
19	0.097483	1.777026	18.229034	0.999922
20	0.097482	1.776992	18.228927	0.999903
21	0.097481	1.776969	18.228851	0.999891
22	0.097481	1.776953	18.228759	0.999883
23	0.097480	1.776943	18.228729	0.999877
24	0.097480	1.776936	18.228683	0.999873
25	0.097480	1.776930	18.228652	0.999870

RUN NO. 54-B (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.922375	0.640039	1.441122	1.374162
2	0.917989	0.512671	1.790602	1.305465
3	0.862988	0.456870	1.888914	1.214706
4	0.813914	0.427408	1.904303	1.143890
5	0.778575	0.409959	1.899153	1.094779
6	0.754693	0.399032	1.891308	1.062022
7	0.738913	0.392024	1.884866	1.040483
8	0.728575	0.387486	1.880261	1.026400
9	0.721823	0.384535	1.877131	1.017208
10	0.717420	0.382614	1.875047	1.011215
11	0.714549	0.381363	1.873673	1.007308
12	0.712678	0.380547	1.872771	1.004763
13	0.711459	0.380016	1.872181	1.003103
14	0.710664	0.379670	1.871796	1.002022
15	0.711146	0.379444	1.871545	1.001317
16	0.709809	0.379297	1.871380	1.000857
17	0.709589	0.379201	1.871273	1.000558
18	0.709445	0.379138	1.871203	1.000363
19	0.709352	0.379098	1.871158	1.000237
20	0.709291	0.379071	1.871128	1.000154
21	0.709252	0.379054	1.871109	1.000099
22	0.709226	0.379043	1.871097	1.000064
23	0.709209	0.379035	1.871088	1.000041
24	0.709198	0.379031	1.871082	1.000027
25	0.709191	0.379028	1.871079	1.000017

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	61.748	38.252	30.609	69.391	3.659
2	73.976	26.024	23.689	76.311	9.157
3	80.814	19.186	20.826	79.174	16.013
4	84.549	15.451	19.442	80.558	22.672
5	86.452	13.548	18.696	81.304	27.749
6	87.353	12.647	18.266	81.734	30.907
7	87.749	12.251	18.009	81.991	32.610
8	87.905	12.095	17.850	82.150	33.449
9	87.957	12.043	17.752	82.248	33.839
10	87.965	12.035	17.690	82.310	34.010
11	87.959	12.041	17.650	82.350	34.082
12	87.949	12.051	17.625	82.375	34.109
13	87.940	12.060	17.609	82.391	34.117
14	87.932	12.068	17.599	82.401	34.118
15	87.927	12.073	17.592	82.408	34.116
16	87.923	12.077	17.588	82.412	34.114
17	87.921	12.079	17.585	82.415	34.112
18	87.919	12.081	17.584	82.416	34.110
19	87.918	12.082	17.582	82.418	34.109
20	87.917	12.083	17.582	82.418	34.109
21	87.917	12.083	17.581	82.419	34.108
22	87.916	12.084	17.581	82.419	34.108
23	87.916	12.084	17.581	82.419	34.108
24	87.916	12.084	17.581	82.419	34.107
25	87.916	12.084	17.580	82.420	34.107

RUN NO. 54-C

THIS IS MODE 6
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 82.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 60.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 135.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 18
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

<u>N</u>	<u>YT(HB)</u>	<u>YB(HB)</u>	<u>SF(HB)</u>	<u>REC(HB)</u>
1	0.569187	1.506017	2.645908	1.362851
2	0.312027	1.722119	5.519106	1.195368
3	0.190113	1.809039	9.515580	1.108212
4	0.132293	1.839912	13.907866	1.061698
5	0.104855	1.847650	17.620910	1.036169
6	0.091826	1.846721	20.111145	1.021745
7	0.085631	1.843218	21.525039	1.013356
8	0.082432	1.839726	22.318038	1.008183
9	0.080846	1.836841	22.720199	1.005040
10	0.080095	1.834654	22.905990	1.003142
11	0.079738	1.833071	22.986662	1.001968
12	0.079567	1.831959	23.023971	1.001229
13	0.079485	1.831189	23.038040	1.000757
14	0.079445	1.830663	23.042999	1.000451
15	0.079426	1.830308	23.044189	1.000253
16	0.079416	1.830068	23.043975	1.000122
17	0.079411	1.829907	23.043426	1.000036
18	0.079409	1.829796	23.042755	0.999978
19	0.079407	1.829723	23.042266	0.999941
20	0.079406	1.829675	23.041885	0.999915
21	0.079406	1.829642	23.041610	0.999899
22	0.079406	1.829621	23.041458	0.999888
23	0.079405	1.829606	23.041290	0.999880
24	0.079405	1.829596	23.041198	0.999875
25	0.079405	1.829591	23.041122	0.999872

RUN NO. 54-C (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.926400	0.687658	1.347180	1.336400
2	0.918474	0.569135	1.616649	1.268147
3	0.869516	0.513108	1.694606	1.188177
4	0.825654	0.483462	1.707795	1.126360
5	0.793795	0.465826	1.704060	1.083406
6	0.772129	0.454786	1.697785	1.054672
7	0.757755	0.447710	1.692512	1.035735
8	0.748314	0.443130	1.688702	1.023329
9	0.742137	0.440151	1.686097	1.015222
10	0.738104	0.438211	1.684358	1.009931
11	0.735471	0.436946	1.683209	1.006477
12	0.733754	0.436121	1.682455	1.004225
13	0.732633	0.435583	1.681961	1.002755
14	0.731913	0.435232	1.681639	1.001797
15	0.731426	0.435003	1.681427	1.001171
16	0.731115	0.434853	1.681290	1.000763
17	0.730912	0.434756	1.681199	1.000498
18	0.730780	0.434693	1.681141	1.000324
19	0.730694	0.434651	1.681103	1.000211
20	0.730637	0.434624	1.681078	1.000137
21	0.730601	0.434607	1.681061	1.000089
22	0.730577	0.434595	1.681050	1.000057
23	0.730561	0.434587	1.681045	1.000037
24	0.730551	0.434583	1.681039	1.000024
25	0.730544	0.434579	1.681036	1.000014

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	61.942	38.058	31.347	68.653	3.565
2	74.642	25.358	24.807	75.193	8.922
3	82.059	17.941	22.096	77.904	16.125
4	86.190	13.810	20.809	79.191	23.752
5	88.332	11.668	20.135	79.865	30.027
6	89.371	10.629	19.760	80.240	34.144
7	89.847	10.153	19.543	80.457	36.431
8	90.077	9.923	19.411	80.589	37.689
9	90.176	9.824	19.330	80.670	38.308
10	90.211	9.789	19.280	80.720	38.582
11	90.219	9.781	19.249	80.751	38.695
12	90.217	9.783	19.229	80.771	38.737
13	90.213	9.787	19.216	80.784	38.749
14	90.208	9.792	19.208	80.792	38.750
15	90.205	9.795	19.203	80.797	38.747
16	90.202	9.798	19.199	80.801	38.744
17	90.200	9.800	19.197	80.803	38.741
18	90.199	9.801	19.196	80.804	38.738
19	90.198	9.802	19.195	80.805	38.736
20	90.197	9.803	19.195	80.805	38.735
21	90.197	9.803	19.194	80.806	38.734
22	90.197	9.803	19.194	80.806	38.734
23	90.196	9.804	19.194	80.806	38.733
24	90.196	9.804	19.194	80.806	38.733
25	90.196	9.804	19.194	80.806	38.733

RUN NO. 54-D

THIS IS MODE 6
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 22.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 15.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 RETARDATION COEFFICIENT = 1.160092

N	YTAL	YBAL	SFAL	REC
1	0.926400	0.453402	2.043218	1.219272
2	0.911431	0.375217	2.429070	1.164140
3	0.826672	0.347007	2.382289	1.059222
4	0.796090	0.336829	2.363486	1.02367
5	0.785056	0.333156	2.356421	1.007709
6	0.781075	0.331831	2.353831	1.002780
7	0.779638	0.331353	2.352893	1.001002
8	0.779120	0.331180	2.352554	1.000360
9	0.778933	0.331118	2.352432	1.000130
10	0.778865	0.331096	2.352387	1.000046
11	0.778841	0.331087	2.352371	1.000015
12	0.778832	0.331084	2.352366	1.000005
13	0.778829	0.331084	2.352363	1.000001
14	0.778828	0.331083	2.352364	1.000000
15	0.778827	0.331083	2.352362	0.999999
16	0.778827	0.331083	2.352363	0.999999
17	0.778827	0.331083	2.352363	0.999999
18	0.778827	0.331083	2.352363	0.999999
19	0.778827	0.331083	2.352363	0.999999
20	0.778827	0.331083	2.352363	0.999999
21	0.778827	0.331083	2.352363	0.999999
22	0.778827	0.331083	2.352363	0.999999
23	0.778827	0.331083	2.352363	0.999999
24	0.778827	0.331083	2.352363	0.999999
25	0.778827	0.331083	2.352363	0.999999

RUN NO. 54-E

THIS IS MODE 6
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 15.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 RETARDATION COEFFICIENT = 1.160092

<u>N</u>	<u>YTAL</u>	<u>YBAL</u>	<u>SFAL</u>	<u>REC</u>
1	0.926400	0.453402	2.043218	1.219272
2	0.919984	0.376980	2.440403	1.174187
3	0.880812	0.358407	2.457573	1.122931
4	0.849799	0.349464	2.431722	1.085230
5	0.827922	0.343727	2.408665	1.058922
6	0.812756	0.339812	2.391777	1.040715
7	0.802271	0.337114	2.379820	1.028131
8	0.795025	0.335250	2.371442	1.019437
9	0.790019	0.333962	2.365596	1.013430
10	0.786560	0.333072	2.361531	1.009278
11	0.784171	0.332457	2.358708	1.006411
12	0.782519	0.332033	2.356751	1.004429
13	0.781378	0.331739	2.355397	1.003059
14	0.780590	0.331530	2.354461	1.002113
15	0.780045	0.331396	2.353813	1.001460
16	0.779669	0.331299	2.353366	1.001078
17	0.779409	0.331232	2.353056	1.000696
18	0.779229	0.331186	2.352841	1.000481
19	0.779105	0.331154	2.352693	1.000331
20	0.779019	0.331132	2.352591	1.000228
21	0.778960	0.331117	2.352520	1.000157
22	0.778919	0.331107	2.352470	1.000108
23	0.778891	0.331099	2.352438	1.000074
24	0.778871	0.331094	2.352415	1.000051
25	0.778857	0.331091	2.352397	1.000034

RUN NO. 54-F

THIS IS MODE 6

VOLUME OF BOTTOM FEED = 35.0 CC

VOLUME OF TOP FEED = 35.0 CC

VOLUME OF BUFFER TO STAGE IV = 40.0 CC

TOP RESERVOIR DEAD VOLUME = 22.5 CC

BOTTOM RESERVOIR DEAD VOLUME = 60.0 CC

STAGE I DISPLACEMENT = 67.5 CC

STAGE V DISPLACEMENT = 60.0 CC

RETARDATION COEFFICIENT = 1.160092

N	YTAL	YBAL	SFAL	REC
1	0.926400	0.687658	1.347180	1.335400
2	0.911430	0.566710	1.608282	1.259886
3	0.824979	0.507562	1.638286	1.135686
4	0.779843	0.470593	1.657150	1.070842
5	0.756277	0.453379	1.668089	1.036985
6	0.743974	0.444392	1.674138	1.019310
7	0.737550	0.439700	1.677393	1.010080
8	0.734196	0.437250	1.679121	1.005262
9	0.732445	0.435971	1.680031	1.002747
10	0.731531	0.435303	1.680509	1.001433
11	0.731053	0.434954	1.680758	1.000748
12	0.730804	0.434772	1.680888	1.000389
13	0.730674	0.434677	1.680957	1.000203
14	0.730606	0.434628	1.680992	1.000105
15	0.730570	0.434602	1.681010	1.000054
16	0.730552	0.434588	1.681020	1.000028
17	0.730542	0.434581	1.681026	1.000013
18	0.730537	0.434577	1.681028	1.000007
19	0.730534	0.434575	1.681029	1.000002
20	0.730533	0.434574	1.681030	1.000001
21	0.730532	0.434574	1.681030	1.000000
22	0.730532	0.434574	1.681030	1.000000
23	0.730532	0.434574	1.681031	0.999999
24	0.730532	0.434574	1.681030	0.999999
25	0.730532	0.434574	1.681030	0.999999

RUN NO. 55-A

THIS IS MODE 7
 VOLUME OF BOTTOM FEED = 52.5 CC
 VOLUME OF TOP FEED = 17.5 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 36
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

<u>N</u>	<u>YT(HB)</u>	<u>YB(HB)</u>	<u>SF(HB)</u>	<u>REC(HB)</u>
1	0.685014	1.119734	1.634613	1.185130
2	0.388364	1.391090	3.581926	0.860967
3	0.235566	1.613396	6.849018	0.714632
4	0.156868	1.803153	11.494703	0.658078
5	0.116344	1.969508	16.928375	0.646116
6	0.095483	2.117755	22.179367	0.655612
7	0.085495	2.250873	26.327423	0.675694
8	0.081328	2.371835	29.151519	0.700178
9	0.078954	2.479671	31.406387	0.724250
10	0.077605	2.578587	33.226974	0.747196
11	0.076841	2.668602	34.729034	0.768690
12	0.076409	2.750567	35.997787	0.788611
13	0.076168	2.825257	37.092376	0.806965
14	0.076035	2.893312	38.052261	0.823803
15	0.075964	2.955360	38.904968	0.839220
16	0.075927	3.011921	39.668716	0.853312
17	0.075909	3.063481	40.357086	0.866179
18	0.075903	3.110498	40.980072	0.877924
19	0.075902	3.153363	41.545227	0.888640
20	0.075904	3.192450	42.058929	0.898414
21	0.075908	3.228082	42.526260	0.907327
22	0.075912	3.260574	42.951812	0.915456
23	0.075917	3.290203	43.339370	0.922870
24	0.075922	3.317213	43.692565	0.929628
25	0.075926	3.341838	44.014328	0.935790
50	0.075969	3.571196	47.008483	0.993186

RUN NO. 55-A (cont.)

