AN EXPERIMENTAL STUDY OF SEMI-CONTINUOUS PH-PARAMETRIC PUMPING WITH A CENTER FEED

BY

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ABSTRACT

Parametric pumping represents a new development in separation science. It has attracted considerable attention both because of its novelty and because it permits continuous operation in small equipment with very high separation factors. The basic principle of parametric pumping is to utilize the coupling of periodic changes in equilibrium conditions caused by periodic changes in some intensive variables (temperature, pH, electric field, etc.), and periodic changes in flow direction to separate the components of fluid which flows past a solid adsorbent. Applications of parametric pumping involving the separation of valuable materials such as proteins would be very attractive and profitable to investigate.

Many proteins are often processed batchwise. Parametric pumping offers the possibility of continuous processing, thereby tending to minimize both processing time and degradation. The overall objective of this research is to determine the feasibility of operating a semi-continuous pH-parametric pump for protein separation. The model system used is hemoglobin-albumin on sephadex ion exchange. It is hoped that the results of this work would be general enough to be invaluable in the