N	YT(AL)	YB(AL)	SF(AL)	REC(AL)
1	0.940324	0.453402	2.073929	1.355922
2	0.935122	0.385835	2.423631	1.332155
3	0.906697	0.341609	2.654193	1.283537
4	0.873794	0.310757	2.811819	1.232345
5	0.843370	0.288241	2.925921	1.186513
6	0.817503	0.271320	3.013061	1.148101
7	0.796348	0.258375	3.082132	1.116910
8	0.779383	0.248369	3.138002	1.091990
9	0.765919	0.240587	3.183539	1.072253
10	0.755294	0.234515	3.220666	1.056695
11	0.746935	0.229767	3.250832	1.044462
12	0.740370	0.226052	3.275225	1.034859
13	0.735219	0.223142	3.294849	1.027324
14	0.731179	0.220863	3.310563	1.021416
15	0.728012	0.219077	3.323096	1.016785
16	0.725530	0.217677	3.333055	1.013154
17	0.723584	0.216580	3.340951	1.011309
18	0.722059	0.215721	3.347192	1.008079
19	0.720864	0.215047	3.352118	1.006331
20	0.719927	0.214519	3.356001	1.004961
21	0.719193	0.214106	3.359056	1.003888
22	0.718618	0.213782	3.361459	1.003047
23	0.718167	0.213527	3.363346	1.002387
24	0.717813	0.213328	3.364830	1.001870
25	0.717537	0.213172	3.365994	1.001466
50	0.716535	0.212608	3.370216	1.000002

N	WT(AL)	WT(HB)	WB(AL)	WB(HB)	ALPHA
1	57.854	42.146	28.822	71.178	3.390
2	70.656	29.344	21.714	78.286	8.681
3	79.377	20.623	17.474	82.526	18.179
4	84.780	15.220	14.701	85.299	32.321
5	87.877	12.123	12.767	87.233	49.531
6	89.542	10.458	11.357	88.643	66.828
7	90.305	9.695	10.297	89.703	81.145
8	90.551	9.449	9.483	90.517	91.478
9	90.655	9.345	8.844	91.156	99.983
10	90.682	9.317	8.337	91.663	107.013
11	90.672	9.328	7.927	92.073	112.898
12	90.645	9.355	7.594	92.406	117.901
13	90.613	9.387	7.320	92.680	122.214
14	90.581	9.419	7.092	92.908	125.974
15	90.551	9.448	6.901	93.099	129.285
16	90.526	9.474	6.740	93.261	132.208
17	90.505	9.495	6.603	93.397	134.831
18	90.488	9.512	6.485	93.515	137.168
19	90.474	9.526	6.384	93.616	139.265
20	90.462	9.538	6.296	93.704	141.150
21	90.453	9.547	6.220	93.780	142.848
22	90.446	9.554	6.153	93.847	144.381
23	90.440	9.560	6.094	93.906	145.765
24	90.435	9.565	6.042	93.958	147.018
25	90.431	9.569	5.996	94.004	148.152
50	90.414	9.586	5.619	94.381	158.429

RUN NO. 55-A

TOP PRODUCT - CONCENTRATION PROFILES

<u>#</u>	<u>VOL</u>	<u><V></u>	<u>N = 1</u>	<u>N = 2</u>	<u>N = 3</u>	<u>N = 4</u>	<u>N = 5</u>
<u>STAGE III</u>							
1	1.25	0.63	0.5514	0.3272	0.2118	0.1524	0.1218
2	2.50	1.88	0.5577	0.3305	0.2134	0.1531	0.1221
3	3.75	3.13	0.5642	0.3337	0.2150	0.1539	0.1224
4	5.00	4.38	0.5708	0.3370	0.2166	0.1546	0.1227
5	6.25	5.63	0.5775	0.3404	0.2183	0.1554	0.1230
6	7.50	6.88	0.5843	0.3438	0.2200	0.1562	0.1233
7	8.75	8.13	0.5913	0.3473	0.2217	0.1570	0.1237
8	10.00	9.38	0.5983	0.3509	0.2234	0.1578	0.1240
9	11.25	10.63	0.6055	0.3545	0.2252	0.1586	0.1244
10	12.50	11.88	0.6127	0.3581	0.2270	0.1595	0.1247
11	13.75	13.13	0.6201	0.3619	0.2289	0.1603	0.1251
12	15.00	14.38	0.6276	0.3656	0.2307	0.1612	0.1254
13	16.25	15.63	0.6352	0.3695	0.2326	0.1621	0.1258
14	17.50	16.88	0.6429	0.3733	0.2345	0.1630	0.1262
15	18.75	18.13	0.6507	0.3773	0.2364	0.1639	0.1265
16	20.00	19.38	0.6586	0.3813	0.2384	0.1648	0.1269
17	21.25	20.63	0.6666	0.3853	0.2404	0.1657	0.1273
18	22.50	21.88	0.6747	0.3894	0.2424	0.1667	0.1277
19	23.75	23.13	0.6829	0.3935	0.2444	0.1676	0.1281
20	25.00	24.38	0.6911	0.3976	0.2464	0.1686	0.1285
21	26.25	25.63	0.6995	0.4018	0.2485	0.1696	0.1289
22	27.50	26.88	0.7078	0.4060	0.2506	0.1705	0.1293
23	28.75	28.13	0.7163	0.4103	0.2527	0.1715	0.1297
24	30.00	29.38	0.7247	0.4145	0.2548	0.1725	0.1301
25	31.25	30.63	0.7332	0.4188	0.2569	0.1735	0.1305
26	32.50	31.88	0.7418	0.4231	0.2590	0.1745	0.1309
27	33.75	33.13	0.7503	0.4274	0.2611	0.1754	0.1313
28	35.00	34.38	0.7588	0.4317	0.2632	0.1764	0.1318
29	36.25	35.63	0.7673	0.4360	0.2653	0.1774	0.1322
30	37.50	36.88	0.7758	0.4402	0.2674	0.1784	0.1326
31	38.75	38.13	0.7842	0.4445	0.2695	0.1794	0.1330
32	40.00	39.38	0.7925	0.4487	0.2716	0.1803	0.1334
33	41.25	40.63	0.8008	0.4528	0.2736	0.1813	0.1338
34	42.50	41.88	0.8089	0.4569	0.2756	0.1823	0.1342
35	43.75	43.13	0.8170	0.4610	0.2776	0.1832	0.1346
36	45.00	44.38	0.8249	0.4650	0.2796	0.1841	0.1349
37	46.25	45.63	0.8327	0.4689	0.2815	0.1850	0.1353
38	47.50	46.88	0.8403	0.4727	0.2834	0.1859	0.1357
39	48.75	48.13	0.8477	0.4765	0.2852	0.1868	0.1360
40	50.00	49.38	0.8550	0.4801	0.2870	0.1876	0.1364
41	51.25	50.63	0.8621	0.4837	0.2888	0.1884	0.1367
42	52.50	51.88	0.8689	0.4871	0.2905	0.1892	0.1371
<u>STAGE IV A</u>							
1	53.75	53.13	0.8755	0.4905	0.2921	0.1900	0.1374
2	55.00	54.38	0.8801	0.4928	0.2933	0.1905	0.1376

RUN NO. 55-A (cont.)TOP PRODUCT - CONCENTRATION PROFILES

<u>#</u>	<u>VOL</u>	<u><V></u>	<u>N = 1</u>	<u>N = 2</u>	<u>N = 3</u>	<u>N = 4</u>	<u>N = 5</u>
3	56.25	55.63	0.8845	0.4950	0.2944	0.1910	0.1378
4	57.50	56.88	0.8888	0.4971	0.2954	0.1915	0.1380
5	58.75	58.13	0.8929	0.4992	0.2965	0.1920	0.1382
6	60.00	59.38	0.8970	0.5013	0.2974	0.1925	0.1384
7	61.25	60.63	0.9008	0.5032	0.2984	0.1929	0.1386
8	62.50	61.88	0.9046	0.5051	0.2993	0.1933	0.1388
9	63.75	63.13	0.9081	0.5069	0.3002	0.1938	0.1389
10	65.00	64.38	0.9116	0.5086	0.3011	0.1942	0.1391
11	66.25	65.63	0.9148	0.5103	0.3019	0.1945	0.1393
12	67.50	66.88	0.9179	0.5118	0.3026	0.1949	0.1394
13	68.75	68.13	0.9209	0.5133	0.3034	0.1952	0.1396
14	70.00	69.38	0.9237	0.5147	0.3041	0.1956	0.1397
15	71.25	70.63	0.9263	0.5160	0.3047	0.1959	0.1398
16	72.50	71.88	0.9287	0.5173	0.3053	0.1962	0.1399
17	73.75	73.13	0.9310	0.5184	0.3059	0.1964	0.1401
18	75.00	74.38	0.9331	0.5195	0.3064	0.1967	0.1402
19	76.25	75.63	0.9350	0.5204	0.3069	0.1969	0.1403
20	77.50	76.88	0.9368	0.5213	0.3073	0.1971	0.1403
21	78.75	78.13	0.9383	0.5221	0.3077	0.1973	0.1404
22	80.00	79.38	0.9397	0.5228	0.3080	0.1974	0.1405
23	81.25	80.63	0.9409	0.5234	0.3083	0.1976	0.1405
24	82.50	81.88	0.9418	0.5239	0.3086	0.1977	0.1406
25	83.75	83.18	0.9426	0.5243	0.3088	0.1978	0.1406
26	85.00	84.38	0.9432	0.5246	0.3089	0.1978	0.1406
27	86.25	85.63	0.9436	0.5247	0.3090	0.1979	0.1407
28	87.50	86.88	0.9438	0.5248	0.3090	0.1979	0.1407
29	88.75	88.13	0.9437	0.5248	0.3090	0.1979	0.1407
30	90.00	89.38	0.9434	0.5247	0.3090	0.1979	0.1407
31	91.25	90.63	0.9429	0.5244	0.3089	0.1978	0.1406
32	92.50	91.88	0.9422	0.5240	0.3087	0.1977	0.1406

RUN NO. 55-A (cont.)TOP PRODUCT - CONCENTRATION PROFILES

<u>#</u>	<u>VOL</u>	<u><V></u>	<u>N = 6</u>	<u>N = 7</u>	<u>N = 8</u>	<u>N = 9</u>	<u>N = 10</u>
<u>STAGE III</u>							
1	1.25	0.63	0.1060	0.0958	0.0872	0.0823	0.0796
2	2.50	1.88	0.1061	0.0958	0.0872	0.0823	0.0796
3	3.75	3.13	0.1062	0.0958	0.0872	0.0823	0.0796
4	5.00	4.38	0.1063	0.0958	0.0872	0.0823	0.0796
5	6.25	5.63	0.1063	0.0958	0.0872	0.0823	0.0796
6	7.50	6.88	0.1064	0.0958	0.0872	0.0823	0.0796
7	8.75	8.13	0.1065	0.0958	0.0872	0.0823	0.0796
8	10.00	9.38	0.1066	0.0958	0.0872	0.0823	0.0796
9	11.25	10.63	0.1067	0.0958	0.0872	0.0823	0.0796
10	12.50	11.88	0.1068	0.0958	0.0872	0.0823	0.0796
11	13.75	13.13	0.1069	0.0958	0.0872	0.0823	0.0796
12	15.00	14.38	0.1070	0.0958	0.0872	0.0823	0.0796
13	16.25	15.63	0.1071	0.0958	0.0872	0.0823	0.0796
14	17.50	16.88	0.1072	0.0958	0.0872	0.0823	0.0796
15	18.75	18.13	0.1073	0.0958	0.0872	0.0823	0.0796
16	20.00	19.38	0.1074	0.0958	0.0872	0.0823	0.0796
17	21.25	20.63	0.1075	0.0958	0.0872	0.0823	0.0796
18	22.50	21.88	0.1076	0.0958	0.0872	0.0823	0.0796
19	23.75	23.13	0.1077	0.0958	0.0872	0.0823	0.0796
20	25.00	24.38	0.1079	0.0958	0.0872	0.0823	0.0796
21	26.25	25.63	0.1080	0.0958	0.0872	0.0823	0.0796
22	27.50	26.88	0.1081	0.0958	0.0872	0.0823	0.0796
23	28.75	28.13	0.1082	0.0958	0.0872	0.0823	0.0796
24	30.00	29.38	0.1083	0.0958	0.0872	0.0823	0.0796
25	31.25	30.63	0.1084	0.0958	0.0872	0.0823	0.0796
26	32.50	31.88	0.1085	0.0958	0.0872	0.0823	0.0796
27	33.75	33.13	0.1086	0.0958	0.0872	0.0823	0.0796
28	35.00	34.38	0.1088	0.0958	0.0872	0.0823	0.0796
29	36.25	35.63	0.1089	0.0958	0.0872	0.0823	0.0796
30	37.50	36.88	0.1090	0.0958	0.0872	0.0823	0.0796
31	38.75	38.13	0.1091	0.0958	0.0872	0.0823	0.0796
32	40.00	39.38	0.1092	0.0958	0.0872	0.0823	0.0796
33	41.25	40.63	0.1093	0.0958	0.0872	0.0823	0.0796
34	42.50	41.88	0.1094	0.0958	0.0872	0.0823	0.0796
35	43.75	43.13	0.1095	0.0958	0.0872	0.0823	0.0796
36	45.00	44.38	0.1096	0.0958	0.0872	0.0823	0.0796
37	46.25	45.63	0.1097	0.1000	0.1000	0.1000	0.1000
38	47.50	46.88	0.1098	0.1000	0.1000	0.1000	0.1000
39	48.75	48.13	0.1099	0.1000	0.1000	0.1000	0.1000
40	50.00	49.38	0.1100	0.1000	0.1000	0.1000	0.1000
41	51.25	50.63	0.1101	0.1000	0.1000	0.1000	0.1000
42	52.50	51.88	0.1102	0.1000	0.1000	0.1000	0.1000
<u>STAGE IV A</u>							
1	53.75	53.13	0.1103	0.1000	0.1000	0.1000	0.1000
2	55.00	54.38	0.1104	0.1000	0.1000	0.1000	0.1000

RUN NO. 55-A (cont.)TOP PRODUCT - CONCENTRATION PROFILES

<u>#</u>	<u>VOL</u>	<u><V></u>	<u>N = 6</u>	<u>N = 7</u>	<u>N = 8</u>	<u>N = 9</u>	<u>N = 10</u>
3	56.25	55.63	0.1104	0.1000	0.1000	0.1000	0.1000
4	57.50	56.88	0.1105	0.1000	0.1000	0.1000	0.1000
5	58.75	58.13	0.1105	0.1000	0.1000	0.1000	0.1000
6	60.00	59.38	0.1106	0.1000	0.1000	0.1000	0.1000
7	61.25	60.63	0.1106	0.1000	0.1000	0.1000	0.1000
8	62.50	61.88	0.1107	0.1000	0.1000	0.1000	0.1000
9	63.75	63.13	0.1107	0.1000	0.1000	0.1000	0.1000
10	65.00	64.38	0.1108	0.1000	0.1000	0.1000	0.1000
11	66.25	65.63	0.1108	0.1000	0.1000	0.1000	0.1000
12	67.50	66.88	0.1109	0.1000	0.1000	0.1000	0.1000
13	68.75	68.13	0.1109	0.1000	0.1000	0.1000	0.1000
14	70.00	69.38	0.1109	0.1000	0.1000	0.1000	0.1000
15	71.25	70.63	0.1110	0.1000	0.1000	0.1000	0.1000
16	72.50	71.88	0.1110	0.1000	0.1000	0.1000	0.1000
17	73.75	73.13	0.1110	0.1000	0.1000	0.1000	0.1000
18	75.00	74.38	0.1111	0.1000	0.1000	0.1000	0.1000
19	76.25	75.63	0.1111	0.1000	0.1000	0.1000	0.1000
20	77.50	76.88	0.1111	0.1000	0.1000	0.1000	0.1000
21	78.75	78.13	0.1111	0.1000	0.1000	0.1000	0.1000
22	80.00	79.38	0.1112	0.1000	0.1000	0.1000	0.1000
23	81.25	80.63	0.1112	0.1000	0.1000	0.1000	0.1000
24	82.50	81.88	0.1112	0.1000	0.1000	0.1000	0.1000
25	83.75	83.13	0.1112	0.1000	0.1000	0.1000	0.1000
26	85.00	84.38	0.1112	0.1000	0.1000	0.1000	0.1000
27	86.25	85.63	0.1112	0.1000	0.1000	0.1000	0.1000
28	87.50	86.88	0.1112	0.1000	0.1000	0.1000	0.1000
29	88.75	88.13	0.1112	0.1000	0.1000	0.1000	0.1000
30	90.00	89.38	0.1112	0.1000	0.1000	0.1000	0.1000
31	91.25	90.63	0.1112	0.1000	0.1000	0.1000	0.1000
32	92.50	91.88	0.1112	0.1000	0.1000	0.1000	0.1000

RUN NO. 55-A

BOTTOM PRODUCT - CONCENTRATION PROFILES

<u>#</u>	<u>VOL</u>	<u>V</u>	<u>N = 1</u>	<u>N = 2</u>	<u>N = 3</u>	<u>N = 4</u>	<u>N = 5</u>
1	1.25	0.63	0	0	0	0	0
2	2.50	1.88	0	0	0	0	0
3	3.75	3.13	0	0	0	0	0
4	5.00	4.38	0	0	0	0	0
5	6.25	5.63	0	0	0	0	0
6	7.50	6.88	0	0	0	0	0
7	8.75	8.13	0	0	0	0	0
8	10.00	9.38	0	0	0	0	0
9	11.25	10.63	0	0	0	0	0
10	12.50	11.88	0	0	0	0	0
11	13.75	13.13	0	0	0	0	0
12	15.00	14.38	0	0	0	0	0.0001
13	16.25	15.63	0.0001	0.0001	0.0002	0.0002	0.0002
14	17.50	16.88	0.0004	0.0006	0.0007	0.0008	0.0009
15	18.75	18.13	0.0014	0.0020	0.0025	0.0029	0.0033
16	20.00	19.38	0.0044	0.0063	0.0078	0.0091	0.0102
17	21.25	20.63	0.0123	0.0177	0.0220	0.0256	0.0288
18	22.50	21.88	0.0311	0.0445	0.0555	0.0648	0.0730
19	23.75	23.13	0.0706	0.1012	0.1225	0.1404	0.1559
20	25.00	24.38	0.1376	0.1895	0.2342	0.2719	0.3046
21	26.25	25.63	0.2418	0.3415	0.4218	0.4895	0.5482
22	27.50	26.88	0.3952	0.5579	0.6887	0.7989	0.8946
23	28.75	28.13	0.5869	0.8283	1.0222	1.1856	1.3277
24	30.00	29.38	0.7970	1.1246	1.3880	1.6099	1.8028
25	31.25	30.63	1.0065	1.4196	1.7516	2.0313	2.2745
26	32.50	31.88	1.2362	1.7354	2.1367	2.4748	2.7688
27	33.75	33.13	1.5701	2.1700	2.6524	3.0591	3.4128
28	35.00	34.38	2.0584	2.7739	3.3501	3.8363	4.2593
29	36.25	35.63	2.6091	3.4277	4.0883	4.6464	5.1324
30	37.50	36.88	3.0756	3.9571	4.6702	5.2736	5.7998
31	38.75	38.13	3.3709	4.2668	4.9935	5.6096	6.1475
32	40.00	39.38	3.4844	4.3538	5.0610	5.6619	6.1871
33	41.25	40.63	3.4496	4.2649	4.9304	5.4969	5.9928
34	42.50	41.88	3.3124	4.0591	4.6706	5.1923	5.6496
35	43.75	43.13	3.1145	3.7875	4.3403	4.8132	5.2282
36	45.00	44.38	2.8873	3.4874	3.9821	4.4063	4.7791
37	46.25	45.63	2.6521	3.1838	3.6724	4.0018	4.3346
38	47.50	46.88	2.4224	2.8919	3.2818	3.6176	3.9136
39	48.75	48.13	2.2059	2.6198	2.9649	3.2628	3.5258
40	50.00	49.38	2.0066	2.3716	2.6770	2.9414	3.1751
41	51.25	50.63	1.8260	2.1483	2.4190	2.6540	2.8620
42	52.50	51.88	1.6643	1.9496	2.1901	2.3993	2.5849
43	53.75	53.13	1.5207	1.7739	1.9883	2.1753	2.3413
44	55.00	54.38	1.3941	1.6197	1.8114	1.9791	2.1283
45	56.25	55.63	1.2831	1.4849	1.6571	1.8081	1.9426
46	57.50	56.88	1.1862	1.3675	1.5229	1.6596	1.7815
47	58.75	58.13	1.1022	1.2658	1.4068	1.5311	1.6422
48	60.00	59.38	1.0296	1.1781	1.3067	1.4204	1.5223

RUN NO. 55-A (cont.)BOTTOM PRODUCT - CONCENTRATION PROFILES

<u>#</u>	<u>VOL</u>	<u>V</u>	<u>N = 6</u>	<u>N = 7</u>	<u>N = 8</u>	<u>N = 9</u>	<u>N = 10</u>
1	1.25	0.63	0	0	0	0	0
2	2.50	1.88	0	0	0	0	0
3	3.75	3.13	0	0	0	0	0
4	5.00	4.38	0	0	0	0	0
5	6.25	5.63	0	0	0	0	0
6	7.50	6.88	0	0	0	0	0
7	8.75	8.13	0	0	0	0	0
8	10.00	9.38	0	0	0	0	0
9	11.25	10.63	0	0	0	0	0
10	12.50	11.88	0	0	0	0	0
11	13.75	13.13	0	0	0	0	0
12	15.00	14.38	0.0001	0.0001	0.0001	0.0001	0.0001
13	16.25	15.63	0.0003	0.0003	0.0003	0.0003	0.0003
14	17.50	16.88	0.0010	0.0011	0.0012	0.0012	0.0013
15	18.75	18.13	0.0036	0.0039	0.0041	0.0044	0.0046
16	20.00	19.38	0.0113	0.0122	0.0130	0.0137	0.0144
17	21.25	20.63	0.0317	0.0343	0.0366	0.0387	0.0406
18	22.50	21.88	0.0802	0.0868	0.0926	0.0979	0.1024
19	23.75	23.13	0.1697	0.1820	0.1930	0.2031	0.2125
20	25.00	24.38	0.3335	0.3595	0.3828	0.4039	0.4231
21	26.25	25.63	0.6004	0.6470	0.6888	0.7268	0.7613
22	27.50	26.88	0.9795	1.0554	1.1237	1.1856	1.2418
23	28.75	28.13	1.4537	1.5663	1.6775	1.7594	1.8428
24	30.00	29.38	1.9738	2.1268	2.2642	2.3890	2.5023
25	31.25	30.63	2.4902	2.6831	2.8564	3.0136	3.1566
26	32.50	31.88	3.0294	3.2626	3.4721	3.6622	3.8350
27	33.75	33.13	3.7265	4.0007	4.2593	4.4881	4.6960
28	35.00	34.38	4.6347	4.9705	5.2724	5.5463	5.7952
29	36.25	35.63	5.5639	5.9502	6.2974	6.6125	6.8988
30	37.50	36.88	6.2671	6.6857	7.0622	7.4037	7.7141
31	38.75	38.13	6.6255	7.0540	7.4395	7.7893	8.1071
32	40.00	39.38	6.6543	7.0732	7.4504	7.7925	8.1035
33	41.25	40.63	6.4343	6.8304	7.1871	7.5108	7.8050
34	42.50	41.88	6.0570	6.4229	6.7525	7.0516	7.3234
35	43.75	43.13	5.5984	5.9309	6.2307	6.5027	6.7499
36	45.00	44.38	5.1119	5.4110	5.6809	5.9257	6.1482
37	46.25	45.63	4.6319	4.8994	5.1408	5.3598	5.5588
38	47.50	46.88	4.1783	4.4167	4.6319	4.8271	5.0045
39	48.75	48.13	3.7612	3.9733	4.1649	4.3387	4.4967
40	50.00	49.38	3.3845	3.5733	3.7439	3.8987	4.0394
41	51.25	50.63	3.0485	3.2168	3.3690	3.5070	3.6325
42	52.50	51.88	2.7515	2.9018	3.0378	3.1613	3.2734
43	53.75	53.13	2.4905	2.6253	2.7473	2.8679	2.9585
44	55.00	54.38	2.2624	2.3836	2.4934	2.5931	2.6836
45	56.25	55.63	2.0637	2.1733	2.2726	2.3626	2.4445
46	57.50	56.88	1.8914	1.9909	2.0811	2.1629	2.2373
47	58.75	58.13	1.7424	1.8332	1.9156	1.9904	2.0583
48	60.00	59.38	1.6142	1.6976	1.7733	1.8419	1.9043

RUN NO. 55-B

THIS IS MODE 7
 VOLUME OF BOTTOM FEED = 52.5 CC
 VOLUME OF TOP FEED = 17.5 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 18
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

N	YT(HB)	YB(HB)	SF(HB)	REC(HB)
1	0.683855	1.000821	1.463499	1.153870
2	0.387729	1.244957	3.211894	0.823595
3	0.240554	1.446634	6.013767	0.679533
4	0.167599	1.620630	9.669712	0.626627
5	0.131613	1.774854	13.485377	0.617631
6	0.114028	1.913722	16.782882	0.629110
7	0.105587	2.039892	19.319473	0.649499
8	0.101678	2.155171	21.195266	0.673136
9	0.100005	2.260584	22.604782	0.697295
10	0.099423	2.357313	23.709915	0.720709
11	0.099365	2.446079	24.617065	0.742824
12	0.099550	2.527575	25.390106	0.763441
13	0.099837	2.602418	26.066589	0.782532
14	0.100160	2.671156	26.668746	0.800144
15	0.100487	2.734297	27.210449	0.816360
16	0.100802	2.792294	27.700881	0.831275
17	0.101098	2.845572	28.146682	0.844986
18	0.101374	2.894508	28.552871	0.857585
19	0.101629	2.939465	28.923522	0.869161
20	0.101864	2.980761	29.262115	0.879796
21	0.102081	3.018695	29.571640	0.889566
22	0.102280	3.053539	29.854721	0.898540
23	0.102463	3.085546	30.113739	0.906784
24	0.102631	3.114952	30.350875	0.914358
25	0.102786	3.141960	30.568008	0.921314

RUN NO. 55-B (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.940324	0.453402	2.073929	1.355922
2	0.935122	0.385835	2.423631	1.332155
3	0.906697	0.341609	2.654193	1.283537
4	0.873794	0.310757	2.811819	1.232345
5	0.843370	0.288241	2.925921	1.186513
6	0.817503	0.271320	3.013061	1.148101
7	0.796348	0.258375	3.082132	1.116910
8	0.779383	0.248369	3.138002	1.091990
9	0.765919	0.240587	3.183539	1.072253
10	0.755294	0.234515	3.220666	1.056695
11	0.746935	0.229767	3.250832	1.044462
12	0.740370	0.226052	3.275225	1.034859
13	0.735219	0.223142	3.294849	1.027324
14	0.731179	0.220863	3.310563	1.021416
15	0.728012	0.219077	3.323096	1.016785
16	0.725530	0.217677	3.333055	1.013154
17	0.723584	0.216580	3.340951	1.010309
18	0.722059	0.215721	3.347192	1.008079
19	0.720864	0.215047	3.352118	1.006331
20	0.719927	0.214519	3.356001	1.004961
21	0.719193	0.214106	3.359056	1.003888
22	0.718618	0.213782	3.361459	1.003047
23	0.718167	0.213527	3.363346	1.002387
24	0.717813	0.213328	3.364830	1.001870
25	0.717537	0.213172	3.365994	1.001466

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	57.895	42.105	31.178	68.822	3.035
2	70.690	29.310	23.659	76.341	7.782
3	79.032	20.968	19.103	80.897	15.962
4	83.906	16.094	16.090	83.910	27.189
5	86.501	13.499	13.971	86.029	39.457
6	87.759	12.241	12.417	87.583	50.568
7	88.293	11.707	11.242	88.758	59.545
8	88.460	11.540	10.334	89.666	66.511
9	88.451	11.549	9.619	90.381	71.963
10	88.368	11.632	9.048	91.952	76.362
11	88.259	11.741	8.587	91.413	80.026
12	88.148	11.852	8.209	91.791	83.158
13	88.044	11.956	7.897	92.103	85.885
14	87.952	12.048	7.637	92.363	88.289
15	87.871	12.129	7.418	92.582	90.423
16	87.801	12.199	7.232	92.768	92.329
17	87.741	12.259	7.073	92.927	94.037
18	87.689	12.311	6.936	93.064	95.572
19	87.644	12.356	6.817	93.183	96.955
20	87.605	12.395	6.714	93.286	98.214
21	87.570	12.430	6.623	93.377	99.333
22	87.540	12.460	6.543	93.457	100.355
23	87.514	12.486	6.472	93.528	101.283
24	87.491	12.509	6.410	93.590	102.126
25	87.470	12.530	6.354	93.646	102.892

RUN NO. 55-C

THIS IS MODE 7

VOLUME OF BOTTOM FEED = 52.5 CC

VOLUME OF TOP FEED = 17.5 CC

VOLUME OF BUFFER TO STAGE IV = 40.0 CC

TOP RESERVOIR DEAD VOLUME = 112.5 CC

BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC

STAGE I DISPLACEMENT = 67.5 CC

STAGE V DISPLACEMENT = 60.0 CC

RETARDATION COEFFICIENT = 1.000000

N	YTAL	YBAL	SFAL	REC
1	1.000000	0.333333	3.000000	1.404761
2	1.000000	0.273368	3.658068	1.389771
3	0.965271	0.236021	4.089771	1.334541
4	0.923796	0.210817	4.381977	1.273435
5	0.885500	0.192800	4.592843	1.218324
6	0.853181	0.179436	4.754790	1.172276
7	0.826974	0.169305	4.884518	1.135112
8	0.806131	0.161529	4.990614	1.105626
9	0.789717	0.155521	5.077893	1.082435
10	0.776858	0.150860	5.149524	1.064277
11	0.766811	0.147238	5.207960	1.050095
12	0.758972	0.144420	5.255295	1.039031
13	0.752860	0.142227	5.293374	1.030407
14	0.748098	0.140519	5.323822	1.023687
15	0.744387	0.139189	5.348040	1.018451
16	0.741497	0.138153	5.367219	1.014372
17	0.739245	0.137346	5.382360	1.011195
18	0.737491	0.136717	5.394274	1.008720
19	0.736124	0.136228	5.403630	1.006792
20	0.735060	0.135846	5.410965	1.005290
21	0.734231	0.135549	5.416707	1.004120
22	0.733585	0.135318	5.421197	1.003209
23	0.733082	0.135138	5.424706	1.002500
24	0.732690	0.134997	5.427443	1.001946
25	0.732385	0.134888	5.429581	1.001515

RUN NO. 56-A

THIS IS MODE 7
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 36
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

N	YT(HB)	YB(HB)	SF(HB)	REC(HB)
1	0.626274	1.041578	1.663134	1.191796
2	0.375592	1.270471	3.382579	1.037655
3	0.237618	1.420339	5.977393	0.964760
4	0.161678	1.522511	9.416943	0.934482
5	0.119884	1.594948	13.304064	0.925921
6	0.096885	1.648167	17.011596	0.927888
7	0.084228	1.688443	20.046005	0.934466
8	0.077271	1.719329	22.250656	0.942455
9	0.073298	1.744016	23.793594	0.951541
10	0.071029	1.763909	24.833541	0.958157
11	0.069735	1.780052	25.526062	0.964741
12	0.068996	1.793217	25.990112	0.971532
13	0.068575	1.803971	26.306488	0.975459
14	0.068335	1.812785	26.527740	0.979619
15	0.068199	1.820024	26.686950	0.983082
16	0.068122	1.825982	26.804733	0.985978
17	0.068078	1.830873	26.893722	0.988377
18	0.068053	1.834904	26.962692	0.991366
19	0.068040	1.838219	27.016845	0.992009
20	0.068032	1.840951	27.059967	0.993367
21	0.068028	1.843199	27.094619	0.994487
22	0.068026	1.845049	27.122741	0.995409
23	0.068025	1.846573	27.145523	0.996171
24	0.068025	1.847832	27.164199	0.996799
25	0.068024	1.848868	27.179534	0.997317

RUN NO. 56-A (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.926400	0.453402	2.043218	1.219272
2	0.919984	0.413372	2.225561	1.192383
3	0.897720	0.389025	2.307613	1.156354
4	0.873686	0.373085	2.341788	1.122633
5	0.852546	0.362065	2.354677	1.094474
6	0.835346	0.354167	2.358620	1.072097
7	0.821849	0.348381	2.359050	1.054743
8	0.811448	0.344087	2.358265	1.041451
9	0.803509	0.340875	2.357192	1.031340
10	0.797481	0.338465	2.356172	1.023675
11	0.792916	0.336650	2.355311	1.017877
12	0.789464	0.335283	2.354621	1.013495
13	0.786857	0.334252	2.354083	1.010186
14	0.784888	0.333474	2.353666	1.007688
15	0.783401	0.332887	2.353352	1.005802
16	0.782279	0.332445	2.353110	1.004378
17	0.781433	0.332111	2.352927	1.003304
18	0.780793	0.331859	2.352790	1.002493
19	0.780311	0.331668	2.352684	1.001881
20	0.779947	0.331525	2.352607	1.001419
21	0.779672	0.331416	2.352547	1.001071
22	0.779465	0.331335	2.352500	1.000808
23	0.779308	0.331273	2.352467	1.000609
24	0.779190	0.331226	2.352441	1.000460
25	0.779101	0.331191	2.352423	1.000346

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	59.665	40.335	30.328	69.672	3.398
2	71.010	28.990	24.549	75.451	7.528
3	79.071	20.929	21.501	78.499	13.794
4	84.384	15.616	19.682	80.318	22.052
5	87.672	12.328	18.501	81.499	31.327
6	89.607	10.393	17.688	82.312	40.124
7	90.704	9.296	17.104	82.896	47.290
8	91.305	8.695	16.676	83.324	52.473
9	91.640	8.360	16.350	83.650	56.086
10	91.822	8.178	16.099	83.901	58.512
11	91.916	8.084	15.904	84.096	60.122
12	91.963	8.037	15.752	84.248	61.197
13	91.984	8.016	15.632	84.368	61.928
14	91.991	8.009	15.537	84.463	62.437
15	91.992	8.008	15.462	84.538	62.804
16	91.989	8.011	15.402	84.598	63.074
17	91.986	8.014	15.354	84.646	63.279
18	91.983	8.017	15.316	84.684	63.438
19	91.980	8.020	15.285	84.715	63.562
20	91.977	8.023	15.260	84.740	63.661
21	91.975	8.025	15.240	84.760	63.741
22	91.973	8.027	15.224	84.776	63.806
23	91.972	8.028	15.211	84.789	63.859
24	91.971	8.029	15.200	84.800	63.902
25	91.970	8.030	15.192	84.808	63.938

RUN NO. 56-C

THIS IS MODE 7
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 22.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 60.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 RETARDATION COEFFICIENT = 1.16E092

N	YTAL	YBAL	SFAL	REC
1	0.926400	0.453402	2.043218	1.219272
2	0.911430	0.402812	2.262668	1.177937
3	0.856586	0.373145	2.295584	1.104342
4	0.824426	0.355749	2.317438	1.061187
5	0.805567	0.345547	2.331278	1.035880
6	0.794507	0.339565	2.339781	1.021039
7	0.788022	0.336057	2.344908	1.012337
8	0.784219	0.334000	2.347963	1.007234
9	0.781989	0.332793	2.349772	1.004242
10	0.780681	0.332086	2.350841	1.002486
11	0.779914	0.331671	2.351469	1.001457
12	0.779465	0.331428	2.351838	1.000854
13	0.779201	0.331285	2.352056	1.000500
14	0.779046	0.331201	2.352183	1.000293
15	0.778956	0.331152	2.352258	1.000171
16	0.778903	0.331124	2.352301	1.000099
17	0.778871	0.331107	2.352326	1.000057
18	0.778853	0.331097	2.352342	1.000033
19	0.778842	0.331091	2.352350	1.000019
20	0.778836	0.331088	2.352355	1.000010
21	0.778832	0.331086	2.352359	1.000006
22	0.778830	0.331084	2.352361	1.000002
23	0.778829	0.331084	2.352361	1.000001
24	0.778828	0.331083	2.352362	1.000000
25	0.778828	0.331083	2.352363	1.000000

RUN NO. 57-B

THIS IS MODE 7
 VOLUME OF BOTTOM FEED = 17.5 CC
 VOLUME OF TOP FEED = 17.5 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 18
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

<u>N</u>	<u>YT(HB)</u>	<u>YB(HB)</u>	<u>SF(HB)</u>	<u>REC(HB)</u>
1	0.571467	0.890274	1.557873	1.383975
2	0.350537	1.055211	3.010269	1.103487
3	0.227366	1.164747	5.122776	0.955904
4	0.158736	1.241966	7.824084	0.881764
5	0.120531	1.299852	10.784380	0.847941
6	0.099294	1.345751	13.553257	0.836000
7	0.087516	1.387871	15.812690	0.835712
8	0.081012	1.416639	17.486831	0.841410
9	0.076443	1.445771	18.913146	0.848470
10	0.073658	1.472099	19.985549	0.857059
11	0.072121	1.496058	20.743713	0.866513
12	0.071299	1.517936	21.289566	0.876103
13	0.070886	1.537951	21.695953	0.885431
14	0.070705	1.556288	22.011108	0.894301
15	0.070652	1.573112	22.265548	0.902628
16	0.070670	1.588553	22.478378	0.910378
17	0.070725	1.602736	22.661453	0.917558
18	0.070798	1.615757	22.822158	0.924189
19	0.070877	1.627720	22.965332	0.930301
20	0.070958	1.638703	23.094039	0.935925
21	0.071036	1.648794	23.210662	0.941099
22	0.071110	1.658068	23.316818	0.945857
23	0.071180	1.666586	23.413635	0.950231
24	0.071245	1.674412	23.502136	0.954251
25	0.071305	1.681602	23.583160	0.957945

RUN NO. 57-B (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.904000	0.453402	1.993814	1.711843
2	0.895631	0.385835	2.321280	1.664311
3	0.849904	0.341609	2.487941	1.567074
4	0.796973	0.310757	2.564613	1.464690
5	0.748030	0.288241	2.595157	1.373026
6	0.706418	0.271320	2.603636	1.296203
7	0.672385	0.258375	2.602357	1.233820
8	0.645094	0.248369	2.597321	1.183980
9	0.623435	0.240587	2.591305	1.144506
10	0.606342	0.234515	2.585518	1.113390
11	0.592896	0.229767	2.580417	1.088925
12	0.582335	0.226052	2.576112	1.069717
13	0.574048	0.223142	2.572569	1.054648
14	0.567549	0.220863	2.569695	1.042832
15	0.562455	0.219077	2.567389	1.033570
16	0.558461	0.217677	2.565548	1.026309
17	0.555331	0.216581	2.564088	1.020618
18	0.552878	0.215721	2.562931	1.016158
19	0.550955	0.215047	2.562018	1.012663
20	0.549448	0.214519	2.561298	1.009923
21	0.548267	0.214106	2.560730	1.007776
22	0.547342	0.213782	2.560285	1.006094
23	0.546616	0.213527	2.559934	1.004775
24	0.546048	0.213328	2.559659	1.003741
25	0.545603	0.213172	2.559443	1.002932

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	61.269	38.731	33.743	66.257	3.106
2	71.871	28.129	26.775	73.225	6.988
3	78.894	21.106	22.678	77.322	12.745
4	83.391	16.609	20.014	79.986	20.066
5	86.123	13.877	18.150	81.850	27.987
6	87.676	12.324	16.778	83.222	35.288
7	88.483	11.517	15.733	84.267	41.150
8	88.843	11.157	14.917	85.083	45.419
9	89.078	10.922	14.267	85.733	49.010
10	89.168	10.832	13.742	86.258	51.673
11	89.155	10.845	13.313	86.687	53.527
12	89.092	10.908	12.962	87.038	54.844
13	89.009	10.991	12.671	87.329	55.814
14	88.922	11.078	12.428	87.572	56.562
15	88.840	11.160	12.224	87.776	57.164
16	88.767	11.233	12.051	87.949	57.669
17	88.703	11.297	11.904	88.096	58.106
18	88.648	11.352	11.779	88.222	58.492
19	88.602	11.398	11.670	88.330	58.838
20	88.563	11.437	11.575	88.425	59.151
21	88.530	11.470	11.493	88.507	59.436
22	88.502	11.498	11.421	88.579	59.698
23	88.478	11.522	11.357	88.643	59.937
24	88.458	11.542	11.301	88.699	60.157
25	88.441	11.558	11.251	88.749	60.360

RUN NO. 58-B

THIS IS MODE 7
 VOLUME OF BOTTOM FEED = 17.5 CC
 VOLUME OF TOP FEED = 52.5 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 18
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

<u>N</u>	<u>YT(HB)</u>	<u>YB(HB)</u>	<u>SF(HB)</u>	<u>REC(HB)</u>
1	0.571467	0.890274	1.557873	1.137124
2	0.350504	1.072706	3.060471	1.092443
3	0.227369	1.169248	5.142503	1.063703
4	0.158739	1.219159	7.680253	1.044762
5	0.120481	1.244021	10.325425	1.031982
6	0.099148	1.255631	12.664201	1.023166
7	0.087248	1.260395	14.446184	1.016963
8	0.080606	1.261754	15.653423	1.012527
9	0.075681	1.261853	16.673263	1.008556
10	0.072688	1.261510	17.355163	1.005839
11	0.070953	1.261018	17.772659	1.004045
12	0.069946	1.260507	18.021133	1.002835
13	0.069361	1.260040	18.166336	1.002005
14	0.069021	1.259641	18.250061	1.001426
15	0.068823	1.259310	18.297821	1.001015
16	0.068707	1.259043	18.324783	1.000719
17	0.068639	1.258832	18.339828	1.000505
18	0.068599	1.258665	18.348037	1.000348
19	0.068576	1.258537	18.352523	1.000233
20	0.068562	1.258439	18.354843	1.000147
21	0.068553	1.258362	18.355957	1.000082
22	0.068548	1.258303	18.356491	1.000033
23	0.068545	1.258257	18.356674	0.999997
24	0.068543	1.258224	18.356704	0.999971
25	0.068542	1.258198	18.356674	0.999950

RUN NO. 58-B (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.904000	0.453402	1.993814	1.082623
2	0.895631	0.447908	2.031332	1.066378
3	0.883278	0.433666	2.136768	1.050799
4	0.871830	0.429114	2.031697	1.037981
5	0.862530	0.426078	2.024345	1.028065
6	0.855376	0.423975	2.017513	1.020611
7	0.850013	0.422485	2.011939	1.015088
8	0.846046	0.421414	2.007635	1.011026
9	0.843130	0.420640	2.004398	1.008050
10	0.840994	0.420078	2.001996	1.005875
11	0.839433	0.419669	2.000228	1.004286
12	0.838294	0.419371	1.998932	1.003125
13	0.837462	0.419154	1.997983	1.002279
14	0.836855	0.418995	1.997289	1.001662
15	0.836412	0.418880	1.996782	1.001212
16	0.836089	0.418796	1.996412	1.000884
17	0.835854	0.418734	1.996143	1.000644
18	0.835682	0.418689	1.995946	1.000469
19	0.835556	0.418657	1.995802	1.000341
20	0.835465	0.418633	1.995698	1.000249
21	0.835398	0.418616	1.995621	1.000181
22	0.835350	0.418603	1.995565	1.000132
23	0.835314	0.418594	1.995524	1.000095
24	0.835288	0.418587	1.995495	1.000070
25	0.835270	0.418582	1.995474	1.000050

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	61.269	38.731	33.743	66.257	3.106
2	71.873	28.127	29.130	70.871	6.217
3	79.528	20.472	27.055	72.945	10.474
4	84.597	15.403	26.034	73.966	15.614
5	87.744	12.256	25.512	74.488	20.902
6	89.613	10.387	25.243	74.757	25.550
7	90.691	9.309	25.105	74.895	29.065
8	91.301	8.699	25.037	74.953	31.426
9	91.763	8.237	25.001	74.999	33.420
10	92.044	7.955	24.981	75.019	34.745
11	92.206	7.794	24.970	75.030	35.549
12	92.299	7.701	24.964	75.036	36.023
13	92.351	7.649	24.962	75.038	36.296
14	92.381	7.619	24.960	75.040	36.451
15	92.397	7.603	24.960	75.040	36.537
16	92.406	7.594	24.960	75.040	36.584
17	92.411	7.589	24.961	75.039	36.609
18	92.414	7.586	24.961	75.039	36.622
19	92.415	7.585	24.962	75.038	36.628
20	92.416	7.584	24.962	75.038	36.631
21	92.416	7.584	24.962	75.037	36.632
22	92.416	7.584	24.963	75.037	36.632
23	92.416	7.584	24.963	75.037	36.631
24	92.416	7.584	24.963	75.037	36.631
25	92.416	7.584	24.963	75.037	36.630

RUN NO. 59-B

THIS IS MODE 7

VOLUME OF BOTTOM FEED = 52.5 CC

VOLUME OF TOP FEED = 52.5 CC

VOLUME OF BUFFER TO STAGE IV = 40.0 CC

TOP RESERVOIR DEAD VOLUME = 112.5 CC

BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC

STAGE I DISPLACEMENT = 67.5 CC

STAGE II DISPLACEMENT = 67.5 CC

STAGE V DISPLACEMENT = 60.0 CC

HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN

ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN

NUMBER OF CELL IN SERIES = 18

HIGH PH = 8.5

THE HIGH PH VALUE FOR K* IS 0.010

LOW PH = 6.0

THE LOW PH VALUE FOR K* IS 3.800

THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

N	YT(HB)	YR(HB)	SF(HB)	REC(HB)
1	0.683855	1.000821	1.463499	1.102854
2	0.387702	1.244851	3.210848	0.963972
3	0.240525	1.407641	5.852363	0.915712
4	0.167483	1.520062	9.075941	0.907575
5	0.131309	1.599868	12.183961	0.915611
6	0.113453	1.657714	14.611417	0.928804
7	0.104684	1.700276	16.242034	0.942359
8	0.100410	1.731926	17.248458	0.954420
9	0.098354	1.755631	17.850051	0.964461
10	0.097365	1.773473	18.210845	0.972528
11	0.096945	1.786948	18.432632	0.978878
12	0.096757	1.797146	18.573715	0.983811
13	0.096688	1.804875	18.666915	0.987615
14	0.096672	1.810737	18.730621	0.990532
15	0.096679	1.815186	18.775436	0.992762
16	0.096692	1.818565	18.807723	0.994464
17	0.096707	1.821133	18.831375	0.995761
18	0.096721	1.823084	18.848876	0.996748
19	0.096732	1.824565	18.861968	0.997499
20	0.096742	1.825689	18.871749	0.998069
21	0.096749	1.826547	18.879180	0.998505
22	0.096755	1.827198	18.884826	0.998835
23	0.096759	1.827691	18.889038	0.999186
24	0.096763	1.828069	18.892272	0.999278
25	0.096765	1.828354	18.894714	0.999422

RUN NO. 59-B (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.940324	0.453402	2.073929	1.055081
2	0.935122	0.440908	2.120899	1.044251
3	0.927443	0.433666	2.138609	1.033866
4	0.920327	0.429114	2.144712	1.025321
5	0.914546	0.426078	2.146425	1.018710
6	0.910099	0.423975	2.146584	1.013741
7	0.906765	0.422485	2.146268	1.011158
8	0.904299	0.421414	2.145867	1.007351
9	0.902486	0.420640	2.145508	1.005366
10	0.901159	0.420076	2.145218	1.003916
11	0.900188	0.419669	2.144997	1.002857
12	0.899480	0.419371	2.144832	1.002184
13	0.898963	0.419154	2.144709	1.001519
14	0.898585	0.418995	2.144618	1.001108
15	0.898310	0.418880	2.144553	1.000808
16	0.898110	0.418796	2.144505	1.000589
17	0.897963	0.418734	2.144470	1.000429
18	0.897856	0.418689	2.144444	1.000313
19	0.897778	0.418657	2.144424	1.000228
20	0.897722	0.418633	2.144411	1.000166
21	0.897680	0.418616	2.144402	1.000120
22	0.897650	0.418603	2.144394	1.000088
23	0.897628	0.418594	2.144389	1.000063
24	0.897612	0.418587	2.144384	1.000046
25	0.897600	0.418582	2.144383	1.000033

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	57.895	42.105	31.178	68.822	3.035
2	70.691	29.309	26.155	73.845	6.810
3	79.407	20.593	23.552	76.448	12.516
4	84.604	15.396	22.015	77.985	19.465
5	87.445	12.555	21.031	78.969	26.152
6	88.916	11.084	20.367	79.633	31.365
7	89.650	10.350	19.903	80.097	34.860
8	90.006	9.994	19.570	80.430	37.013
9	90.173	9.827	19.328	80.672	38.297
10	90.247	9.753	19.151	80.849	39.066
11	90.278	9.722	19.019	80.981	39.538
12	90.288	9.712	18.920	81.080	39.837
13	90.289	9.711	18.847	81.153	40.035
14	90.287	9.713	18.791	81.209	40.170
15	90.283	9.717	18.750	81.250	40.265
16	90.280	9.720	18.718	81.282	40.333
17	90.277	9.723	18.695	81.305	40.383
18	90.275	9.725	18.677	81.323	40.420
19	90.273	9.727	18.663	81.337	40.448
20	90.272	9.728	18.653	81.347	40.469
21	90.271	9.729	18.645	81.355	40.485
22	90.270	9.730	18.639	81.361	40.497
23	90.269	9.731	18.635	81.365	40.505
24	90.269	9.731	18.632	81.368	40.512
25	90.269	9.731	18.629	81.371	40.518

RUN NO. 60-A

THIS IS MODE 8
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE II DISPLACEMENT = 60.0 CC
 STAGE V DISPLACEMENT = 45.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 36
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

<u>N</u>	<u>YT(HB)</u>	<u>YB(HB)</u>	<u>SF(HB)</u>	<u>REC(HB)</u>
1	0.663502	2.331405	3.513789	1.876596
2	0.403583	2.176738	5.393538	1.521778
3	0.256918	2.072013	8.064878	1.311275
4	0.174156	2.001285	11.491364	1.187237
5	0.127453	1.953620	15.328120	1.113366
6	0.101101	1.921556	19.006286	1.069099
7	0.086230	1.900017	22.034210	1.042397
8	0.078294	1.887891	24.112838	1.027831
9	0.073823	1.878859	25.450836	1.018524
10	0.071268	1.871924	26.266036	1.012320
11	0.069807	1.866779	26.741821	1.008183
12	0.068973	1.863054	27.011367	1.005426
13	0.068496	1.860398	27.160675	1.003587
14	0.068223	1.858531	27.241897	1.002361
15	0.068067	1.857231	27.285308	1.001544
16	0.067978	1.856334	27.307922	1.000999
17	0.067927	1.855716	27.319259	1.000636
18	0.067898	1.855295	27.324813	1.000395
19	0.067881	1.855005	27.327209	1.000232
20	0.067872	1.854811	27.328216	1.000124
21	0.067866	1.854675	27.328399	1.000051
22	0.067863	1.854589	27.328414	1.000004
23	0.067861	1.854529	27.328308	0.999973
24	0.067860	1.854490	27.328125	0.999952
25	0.067860	1.854465	27.327926	0.999939

RUN NO. 60-A (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.926400	0.271203	3.415889	1.128173
2	0.919984	0.258240	3.562521	1.114817
3	0.906249	0.249722	3.629036	1.095843
4	0.891784	0.243773	3.658247	1.077369
5	0.878898	0.239440	3.670638	1.061396
6	0.868169	0.236196	3.675622	1.048278
7	0.859518	0.233728	3.677423	1.037776
8	0.852658	0.231833	3.677902	1.029478
9	0.847266	0.230369	3.677870	1.022968
10	0.843047	0.229234	3.677664	1.017881
11	0.839756	0.228354	3.677426	1.013914
12	0.837191	0.227670	3.677206	1.010825
13	0.835194	0.227139	3.677018	1.008420
14	0.833641	0.226726	3.676867	1.006549
15	0.832432	0.226405	3.676744	1.005093
16	0.831492	0.226155	3.676648	1.003961
17	0.830760	0.225961	3.676573	1.003080
18	0.830192	0.225809	3.676514	1.002395
19	0.829749	0.225692	3.676468	1.001863
20	0.829405	0.225601	3.676432	1.001448
21	0.829138	0.225529	3.676405	1.001126
22	0.828930	0.225474	3.676382	1.000875
23	0.828768	0.225431	3.676366	1.000681
24	0.828642	0.225398	3.676353	1.000528
25	0.828544	0.225372	3.676343	1.000411

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	58.268	41.732	10.420	89.580	12.003
2	69.508	30.492	10.605	89.395	19.215
3	77.912	22.088	10.756	89.244	29.268
4	83.662	16.338	10.858	89.142	42.038
5	87.335	12.665	10.918	89.082	56.264
6	89.569	10.431	10.946	89.054	69.863
7	90.882	9.118	10.954	89.046	81.029
8	91.590	8.410	10.937	89.063	88.685
9	91.985	8.015	10.922	89.078	93.635
10	92.205	7.795	10.910	89.091	96.598
11	92.325	7.675	10.899	89.101	98.341
12	92.388	7.612	10.890	89.110	99.326
13	92.420	7.580	10.881	89.119	99.870
14	92.435	7.565	10.873	89.127	100.165
15	92.441	7.559	10.866	89.134	100.321
16	92.442	7.558	10.860	89.140	100.402
17	92.442	7.558	10.855	89.145	100.441
18	92.440	7.560	10.850	89.150	100.460
19	92.438	7.562	10.847	89.153	100.468
20	92.436	7.564	10.844	89.156	100.470
21	92.434	7.566	10.842	89.158	100.470
22	92.433	7.567	10.840	89.160	100.470
23	92.432	7.568	10.838	89.162	100.469
24	92.431	7.569	10.837	89.163	100.468
25	92.430	7.570	10.836	89.164	100.467

RUN NO. 61

THIS IS MODE 7

VOLUME OF BOTTOM FEED = 60.0 CC

VOLUME OF TOP FEED = 10.0 CC

VOLUME OF BUFFER TO STAGE IV = 40.0 CC

TOP RESERVOIR DEAD VOLUME = 112.5 CC

BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC

STAGE I DISPLACEMENT = 67.5 CC

STAGE II DISPLACEMENT = 67.5 CC

STAGE V DISPLACEMENT = 60.0 CC

HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN

ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN

NUMBER OF CELL IN SERIES = 36

HIGH PH = 8.5

THE HIGH PH VALUE FOR K* IS 0.010

LOW PH = 6.0

THE LOW PH VALUE FOR K* IS 3.800

THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436

THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

N	YT(HB)	YB(HB)	SF(HB)	REC(HB)
1	0.703763	1.149755	1.633724	1.169625
2	0.385889	1.445198	3.745111	0.757727
3	0.229270	1.705522	7.438931	0.571174
4	0.152111	1.942748	12.771940	0.494836
5	0.114110	2.163096	18.956207	0.472228
6	0.095405	2.369905	24.840423	0.474850
7	0.086981	2.564823	29.487075	0.490662
8	0.083882	2.748819	32.770050	0.512520
9	0.082122	2.923092	35.594543	0.534901
10	0.081127	3.088321	38.067779	0.557084
11	0.080568	3.245049	40.277160	0.578675
12	0.080258	3.393795	42.286239	0.599481
13	0.080089	3.534986	44.137954	0.619411
14	0.080002	3.669034	45.861862	0.638436
15	0.079960	3.796300	47.477737	0.656556
16	0.079943	3.917125	48.998886	0.673793
17	0.079941	4.031846	50.435424	0.691179
18	0.079946	4.140768	51.794418	0.705747
19	0.079956	4.244205	53.081863	0.720537
20	0.079967	4.342415	54.302246	0.734584
21	0.079980	4.435677	55.459686	0.747925
22	0.079993	4.524222	56.557800	0.760593
23	0.080005	4.608296	57.599884	0.772621
24	0.080017	4.688127	58.588882	0.784043
25	0.080029	4.763927	59.527282	0.794888

RUN NO. 61 (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.944800	0.453402	2.083800	1.414485
2	0.939988	0.374034	2.513109	1.396273
3	0.909582	0.320439	2.838554	1.345180
4	0.873533	0.282014	3.097480	1.288191
5	0.839544	0.253294	3.314507	1.235532
6	0.810113	0.231247	3.503237	1.191340
7	0.785614	0.214049	3.670252	1.152884
8	0.765622	0.200507	3.818439	1.122389
9	0.749479	0.189785	3.949100	1.097795
10	0.736519	0.181270	4.063090	1.078064
11	0.726146	0.174497	4.161353	1.062279
12	0.717858	0.169105	4.245056	1.049669
13	0.711243	0.164808	4.315586	1.039605
14	0.705966	0.161384	4.374448	1.031578
15	0.701758	0.158655	4.423165	1.025176
16	0.698402	0.156480	4.463211	1.020071
17	0.695726	0.154746	4.495937	1.016001
18	0.693593	0.153363	4.522553	1.012756
19	0.691893	0.152261	4.544118	1.010169
20	0.690537	0.151383	4.561535	1.008107
21	0.689456	0.150682	4.575564	1.006462
22	0.688594	0.150124	4.586843	1.005152
23	0.687907	0.149679	4.595896	1.004107
24	0.687360	0.149324	4.603150	1.003273
25	0.686923	0.149041	4.608960	1.002609

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	57.311	42.689	28.282	71.718	3.404
2	70.896	29.104	20.560	79.440	9.412
3	79.868	20.132	15.817	84.183	21.116
4	85.169	14.831	12.676	87.324	39.561
5	88.034	11.966	10.482	89.518	62.830
6	89.464	10.536	8.890	91.110	87.022
7	90.032	9.968	7.703	92.297	108.225
8	90.126	9.874	6.798	93.202	125.130
9	90.125	9.875	6.097	93.903	140.566
10	90.078	9.922	5.544	94.456	154.673
11	90.013	9.987	5.103	94.897	167.607
12	89.944	10.056	4.746	95.254	179.507
13	89.879	10.121	4.455	95.545	191.481
14	89.821	10.179	4.213	95.787	200.620
15	89.771	10.229	4.012	95.988	210.002
16	89.729	10.271	3.841	96.159	218.692
17	89.694	10.306	3.696	96.304	226.755
18	89.665	10.335	3.571	96.429	234.243
19	89.641	10.359	3.463	96.537	241.210
20	89.621	10.379	3.369	96.631	247.702
21	89.605	10.395	3.285	96.715	253.759
22	89.592	10.408	3.212	96.788	259.422
23	89.581	10.419	3.146	96.854	264.723
24	89.573	10.427	3.087	96.913	269.693
25	89.565	10.435	3.034	96.966	274.359

RUN NO. 62 (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.961931	0.453402	2.121583	1.109635
2	0.958612	0.413372	2.319008	1.096191
3	0.947096	0.389025	2.434538	1.078176
4	0.934665	0.373085	2.505235	1.061316
5	0.923731	0.362065	2.551285	1.047236
6	0.914834	0.354167	2.583055	1.036048
7	0.907853	0.348381	2.605917	1.027371
8	0.902473	0.344087	2.622807	1.020725
9	0.898367	0.340875	2.635468	1.015670
10	0.895249	0.338465	2.645028	1.011837
11	0.892887	0.336650	2.652271	1.008938
12	0.891102	0.335283	2.657762	1.006747
13	0.889754	0.334252	2.661924	1.005093
14	0.888735	0.333474	2.665076	1.003843
15	0.887966	0.332887	2.667466	1.002900
16	0.887386	0.332445	2.669273	1.002189
17	0.886948	0.332111	2.670638	1.001652
18	0.886617	0.331859	2.671672	1.001246
19	0.886368	0.331668	2.672452	1.000940
20	0.886180	0.331525	2.673042	1.000710
21	0.886037	0.331416	2.673488	1.000535
22	0.885930	0.331335	2.673822	1.000403
23	0.885849	0.331273	2.674077	1.000304
24	0.885788	0.331226	2.674268	1.000230
25	0.885742	0.331191	2.674415	1.000173

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	57.133	42.867	26.768	73.232	3.646
2	72.869	27.131	20.428	79.572	10.461
3	80.333	19.667	16.912	83.088	20.067
4	83.179	16.821	14.740	85.260	28.603
5	84.123	15.877	13.296	86.704	34.549
6	84.381	15.619	12.288	87.712	38.561
7	84.413	15.587	11.559	88.441	41.435
8	84.380	15.620	11.019	88.981	43.624
9	84.334	15.666	10.610	89.390	45.356
10	84.293	15.707	10.296	89.704	46.754
11	84.259	15.741	10.053	89.947	47.892
12	84.232	15.768	9.863	90.137	48.821
13	84.211	15.789	9.713	90.287	49.581
14	84.196	15.804	9.594	90.406	50.203
15	84.184	15.816	9.499	90.501	50.712
16	84.175	15.825	9.423	90.577	51.129
17	84.168	15.832	9.362	90.638	51.470
18	84.163	15.837	9.313	90.687	51.750
19	84.159	15.841	9.273	90.727	51.979
20	84.156	15.844	9.241	90.759	52.167
21	84.153	15.847	9.215	90.785	52.321
22	84.152	15.848	9.193	90.807	52.447
23	84.150	15.850	9.176	90.824	52.551
24	84.149	15.851	9.162	90.838	52.636
25	84.149	15.851	9.150	90.850	52.706

RUN NO. 63

THIS IS MODE 8
 VOLUME OF BOTTOM FEED = 35.0 CC
 VOLUME OF TOP FEED = 35.0 CC
 VOLUME OF BUFFER TO STAGE IV = 47.5 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 60.0 CC
 STAGE II DISPLACEMENT = 60.0 CC
 STAGE V DISPLACEMENT = 45.0 CC
 HEMOGLOBIN MIGRATION VELOCITY = -0.048 CM/MIN
 ALBUMIN MIGRATION VELOCITY = -0.023 CM/MIN
 NUMBER OF CELL IN SERIES = 36
 HIGH PH = 8.5
 THE HIGH PH VALUE FOR K* IS 0.010
 LOW PH = 6.0
 THE LOW PH VALUE FOR K* IS 3.800
 THE VALUE FOR B WITH ZERO ELECTRIC FIELD IS 0.436
 THE VALUE FOR B WITH FINITE ELECTRIC FIELD IS 0.371

<u>N</u>	<u>YT(HB)</u>	<u>YB(HB)</u>	<u>SF(HB)</u>	<u>REC(HB)</u>
1	0.674307	2.136995	3.169172	1.863215
2	0.406715	2.037986	5.010848	1.498335
3	0.257166	1.972734	7.671064	1.289454
4	0.173582	1.929435	11.115407	1.169295
5	0.126867	1.900529	14.980496	1.099786
6	0.100760	1.881131	18.669357	1.059319
7	0.086170	1.868059	21.678833	1.035586
8	0.078926	1.860775	23.576171	1.023407
9	0.074860	1.855600	24.787551	1.016027
10	0.072343	1.851420	25.592208	1.010971
11	0.070785	1.848162	26.109634	1.007504
12	0.069820	1.845680	26.434967	1.005127
13	0.069222	1.843826	26.636398	1.003496
14	0.068852	1.842457	26.759643	1.002375
15	0.068623	1.841460	26.834396	1.001607
16	0.068481	1.840736	26.879409	1.001078
17	0.068393	1.840217	26.906341	1.000714
18	0.068339	1.839849	26.922393	1.000465
19	0.068305	1.839582	26.931777	1.000293
20	0.068284	1.839395	26.937255	1.000175
21	0.068272	1.839265	26.940429	1.000094
22	0.068264	1.839172	26.942214	1.000039
23	0.068259	1.839107	26.943191	1.000000
24	0.068256	1.839059	26.943679	0.999973
25	0.068254	1.839027	26.943984	0.999955

RUN NO. 63 (cont.)

<u>N</u>	<u>YT(AL)</u>	<u>YB(AL)</u>	<u>SF(AL)</u>	<u>REC(AL)</u>
1	0.920546	0.104537	8.805964	1.137197
2	0.918297	0.098253	9.346260	1.131435
3	0.905394	0.094192	9.612261	1.114167
4	0.889967	0.091431	9.733772	1.094634
5	0.875359	0.089475	9.783260	1.076410
6	0.862734	0.088046	9.798644	1.063816
7	0.852300	0.086979	9.798932	1.047986
8	0.843886	0.086170	9.793293	1.037664
9	0.837196	0.085551	9.785975	1.029469
10	0.831919	0.085074	9.778824	1.023012
11	0.827779	0.084705	9.772533	1.017948
12	0.824540	0.084419	9.767274	1.013988
13	0.822011	0.084197	9.763002	1.011897
14	0.820039	0.084024	9.759580	1.008486
15	0.818502	0.083890	9.756866	1.006637
16	0.817305	0.083785	9.754737	1.005144
17	0.816372	0.083704	9.753064	1.004034
18	0.815646	0.083641	9.751757	1.003117
19	0.815081	0.083592	9.750738	1.002426
20	0.814641	0.083553	9.749936	1.001888
21	0.814298	0.083524	9.749311	1.001470
22	0.814031	0.083500	9.748827	1.001143
23	0.813824	0.083482	9.748455	1.000890
24	0.813662	0.083468	9.748161	1.000692
25	0.813536	0.083457	9.747928	1.000539

<u>N</u>	<u>WT(AL)</u>	<u>WT(HB)</u>	<u>WB(AL)</u>	<u>WB(HB)</u>	<u>ALPHA</u>
1	57.720	42.280	4.664	95.336	27.908
2	69.305	30.695	4.599	95.401	46.833
3	77.879	22.121	4.557	95.443	73.736
4	83.679	16.321	4.524	95.476	108.195
5	87.342	12.659	4.496	95.504	146.558
6	89.542	10.458	4.471	95.529	182.934
7	90.818	9.182	4.449	95.551	212.429
8	91.447	8.553	4.426	95.574	230.888
9	91.792	8.208	4.407	95.593	242.573
10	92.000	8.000	4.393	95.607	250.262
11	92.122	7.878	4.382	95.618	255.157
12	92.193	7.807	4.374	95.626	258.198
13	92.233	7.767	4.367	95.633	261.051
14	92.254	7.746	4.362	95.638	261.163
15	92.265	7.735	4.357	95.643	261.820
16	92.269	7.731	4.354	95.646	262.231
17	92.270	7.730	4.351	95.649	262.419
18	92.269	7.731	4.348	95.652	262.541
19	92.268	7.732	4.347	95.653	262.604
20	92.266	7.734	4.345	95.655	262.636
21	92.264	7.736	4.344	95.656	262.651
22	92.263	7.737	4.343	95.657	262.655
23	92.262	7.738	4.342	95.658	262.654
24	92.261	7.739	4.342	95.658	262.651
25	92.260	7.740	4.341	95.659	262.648

RUN NO. 64

THIS IS MODE 9
 VOLUME OF BOTTOM FEED = 52.5 CC
 VOLUME OF TOP FEED = 17.5 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 RETARDATION COEFFICIENT = 1.160092

<u>N</u>	<u>YTAL</u>	<u>YBAL</u>	<u>SFAL</u>	<u>REC</u>
1	0.940324	0.893219	1.052735	1.465876
2	0.900530	0.770899	1.168156	1.382710
3	0.859138	0.684109	1.255850	1.306316
4	0.822251	0.620556	1.325023	1.241685
5	0.791482	0.573034	1.381212	1.189144
6	0.766653	0.537021	1.427603	1.147331
7	0.746973	0.509503	1.466082	1.114446
8	0.731531	0.488370	1.497903	1.088758
9	0.719485	0.472092	1.524035	1.068769
10	0.710119	0.459530	1.545316	1.053253
11	0.702851	0.449826	1.562497	1.041224
12	0.697219	0.442324	1.576262	1.031905
13	0.692856	0.436523	1.587215	1.024690
14	0.689479	0.432036	1.595882	1.019105
15	0.686865	0.428565	1.602708	1.014783
16	0.684841	0.425879	1.608064	1.011438
17	0.683276	0.423802	1.612253	1.008850
18	0.682064	0.422194	1.615522	1.006847
19	0.681127	0.420950	1.618069	1.005298
20	0.680402	0.419988	1.620049	1.004098
21	0.679840	0.419243	1.621589	1.003171
22	0.679406	0.418667	1.622784	1.002453
23	0.679070	0.418222	1.623709	1.001897
24	0.678810	0.417877	1.624427	1.001468
25	0.678609	0.417610	1.624984	1.001135

RUN NO. 65

THIS IS MODE 10
 VOLUME OF BOTTOM FEED = 52.5 CC
 VOLUME OF TOP FEED = 17.5 CC
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 STAGE V DISPLACEMENT = 60.0 CC
 RETARDATION COEFFICIENT = 1.160092

N	YTAL	YBAL	SFAL	REC
1	0.940324	1.120069	0.839524	1.522588
2	0.811570	1.114010	0.728512	1.350934
3	0.737741	1.085010	0.679939	1.246123
4	0.694329	1.048157	0.662429	1.179544
5	0.667968	1.010799	0.660832	1.135371
6	0.651329	0.976247	0.667177	1.104746
7	0.640363	0.945743	0.677101	1.082629
8	0.632810	0.919513	0.686201	1.066092
9	0.627388	0.897312	0.699186	1.053376
10	0.623353	0.878703	0.709401	1.043392
11	0.620263	0.863201	0.718561	1.035432
12	0.617843	0.850337	0.726586	1.029018
13	0.615917	0.839690	0.733505	1.023812
14	0.614368	0.830893	0.739407	1.019566
15	0.613111	0.823632	0.744400	1.016090
16	0.612087	0.817642	0.748600	1.013239
17	0.611249	0.812704	0.752118	1.010898
18	0.610562	0.808634	0.755053	1.008972
19	0.609998	0.805281	0.757497	1.007388
20	0.609534	0.802517	0.759527	1.006084
21	0.609152	0.800241	0.761211	1.005011
22	0.608838	0.798365	0.762606	1.004127
23	0.608580	0.796821	0.763760	1.003399
24	0.608367	0.795548	0.764714	1.002800
25	0.608191	0.794500	0.765502	1.002306

RUN NO. 66-A

HEMOGLOBIN OPTIMIZATION - Mode 7 ($F_0 = 40.0$ cc)

F_T	F_B	FEED	N	% cal	y_T (HB)	y_B (HB)	SF(HB)	α
3.0	1.0	4.0	25	130	0.0461	1.109	24.05	54.7
			50	123	0.0461	1.011	21.96	47.5
			-	100	0.0461	0.703	15.26	33.0
6.0	2.0	8.0	25	105	0.0465	1.078	23.17	50.9
			42	100	0.0465	1.008	21.69	46.7
9.0	3.0	12.0	25	101	0.0474	1.126	23.76	51.4
18.0	6.0	24.0	25	97	0.0494	1.174	23.74	50.1
			50	96	0.0494	1.160	23.46	49.4
			-	100	0.0494	1.207	24.43	51.5
33.0	11.0	44.0	25	98	0.0531	1.229	23.14	47.5
			-	100	0.0531	1.251	23.56	48.4
52.5	17.5	70.0	25	100	0.0580	1.270	21.91	43.7
87.0	29.0	116.0	25	100	0.0649	1.286	19.83	37.0

RUN NO. 66-B

OPTIMIZATION OF MODE 7
 FR = 0.33333 TIMES FT
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 RETARDATION COEFFICIENT = 1.160002

FT	FR	FEEED	YTAL	YBAL	SFAL	REC
1.0	0.3	1.33	0.033028	0.015171	2.177086	1.10465
2.0	0.7	2.67	0.0664400	0.029689	2.169120	1.004359
3.0	1.0	4.00	0.094598	0.043716	2.163933	1.002419
4.0	1.3	5.33	0.123684	0.057276	2.159446	1.001508
5.0	1.7	6.67	0.151713	0.070393	2.155235	1.000998
10.0	3.3	13.33	0.277739	0.130058	2.135496	1.000194
15.0	5.0	20.00	0.383989	0.181429	2.116474	1.000046
20.0	6.7	26.67	0.474499	0.226184	2.097841	1.000010
25.0	8.3	33.33	0.552288	0.265578	2.079572	1.000000
30.0	10.0	40.00	0.619662	0.300562	2.061681	0.999999
35.0	11.7	46.67	0.678943	0.331083	2.050673	0.999998
40.0	13.3	53.33	0.731008	0.358654	2.038194	0.999998
45.0	15.0	60.00	0.776691	0.384043	2.022403	0.999998
50.0	16.7	66.67	0.816912	0.407499	2.004699	0.999999
55.0	18.3	73.33	0.852437	0.429233	1.985952	0.999999
60.0	20.0	80.00	0.883903	0.449429	1.966723	0.999999
65.0	21.7	86.67	0.914210	0.4691421	1.805823	0.999999
70.0	23.3	93.33	0.940702	0.527459	1.688664	0.999999
75.0	25.0	100.00	0.963776	0.558726	1.599668	0.999999
80.0	26.7	106.67	0.986666	0.586111	1.529855	0.999999
85.0	28.3	113.33	0.999388	0.610295	1.473693	0.999999
90.0	30.0	120.00	0.901960	0.631808	1.427585	0.999999

RUN NO. 67-A

HEMOGLOBIN OPTIMIZATION - Mode 7 (F₀ = 40.0 cc)

F_T	F_B	FEED	N	δ_{cal}	$y_T(HB)$	$y_B(HB)$	SF(HB)	α
1.0	1.0	2.0	25	151	0.0461	1.122	24.34	67.3
			50	146	0.0460	1.024	22.24	58.6
			-	100	0.0460	0.114	2.48	6.5
3.0	3.0	6.0	25	95	0.0474	1.230	25.94	68.7
			50	95	0.0474	1.225	25.83	67.4
			-	100	0.0474	1.321	27.86	72.7
6.0	6.0	12.0	25	89	0.0495	1.394	28.16	72.8
			50	93	0.0495	1.477	29.83	76.7
			-	100	0.0495	1.621	32.74	84.2
9.0	9.0	18.0	25	92	0.0521	1.557	29.90	76.0
			50	97	0.0521	1.660	31.86	80.8
			-	100	0.0521	1.716	32.94	83.5
17.5	17.5	35.0	25	97	0.0581	1.752	30.16	77.2
			50	100	0.0581	1.803	31.03	79.4
35.0	35.0	70.0	25	100	0.0680	1.849	27.18	63.9
52.5	52.5	105.0	25	100	0.0757	1.866	24.64	52.8
89.0	89.0	178.0	25	100	0.1191	1.829	15.35	28.4

RUN NO. 67-B

OPTIMIZATION OF MODE 7
 FB = 1.000000 TIMES FT
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 RETARDATION COEFFICIENT = 1.160000

FT	FB	FEED	YIAL	YBAL	SFAL	REC
0.5	0.5	1.00	0.024928	0.009423	2.645468	1.014307
1.0	1.0	2.00	0.048629	0.018448	2.636078	1.006120
1.5	1.5	3.00	0.071557	0.027219	2.628961	1.003487
2.0	2.0	4.00	0.093749	0.035749	2.622408	1.002234
2.5	2.5	5.00	0.115236	0.044049	2.616060	1.001527
3.0	3.0	10.00	0.213138	0.082446	2.585166	1.000342
3.5	3.5	15.00	0.297438	0.116431	2.554636	1.000101
4.0	4.0	20.00	0.370648	0.146825	2.524411	1.000031
4.5	4.5	30.00	0.493050	0.192169	2.565715	1.000008
5.0	5.0	40.00	0.589322	0.232039	2.539755	1.000002
5.5	5.5	50.00	0.666093	0.268159	2.483948	1.000000
6.0	6.0	60.00	0.728128	0.301034	2.418754	0.999999
6.5	6.5	70.00	0.778827	0.331083	2.352363	0.999999
7.0	7.0	80.00	0.820672	0.358654	2.288196	0.999999
7.5	7.5	90.00	0.855506	0.384043	2.227628	0.999999
8.0	8.0	100.00	0.884723	0.407499	2.171105	0.999999
8.5	8.5	110.00	0.909391	0.429233	2.118641	0.999999
9.0	9.0	120.00	0.930342	0.449429	2.070051	0.999999
9.5	9.5	130.00	0.933882	0.491421	1.900369	1.000000
10.0	10.0	140.00	0.937071	0.527459	1.776574	0.999999
10.5	10.5	150.00	0.939961	0.558726	1.682326	0.999999
11.0	11.0	160.00	0.942592	0.586111	1.608212	1.000000
11.5	11.5	170.00	0.944999	0.610295	1.548429	1.000000
12.0	12.0	180.00	0.947209	0.631808	1.499203	1.000000

RUN NO. 68-A

HEMOGLOBIN OPTIMIZATION - Mode 7 (F₀ = 40.0 cc)

F_T	F_B	FEED	N	% cal	v_T (HB)	v_B (HB)	SF(HB)	α
<u>1.0</u>	<u>3.0</u>	<u>4.0</u>	25	<u>82</u>	<u>0.0474</u>	<u>1.252</u>	<u>26.41</u>	<u>87.5</u>
			50	83	0.0474	1.269	26.76	89.8
			-	100	0.0474	1.962	41.39	138.9
3.0	9.0	12.0	25	67	0.0521	1.830	35.10	115.5
			50	78	0.0523	2.284	43.70	144.3
			-	100	0.0523	3.146	60.15	198.6
6.0	18.0	24.0	25	75	0.0583	2.433	41.70	153.5
			50	92	0.0585	3.100	52.99	195.9
			-	100	0.0585	3.435	58.71	217.0
9.0	27.0	36.0	25	81	0.0639	2.748	43.04	160.7
			50	94	0.0640	3.266	51.05	191.2
			-	100	0.0640	3.524	55.06	206.2
17.5	52.5	70.0	25	94	0.0759	3.342	44.01	148.2
			50	99	0.0760	3.571	47.01	158.4
30.0	90.0	120.0	25	99	0.1224	3.425	27.98	79.3
35.0	105.0	140.0	25	99	0.1668	3.288	19.71	52.7
45.0	135.0	180.0	25	100	0.2765	2.920	10.56	25.6
60.0	180.0	240.0	25	100	0.4190	2.463	5.88	12.7
89.0	267.0	356.0	25	100	0.5824	1.992	3.42	6.3

RUN NO. 68-B

OPTIMIZATION OF MODE 7

FR = 3.00000 TIMES FT
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 RETARDATION COEFFICIENT = 1.160092

FT	FR	FEED	YIAL	YBAL	SFAL	REC
0.3	0.8	1.00	0.024839	0.007372	3.369416	1.14026
0.5	1.5	2.00	0.048304	0.014351	3.365967	1.005887
0.8	2.3	3.00	0.070866	0.021085	3.360949	1.003295
1.0	3.0	4.00	0.092575	0.027590	3.355371	1.002074
1.3	3.8	5.00	0.113477	0.033879	3.349461	1.001393
2.5	7.5	10.00	0.207299	0.062499	3.316816	1.000292
3.8	11.3	15.00	0.286467	0.085327	3.357301	1.000093
5.0	15.0	20.00	0.354697	0.098573	3.598326	1.000057
7.5	22.5	30.00	0.465140	0.123947	3.752728	1.000027
10.0	30.0	40.00	0.550304	0.147932	3.719965	1.000013
12.5	37.5	50.00	0.617644	0.170640	3.619576	1.000008
15.0	45.0	60.00	0.671973	0.192169	3.496791	1.000004
17.5	52.5	70.00	0.716535	0.212608	3.370216	1.000002
20.0	60.0	80.00	0.753593	0.232039	3.247702	1.000001
22.5	67.5	90.00	0.784772	0.250534	3.132399	1.000000
25.0	75.0	100.00	0.811270	0.268159	3.025331	1.000000
27.5	82.5	110.00	0.833985	0.284974	2.926527	1.000000
30.0	90.0	120.00	0.853607	0.301034	2.835581	1.000000
32.5	97.5	130.00	0.870671	0.316389	2.751904	1.000000
35.0	105.0	140.00	0.885600	0.331083	2.674859	0.999999
37.5	112.5	150.00	0.898731	0.345159	2.603818	0.999999
40.0	120.0	160.00	0.910336	0.358654	2.538197	0.999999
42.5	127.5	170.00	0.920637	0.371605	2.477459	1.000000
45.0	135.0	180.00	0.929817	0.384043	2.421124	0.999999

RUN NO. 68-B (cont.)

OPTIMIZATION OF MODE 7
 FB = 3.00000 TIMES FT
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 RETARDATION COEFFICIENT = 1.160002

FT	FB	FEED	YTAL	YBAL	SFAL	REC
1.0	3.0	4.00	0.092575	0.027590	3.355371	1.002074
2.0	6.0	8.00	0.171761	0.051574	3.330374	1.000517
3.0	9.0	12.00	0.240482	0.072813	3.302756	1.000173
4.0	12.0	16.00	0.300949	0.088007	3.419606	1.000085
5.0	15.0	20.00	0.354697	0.098573	3.598326	1.000057
10.0	30.0	40.00	0.550304	0.147932	3.719965	1.000013
15.0	45.0	60.00	0.671973	0.192169	3.496791	1.000004
20.0	60.0	80.00	0.753593	0.232039	3.247702	1.000001
25.0	75.0	100.00	0.811270	0.268159	3.025331	1.000000
30.0	90.0	120.00	0.853607	0.301034	2.835581	1.000000
35.0	105.0	140.00	0.885600	0.331083	2.674859	0.999999
40.0	120.0	160.00	0.910336	0.358654	2.538197	0.999999
45.0	135.0	180.00	0.929817	0.384043	2.421124	0.999999
50.0	150.0	200.00	0.945395	0.407499	2.319995	1.000000
55.0	165.0	220.00	0.958010	0.429233	2.231912	1.000000
60.0	180.0	240.00	0.968337	0.449429	2.154593	1.000000
65.0	195.0	260.00	0.977458	0.471421	1.974798	0.999999
70.0	210.0	280.00	0.972311	0.527459	1.843386	0.999999
75.0	225.0	300.00	0.973945	0.558726	1.743152	0.999998
80.0	240.0	320.00	0.975396	0.586111	1.664181	0.999998
85.0	255.0	340.00	0.976694	0.610295	1.600363	0.999999
90.0	270.0	360.00	0.977862	0.631808	1.547719	0.999999

RUN NO. 69-A

HEMOGLOBIN OPTIMIZATION - Mode 7 (F₀ = 40.0 cc)

F_T	F_B	FEED	N	δ_{cal}	y_T (HB)	y_B (HB)	SF(HB)	α
<u>1.0</u>	<u>7.0</u>	<u>8.0</u>	<u>25</u>	<u>50</u>	<u>0.0504</u>	<u>1.640</u>	<u>32.51</u>	<u>125.0</u>
			50	55	0.0505	1.999	39.57	154.9
			-	100	0.0505	5.627	111.4	436.1
3.0	21.0	24.0	25	52	0.0602	2.898	48.16	239.8
			50	68	0.0605	4.170	68.94	348.0
			-	100	0.0605	6.770	111.9	564.8
6.0	42.0	48.0	25	67	0.0712	4.380	61.52	323.1
			50	89	0.0715	6.142	85.88	455.3
			-	100	0.0715	7.023	98.22	520.7
9.0	63.0	72.0	25	76	0.0817	5.169	63.30	312.1
			50	93	0.0819	6.527	79.73	395.5
			-	100	0.0819	7.063	86.24	427.8
13.0	91.0	104.0	25	87	0.1228	5.724	46.61	206.0
			50	99	0.1230	6.679	54.31	240.9
17.5	122.5	140.0	25	94	0.2302	5.355	23.26	91.6
			50	99	0.2303	5.807	25.21	99.5
22.5	157.5	180.0	25	97	0.3541	4.686	13.23	46.6
			-	100	0.3541	4.892	13.81	48.7
35.0	245.0	280.0	25	100	0.5514	3.487	6.32	18.0
60.0	420.0	480.0	25	100	0.7220	2.464	3.41	7.5
89.0	623.0	712.0	25	100	0.8069	1.992	2.47	4.5

RUN NO. 69-B

OPTIMIZATION OF MODE 7
 FB = 7.00000 TIMES FT
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TCP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 RETARDATION COEFFICIENT = 1.160092

FT	FB	FEED	YTAL	YBAL	SFAL	RFC
0.1	7.9	1.00	0.024785	0.006341	3.908555	1.013889
0.3	1.8	2.00	0.048107	0.012283	3.916656	1.005776
0.4	2.6	3.00	0.070449	0.017976	3.919081	1.003206
0.5	3.5	4.00	0.091869	0.023438	3.919733	1.002004
0.6	4.4	5.00	0.112423	0.028683	3.919433	1.001335
1.3	8.8	10.00	0.203848	0.052133	3.910151	1.000275
1.9	13.1	15.00	0.280103	0.064711	4.328502	1.000121
2.5	17.5	20.00	0.344739	0.071686	4.809036	1.000082
3.8	26.3	30.00	0.448022	0.085327	5.250669	1.000046
5.0	35.0	40.00	0.526778	0.098573	5.344051	1.000029
6.3	43.8	50.00	0.588710	0.111441	5.282713	1.000019
7.5	52.5	60.00	0.638608	0.123947	5.152264	1.000013
8.8	61.3	70.00	0.679603	0.136106	4.993181	1.000010
10.0	70.0	80.00	0.713830	0.147932	4.825374	1.000007
11.3	78.8	90.00	0.742794	0.159439	4.658787	1.000005
12.5	87.5	100.00	0.767587	0.170640	4.498290	1.000004
13.8	96.3	110.00	0.789021	0.181546	4.346126	1.000002
15.0	105.0	120.00	0.807700	0.192169	4.203125	1.000002
16.3	113.8	130.00	0.824125	0.202519	4.069367	1.000001
17.5	122.5	140.00	0.838643	0.212608	3.944550	1.000001
18.8	131.3	150.00	0.851558	0.222445	3.828175	1.000000
20.0	140.0	160.00	0.863107	0.232039	3.719666	1.000000
21.3	148.8	170.00	0.873485	0.241399	3.618426	1.000000
22.5	157.5	180.00	0.882851	0.250534	3.523876	1.000000

RUN NO. 69-B (cont.)

OPTIMIZATION OF MODE 7
 FB = 7.00000 TIMES FT
 VOLUME OF BUFFER TO STAGE IV = 40.0 CC
 TOP RESERVOIR DEAD VOLUME = 112.5 CC
 BOTTOM RESERVOIR DEAD VOLUME = 90.0 CC
 STAGE I DISPLACEMENT = 67.5 CC
 RETARDATION COEFFICIENT = 1.160092

FT	FB	FEED	YTAL	YBAL	SFAL	REC
1.0	7.0	8.00	0.169376	0.043262	3.915080	1.000489
2.0	14.0	16.00	0.293881	0.066115	4.445022	1.000112
3.0	21.0	24.00	0.389672	0.077190	5.048187	1.000064
4.0	28.0	32.00	0.465431	0.088007	5.288581	1.000042
5.0	35.0	40.00	0.526778	0.098573	5.344051	1.000029
10.0	70.0	80.00	0.713830	0.147932	4.825374	1.000007
15.0	105.0	120.00	0.807709	0.192169	4.203125	1.000002
20.0	140.0	160.00	0.863107	0.232039	3.719666	1.000000
25.0	175.0	200.00	0.899051	0.268159	3.352680	1.000000
30.0	210.0	240.00	0.923876	0.301034	3.069005	1.000000
35.0	245.0	280.00	0.941796	0.331083	2.844592	0.999998
40.0	280.0	320.00	0.955167	0.358654	2.663196	0.999998
45.0	315.0	360.00	0.965402	0.384043	2.513783	0.999998
50.0	350.0	400.00	0.973397	0.407499	2.388713	0.999999
55.0	385.0	440.00	0.979746	0.429233	2.282550	0.999999
60.0	420.0	480.00	0.984857	0.449429	2.191350	0.999999
65.0	455.0	520.00	0.985974	0.4691421	2.06373	0.999999
70.0	490.0	560.00	0.986939	0.527459	1.871119	0.999999
75.0	525.0	600.00	0.987780	0.558726	1.767913	0.999999
80.0	560.0	640.00	0.988518	0.586111	1.686570	0.999999
85.0	595.0	680.00	0.989173	0.610295	1.620810	0.999999
90.0	630.0	720.00	0.989757	0.631808	1.566545	0.999999

REFERENCES

- Ahmed, Z.M., Paper F 2-2, AIChE-GVC joint meeting, Munich, Germany, Vol IV of preprints, September, 1974.
- Ahmed, Z.M., "Parametric Pumping with pH and Ionic Strength, Enzyme Purification," D. Eng. Sc. Dissertation, New Jersey Institute of Technology, 1981.
- Alexis, R.W., "Upgrading Hydrogen via Heatless Adsorption," Chemical Engineering Progress, 63, No. 5, 1967, pp. 69-71.
- Baker, B. and Pigford, R.L., "Cycling Zone Adsorption: Quantitative Theory and Experimental Results," I&EC Fundamentals, 10, 1971, pp. 283-292.
- Breck, D.W., Zeolite Molecular Sieves, Structure, Chemistry and Use, New York: John Wiley & Sons, Inc., 1974, p. 710.
- Bird, R.B., Stewart, W.E. and Lightfoot, E.N., Transport Phenomena, New York: John Wiley & Sons, Inc., 1960, pp. 702-705.
- Broughton, D.B., Bieser, H.J. and Anderson, M.C., "SORBEX The Continuous Countercurrent Adsorption Technique," U.O.P. Process Division Technology Conference, Des Plaines, Illinois, September-November, 1975.
- Busbice, M.F. and Wankat, P.C., "pH Cycling Zone Separation of Sugars. A Preparative Separation Technique for Counter Current Distribution and Chromatography," Journal of Chromatography, 114, 1975, pp. 369-381.
- Butts, T.J., Sweed, N.H. and Camero, H.H., "Batch Fractionation of Ionic Mixtures by Parametric Pumping," I&EC Fundamentals, 12, 1973, pp. 467-472.
- Chan, Y.N.I., Hill, F.B. and Wong, Y.W., "Equilibrium Theory of a Pressure Swing Adsorption Process," Chemical Engineering Science, 36, 1981, pp. 243-251.
- Chao, J.F., Huang, J.J. and Huang, C.R., "Continuous Multiafinity Separation of Proteins: Cyclic Processes," Paper No. 42f, AIChE Meeting, New Orleans, Louisiana, November, 1981.
- Chao, R. and Hoelscher, H.E., "Simultaneous Axial Dispersion and Adsorption in a Packed Bed," AIChE Journal, 12, 1966, pp. 271-278.
- Chen, H.T. and Hill, F.B., "Characteristics of Batch, Semi-continuous, and Continuous Equilibrium Parametric Pumps," Separation Science, 6, 1971, pp. 411-434.

- Chen, H.T., Rak, J.L., Stokes, J.D. and Hill, F.B. "Separations via Continuous Parametric Pumping," AICHE Journal, 18, 1972a, pp. 356-361.
- Chen, H.T., Jaferi, J. and Stokes, J.D., Paper 9e presented at the 73rd AIChE National Meeting Minneapolis, MN, August, 1972b.
- Chen, H.T., Reise, E.H., Stokes, J.D. and Hill, F.B., "Separations via Semicontinuous Parametric Pumping," AICHE Journal, 19, 1973, pp. 589-595.
- Chen, H.T., Park, J.A. and Rak, J.L., "Equilibrium Parametric Pumps," Separation Science, 2, 1974a, pp. 35-45.
- Chen, H.T. and Manganaro, J.A., "Optional Performance of Equilibrium Parametric Pumps," AICHE Journal, 20, 1974c, pp. 1020-1022.
- Chen, H.T. and D'Emidio, V.J., "Separation of Isomers via Thermal Parametric Pumping," AICHE Journal, 21, 1975, pp. 813-815.
- Chen, H.T. Lin, W.W., Stokes, J.D. and Fabrisiak, W.R., "Separation of Multicomponent Mixtures via Thermal Parametric Pumping," AICHE Journal, 20, 1974b, pp. 306-310.
- Chen, H.T., Rastogi, A.K., Kim, C.Y. and Rak, J.L., "Nonequilibrium Parametric Pumps," Separation Science, 11, 1976, pp. 335-346.
- Chen H.T., Hsieh, T.K., Lee, H.C. and Hill, F.B., "Separation of Proteins via Semicontinuous pH Parametric Pumping," AICHE Journal, 23, 1977, pp. 695-701.
- Chen, H.T., Wong, Y.W. and Wu, S., "Continuous Fractionation of Protein Mixtures by pH Parametric Pumping," AICHE Journal, 25, 1979a, pp. 320-327.
- Chen, H.T., "Parametric Pumping," in P.A. Schweitzer (ed.), Handbook of Separation Techniques for Chemical Engineers, New York: McGraw-Hill, 1979b, Section 1.15, pp. 467-486.
- Chen, H.T., Yang, W.T., Pancharoen, U. and Parisi, R., "Separation of Proteins via Multicolumn pH Parametric Pumping," AICHE Journal, 26, 1980a, pp. 839-849.
- Chen, H.T., Pancharoen, U., Yang, W.T., Kerobo, C.O. and Parisi, R.J., "Separation of Proteins via pH Parametric Pumping," Separation Science and Technology, 15, 1980b, pp. 1377-1391.

- Chen, H.T., "Protein Separation Using Parametric Pumping," Gordon Research Conference on Separation and Purification, New London, New Hampshire, August, 1980c.
- Chen, H.T., Yang, W.T., Wu, C.M., Kerobo, C.O. and Jajalla, V. "Semicontinuous pH Parametric Pumping: Process Characteristics and Protein Separations," Separation Science and Technology, 16, 1981a, pp. 43-61.
- Chen, H.T., Ahmed, Z.M. and Rollan, V., "Parametric Pumping with pH and Ionic Strength: Enzyme Purification," I&EC Fundamentals, 20, 1981b, pp. 171-174.
- Chen, H.T., Hollein, H.C. (speaker) and Ma, H.C., "Parametric Pumping with pH and Electric Field: Protein Separations," Presented at the 2nd World Congress of Chemical Engineering, Montreal, Canada, Vol. I of Proceedings, October, 1981c, pp. 222-226.
- Chen, H.T., Kerobo, C.O., Hollein, H.C. and Huang, C.R., "Research on Parametric Pumping," Chemical Engineering Education, 15, No. 4, 1981d, pp. 166-171.
- Chiang, A.S., Kmietek, E.H., Langan, S.M., Noble, P.T., Reis, J.F.G. and Lightfoot, E.N., "Preliminary Experimental Survey of Hollow-Fiber Electropolarization Chromatography (Electrical Field-Flow Fractionation) for Protein Fractionation," Separation Science and Technology, 14, 1979, pp. 453-474.
- Chin, H.P., Cellulose Acetate Electrophoresis: techniques and applications, Ann Arbor: Humphrey Science Publishers, 1940, p. 12.
- Collins, J.J., "Where to Use Molecular Sieves," Chemical Engineering Progress, 64, no. 8, 1968, pp. 66-71.
- Colowick, S.P. and Kaplan, N.O., Methods in Enzymology, New York: Academic Press, 1955, p. 143.
- Costa, C.A.V., Rodrigues, A.E., Grevillot, G. and Tondeur, D., "Purification of Phenolic Wastewater by Parametric Pumping: Nonmixed Dead Volume Equilibrium Model," AIChE Journal, 28, 1982, pp. 73-85.
- Dore, J.C. and Wankat, P.C., "Multicomponent Cycling Zone Adsorption," Chemical Engineering Science, 31, 1976, pp. 921-927.
- Doshi, K.J., Katira, C.H. and Stewart, H.A., "Optimization of a Pressure Swing Cycle," in M.N.Y. Lee and I. Zwiebel (eds.), Adsorption Technology, AIChE Symposium Series, 67, No. 117, 1971, pp. 90-97.

- Foo, S.C. and Rice, R.G., "On the Prediction of Ultimate Separation in Parametric Pumps," AICHE Journal, 21, 1975, pp. 1149-1158.
- Foo, S.C. and Rice, R.G., "Steady State Predictions for Non-equilibrium Parametric Pumps," AICHE Journal, 23, 1977, pp. 120-123.
- Foo, S.C., Bergsman, K.H. and Wankat, P.C., "Multicomponent Fractionation by Direct Thermal Mode Cycling Zone Adsorption," I&EC Fundamentals, 19, 1980, pp. 86-93.
- Fox, C.R., "Removing Toxic Organics from Waste Water," Chemical Engineering Progress, 75, No. 8, 1979, pp. 70-77.
- Geankoplis, C.J., Transport Processes and Unit Operations, Boston: Allyn and Bacon, Inc., 1978, pp. 307-308.
- Graham, E.E. and Fook, C.F., "Rate of Protein Absorption and Desorption on Cellulostic Ion Exchangers," AICHE Journal, 28, 1982, pp. 245-250.
- Gregory, R.A. and Sweed, N.H., "Parametric Pumping: Behavior of Open Systems. Part II. Experiment and Computation," Chemical Engineering Journal, 4, 1972, pp. 139-148.
- Gregory, R.A., "Comparison of Parametric Pumping with Conventional Adsorption," AICHE Journal, 20, 1974, pp. 294-300.
- Grevillot, G. and Tondeur, D., "Equilibrium Staged Parametric Pumping. I. Single Transfer Step per Half-cycle and Total Reflux - the Analogy with Distillation," AICHE Journal, 22, 1976, pp. 1055-1063.
- Grevillot, G. and Tondeur, D., "Equilibrium Staged Parametric Pumping. II. Multiple Transfer Steps per Half-cycle and Reservoir Staging," AICHE Journal, 23, 1977, pp. 840-851.
- Grevillot, G., "Equilibrium Staged Parametric Pumping: Part III. Open Systems at Steady-State-McCabe-Thiele Diagrams," AICHE Journal, 26, 1980, pp. 120-131.
- Griesmer, G.J., Avery, W.F. and Lee, M.N.Y., "Separate n-Paraffins with ISOSIV," Hydrocarbon Processing, 44, No. 6, 1965, pp. 147-150.
- Guccione, E., "Recovery of n-Paraffins by Molecular Sieve Adsorption," Chemical Engineering, April 26, 1965, pp. 104-106.

- Gupta, R. and Sweed, N.H., "Equilibrium Theory of Cycling Zone Adsorption," I&EC Fundamentals, 10, 1971, pp. 280-283.
- Hill, F.B., Wong, Y.W. and Chan, Y.N.I., "A Temperature Swing Process for Hydrogen Isotope Separation," AICHE Journal, 28, 1982, pp. 1-6.
- Hollein, H.C., Ma, H.C., Huang, C.R. and Chen, H.T., "Parametric Pumping with pH and Electric Field: Protein Separations," I&EC Fundamentals, in press, accepted January, 1982.
- Houghton, G., "Band Shapes in Non-Linear Chromatography with Axial Dispersion," Journal of Physical Chemistry, 67, 1963, pp. 84-88.
- Jeczewski, T.J. and Myers, A.L., "Separation of Gas Mixtures by Pulsed Adsorption," I&EC Fundamentals, 9, 1970, pp. 216-221.
- Keller, K.H., Canales, E.R. and Yum, S.II., "Tracer and Mutual Diffusion Coefficients of Proteins," Journal of Physical Chemistry, 75, 1971, pp. 379-387.
- Lapidus, L. and Amundson, N.R., "Mathematics of Adsorption in Beds. VI. The Effect of Longitudinal Diffusion in Ion Exchange and Chromatographic Columns," Journal of Physical Chemistry, 56, 1952, pp. 984-988.
- Lee, H-L., Reis, J.F.G., Dohner, J. and Lightfoot, E.N., "Single-phase Chromatography: Solute Retardation by Ultrafiltration and Electrophoresis," AICHE Journal, 20, 1974, pp. 776-784.
- Mickley, H.S., Sherwood, T.K. and Reed, C.E., Applied Mathematics in Chemical Engineering, New York: McGraw-Hill, 1957, pp. 303-307.
- Möller, W.J.H.M., van Os, G.A.J. and Overbeek, J.Th.G., "Electric Conductivity and Transference of Alkali Albuminates," Transactions of the Faraday Society, 57, 1961, pp. 312-324.
- Mysels, E.K. and Mysels, K.J., "Tracer Electrophoresis. V. The Mobility and Charge of Human Serum Albumin at Low Concentrations and Low Ionic Strengths," American Chemical Society Journal, 83, 1961, pp. 2049-2055.
- Nelson, W.C., Silarski, D.F. and Wankat, P.C., "Continuous Flow Equilibrium Staged Model for Cycling Zone Adsorption," I&EC Fundamentals, 17, 1978, pp. 32-38.

- Ohman, J., "Preparative Protein Separation: I. Polyacrylamide Gel Electrophoresis in the LKB UNIPHOR," LKB Application Note, 64, March 5, 1973, pp. 1-8.
- Oren, Y. and Soffer, A., "Electrochemical Parametric Pumping," Journal of the Electrochemical Society, 125, 1978, pp. 869-875.
- Pancharoen, U., D. Eng. Sc. Dissertation, New Jersey Institute of Technology, 1982.
- Pauling, L., Itano, H.A., Singer, S.J. and Wells, I.C., "Sickle Cell Anemia, a Molecular Disease," Science, 110, 1949, pp. 543-548.
- Pharmacia Fine Chemicals, "Ion Exchange Chromatography Principles and Methods," 1980.
- Pharmacia Fine Chemicals, "A Process for Human Albumin."
- Pigford, R.L., Baker, B. and Blum, D.E., "Cycling Zone Adsorption, A New Separation Process," I&EC Fundamentals, 8, 1969a, pp. 848-51.
- Pigford, R.L., Baker, B. and Blum, D.E., "An Equilibrium Theory of the Parametric Pump," I&EC Fundamentals, 8, 1969b, pp. 144-149.
- Rak, J.L., "The Continuous Nonequilibrium Parametric Pump: A Theoretical and Experimental Study," D. Eng. Sc. Dissertation, New Jersey Institute of Technology, 1978.
- Rasmuson, A., "Exact Solution of a Model for Diffusion and Transient Adsorption in Particles and Longitudinal Dispersion in Packed Beds," AIChE Journal, 27, 1981, pp. 1032-1035.
- Reis, J.F.G., Lightfoot, E.N. and Lee, H.L., "Concentration Profiles in Free-Flow Electrophoresis," AIChE Journal, 20, 1974, pp. 362-368.
- Reis, J.F.G. and Lightfoot, E.N., "Electropolarization Chromatography," AIChE Journal, 22, 1976, pp. 779-785.
- Rice, R.G., "Progress in Parametric Pumping," Separation and Purification Methods, 5, 1976, pp. 139-188.
- Rice, R.G., "Dispersion and Ultimate Separation in the Parametric Pump," I&EC Fundamentals, 12, 1973, pp. 406-412.
- Rice, R.G. and Foo, S.C., "Continuous Desalination Using Cyclic Mass Transfer on Bifunctional Resins," I&EC Fundamentals, 20, 1981, pp. 150-155.

- Righetti, P.G. and Caravaggio, T., "Isoelectric Points and Molecular Weights of Proteins, A Table," Journal of Chromatography, 127, 1976, pp. 1-28.
- Rolke, R.W. and Wilhelm, R.H., "Recuperative Parametric Pumping: Model Development and Experimental Evaluation," I&EC Fundamentals, 8, 1969, pp. 235-246.
- Sabadell, J.E. and Sweed, N.H., "Parametric Pumping with pH," Separation Science, 5, 1970, pp. 171-181.
- Shafer, J.A., "Protein Interactions and Their Application to Problems in Protein Separation," Paper No. 5a, AIChE National Meeting, Detroit, Michigan, August, 1981.
- Shaffer, A.G. and Hamrin, C.E., "Enzyme Separation by Parametric Pumping," AIChE Journal, 21, 1975, pp. 782-86.
- Shah, A.B., Reis, J.F.G., Lightfoot, E.N. and Moore, R.E., "Modeling Electroretention of Proteins during Electropolarization Chromatography," Separation Science and Technology, 14, 1979, pp. 475-497.
- Shendalman, L.H. and Mitchell, J.E., "A Study of Heatless Adsorption in the Model System CO₂ in He, I," Chemical Engineering Science, 27, 1972, pp. 1449-58.
- Skarstrom, C.W., Annals of the New York Academy of Sciences, 72, 1959, p. 715.
- Soffer, A. and Folman, M. "The Electrical Double Layer of High Surface Porous Carbon Electrode," Journal of Electroanalytical Chemistry, 38, 1972, pp. 25-43.
- Stokes, J.D., "Separations of Multicomponent Mixtures via Thermal Parametric Pumping," D. Eng. Sc. Dissertation, New Jersey Institute of Technology, 1976.
- Stokes, J.D. and Chen, H.T., "Design and Scale-up of a Continuous Thermal Parametric Pumping System," I&EC Process Design and Development, 18, 1979, pp. 147-154.
- Sweed, N.H. and Wilhelm, R.H., "Parametric Pumping. Separations via Direct Thermal Mode," I&EC Fundamentals, 8, 1969, pp. 221-231.
- Sweed, N.H. and Gregory, R.H., "Parametric Pumping: Modeling Direct Thermal Separations of Sodium Chloride-Water in Open and Closed Systems," AIChE Journal, 17, 1971, pp. 171-176.

- Sweed, N.H. and Rigaudeau, J.M., "Equilibrium Theory and Scale-Up of Parametric Pump," in I. Zwiebel and N.H. Sweed, Adsorption and Ion Exchange, AIChE Symposium Series, 71, No. 152, 1975, pp. 1-5.
- Thompson, D.W. and Bowen, B.D., "Equilibrium Theory of the Parametric Pump. Effect of Boundary Conditions," I&EC Fundamentals, 11, 1972, pp. 415-417.
- Thompson, D.W. and Bass, D., "Cyclic Electrodialysis: Experimental Results in a Closed System," Canadian Journal of Chemical Engineering, 52, 1974a, pp. 345-54.
- Thompson, D.W., Bass, D. and Abu-Goukh, M.E., "Rate Models for Cyclic Separation Processes - Comparison with Experimental Results in Cyclic Electrodialysis," Canadian Journal of Chemical Engineering, 52, 1974b, pp. 479-86.
- Union Carbide Corporation, Linde Division, "ISOSIV," Hydrocarbon Processing, 55, No. 9, 1976, p. 222.
- van Oss, C.J., "Electrokinetic Separation Methods," Separation and Purification Methods, 8, 1949, pp. 119-198.
- Varuntanya, C., M.S. Thesis, New Jersey Institute of Technology, 1982.
- Wankat, P.C., "Liquid-Liquid Extraction Parametric Pumping," I&EC Fundamentals, 12, 1973, pp. 372-81.
- Wankat, P.C., "Cyclic Separation Processes," Separation Science, 2, 1974a, pp. 85-116.
- Wankat, P.C., "Thermal Wave Cycling Zone Separation. A Preparative Separation Technique for Counter-Current Distribution and Chromatography," Journal of Chromatography, 88, 1974b, pp. 211-228.
- Wankat, P.C., "Multicomponent Cycling Zone Separations," I&EC Fundamentals, 14, 1975, pp. 96-102.
- Wankat, P.C. and Ross, J.W., "Partial Fractionation of Dyes by Cycling Zone Separation," Separation Science, 11, 1976, pp. 207-213.
- Wankat, P.C., "Fractionation by Cycling Zone Adsorption," Chemical Engineering Science, 32, 1977, pp. 1283-1287.
- Wankat, P.C., "Cyclic Separation Techniques," in A.E. Rodrigues (ed.), Proceedings of the NATO Advanced Study Institute on Percolation Processes, Espinho, Portugal, July, 1978a.

- Wankat, P.C., "Continuous Recuperative Mode Parametric Pumping," Chemical Engineering Science, 33, 1978b, pp. 723-733.
- Wankat, P.C., "Cyclic Separations: Parametric Pumping, Pressure Swing Adsorption and Cycling Zone Adsorption," CHEM I module to be published by AIChE.
- Weaver, K. and Hamrin, C.E., "Separation of Isotopes by Heatless Adsorption," Chemical Engineering Science, 29, 1974, pp. 1873-1882.
- Wilhelm, R.H., Rice, A.W. and Bendelius, A.R., "Parametric Pumping: A Dynamic Principle for Separating Fluid Mixtures," I&EC Fundamentals, 5, 1966, pp. 141-43.
- Wilhelm, R.H., Rice, A.W., Rolke, R.W. and Sweed, N.H., "Parametric Pumping: A Dynamic Principle for Separating Fluid Mixtures," I&EC Fundamentals, 7, 1968, pp. 337-49.
- Wilhelm, R.H. and Sweed, N.H., "Parametric Pumping: Separation of Mixture of Toluene and n-Heptane," Science, 159, 1968, pp. 522-524.
- Wilson, D.J., "Theory of Adsorption by Activated Carbon. II. Continuous Flow Columns," Separation Science and Technology, 14, 1979, pp. 415-430.
- Wong, Y.W. and Hill, F.B., "Equilibrium and Kinetics Studies of Hydrogen Isotope Exchange on Vanadium Hydride," AIChE Journal, 25, 1979, pp. 592-599.
- Wong, Y.W., Hill, F.B. and Chan, Y.N.I., "Studies of the Separation of Hydrogen Isotopes by a Pressure Swing Adsorption Process," Separation Science and Technology, 15, 1980, pp. 423-455.
- Wu, S.I., "Separation of Proteins via Continuous pH Parametric Pumping," D. Eng. Sc. Dissertation, New Jersey Institute of Technology, 1981.
- Yang, W.T., "Separation via Multi-Column pH Parametric Pumping," D. Eng. Sc. Dissertation, New Jersey Institute of Technology, 1981.
- Worthington Enzyme Manual, Freehold, New Jersey: Worthington Biochemical Corporation, 1977, p. 289.

NOMENCLATURE

A^+ , A^-	: Protein A or the adsorbed protein
b	: constant in equation for adsorption isotherm, i.e., Equation 6-3
B	: dimensionless pH wave velocity defined by Equation 6-25 or 6-28
B^-	: Protein B or the unadsorbed protein
BP	: bottom product
BR	: bottom reservoir
$C_{H^+,F}$: hydrogen ion capacity of the fluid, moles H^+ / mole HA • pH unit
$C_{H^+,S}$: hydrogen ion capacity of solid, moles H^+ / mole sites • pH unit
CALC	: calculated value
C_L	: concentration of solute in the bulk fluid, g mole / cc
C_L^*	: concentration of solute at the fluid - solid interface in equilibrium with C_S , g mole / cc
C_0	: concentration of solute in the feed, g mole / cc
C_S	: concentration of solute in the solid phase, g mole / cc
\mathcal{D}	: molecular diffusivity, cm^2 / sec
D_p	: diameter of individual particle in packed bed, cm
E	: strength of electric field, volts / cm
EXP	: experiment
F_B	: volume of bottom feed, cc
F_T	: volume of top feed, cc
F_0	: feed volume with zero protein concentration, cc
i	: cell or position along the axis of the column
I_A	: isoelectric point of the adsorbed protein or hemoglobin

I_B	:	isoelectric point of Protein B or albumin
I.C.	:	initial conditions
I.S.	:	ionic strength or molarity, moles / liter
j	:	transfer step or time unit
J	:	quantity of heat, Joules
J_D	:	dimensionless mass-transfer factor defined by Equation 6-21
k_{pH}	:	equilibrium constant defined by Equation 2-2 or 6-3
M	:	total number of cells-in-series into which the column is divided for calculational purposes
\bar{M}_{Al}	:	total elution volume at the center of mass of the albumin concentration wave, cc
M_{HA}	:	molarity of the buffer acid, moles HA / liter
\bar{M}_{Hb}	:	total elution volume at the center of mass of the hemoglobin concentration wave, cc
n	:	cycle number
N	:	total number of cycles
N_{Re}	:	Reynolds number
N_{Sc}	:	Schmidt number
P_B	:	volume of bottom product, cc
P_T	:	volume of top product, cc
P_1	:	high pH level
P_2	:	low pH level
Q_o	:	high bulk displacement rate, cc / min
Q_p	:	low bulk displacement rate, cc / min
r_{Al}	:	measured albumin concentration defined by Equation A-5, dimensionless
r_{Hb}	:	measured hemoglobin concentration defined by Equation A-1, dimensionless
R_{Al}	:	retardation coefficient for albumin as defined by Equation 3-1, dimensionless

R_B	:	spectrometer reading for buffer
R_F	:	spectrometer reading for feed
R_{Hb}	:	retardation coefficient for hemoglobin as defined by Equation 3-2, dimensionless
R_S	:	spectrometer reading for sample
S^+	:	counter ion on the cation exchanger
S.F.	:	protein separation factor defined by Equations I-1, 2-28, and 2-29
t	:	time, min
t_c	:	circulation time, min
TP	:	top product
TR	:	top reservoir
V	:	volume of the fluid phase in the column, cc
\bar{V}	:	volume of the solid phase in the column, cc
V_B	:	volume of bottom reservoir, cc
V_e	:	volume of effluent fluid, cc
V_T	:	volume of top reservoir, cc
x	:	dimensionless concentration of solute in the solid phase defined by Equation 2-1
x_0	:	initial concentration of solute in the solid phase, dimensionless
y	:	dimensionless concentration of solute in the bulk fluid defined by Equation 2-1
y_0	:	dimensionless concentration of solute in the feed
y^*	:	dimensionless concentration of solute at the fluid - solid interface in equilibrium with x
y_{BR}^*	:	intermediate value for concentration of solute in the bottom reservoir in the n 'th cycle of operation, dimensionless
y_{BR}'	:	intermediate value for concentration of solute in the bottom reservoir in the n 'th cycle of operation, dimensionless

Y	:	weight fraction of solute in the fluid phase, g/g
Y ₀	:	weight fraction of solute in the feed, g/g
z	:	axial direction
Z	:	length of packed bed, cm
<>	:	average value

Greek Letters

α	:	overall separation factor for protein mixture defined by Equations 2-30 and 2-31
Γ	:	total number of transfer steps defined by Equation 6-29
δ	:	recovery defined by Equation 5-52
ϵ	:	void fraction in packing, dimensionless
θ_F	:	correction factor at feed pH from Figure 84
θ_S	:	correction factor at sample pH from Figure 84
λ	:	mass-transfer coefficient through the film as defined by Equation 2-1, min ⁻¹
μ	:	protein mobility, cm ² /volt-sec
ν_E	:	migration velocity of the protein in the fluid phase due to the electric field, cm/min
ν_0	:	bulk velocity, cm/min
ν_{pH}	:	pH wave velocity, cm/min
ν_Z	:	net velocity in the axial direction, cm/min
ξ	:	ratio of experimental and calculated recoveries defined by Equation 5-53
ρ_S	:	moles of hydrogen ion sites per liter of gel
Φ	:	slope of titration curve for the buffer acid, moles HA • pH units / mole OH ⁻
ψ	:	concentration ratio defined by Equation 4-11

Subscripts

Al	:	albumin
B	:	bottom product
BP	:	bottom product
BR	:	bottom reservoir
C	:	column
EXP	:	experimental
Hb	:	hemoglobin
n	:	n'th cycle of operation
p	:	product
T	:	top product
TP	:	top product
TR	:	top reservoir
∞	:	steady-state condition
1, 2, etc.	:	cycle number
I, II, etc.	:	stage number

VITA

Name: Helen Conway Faris Hollein.

Permanent address: 49 Poplar Drive, Morris Plains, New Jersey.

Degree and date to be conferred: D. Eng. Sc., 1982.

Secondary education: Douglas MacArthur High School,
San Antonio, Texas, 1961.

<u>Collegiate institutions attended</u>	<u>Dates</u>	<u>Degree</u>	<u>Date</u>
University of South Carolina	1961-65	B.S.Ch.E.	1965
New Jersey Institute of Technology	1976-78	M.S.	1979
New Jersey Institute of Technology	1978-82	D.Eng.Sc.	1982

Major: Chemical Engineering.

Minor: Chemistry.

Publications: "The 'cis Effect' in β -Substituted Vinyl Ethers and Halides," Journal of Molecular Structure, in press.

"Parametric Pumping with pH and Electric Field: Protein Separations," Industrial & Engineering Chemistry Fundamentals, 21, 1982, pp. 205-214.

"The Dipropenyl Ethers. Vibrational and ^{13}C NMR Spectra," Journal of Molecular Structure, 82, 1982, pp. 187-204.

"Research on Parametric Pumping," Chemical Engineering Education, Vol. 15, No. 4, Fall, 1981, pp. 166-171.

"Parametric Pumping with pH and Electric Field," Proceedings of the 2nd World Congress of Chemical Engineering, Montreal, Canada, Vol. I, October, 1981, pp. 222-226.

Positions held: Assistant Professor of Chemical Engineering, Manhattan College, Riverdale, New York (present).

Adjunct Professor of Chemistry, New Jersey Institute of Technology, Newark, New Jersey (1977-81).

High School Teacher, Chemistry and Physics, Livingston High School, Livingston, New Jersey (1967-69).

Process Engineer, Chemicals Division, Exxon Research and Engineering Company, Florham Park, New Jersey (1965-67).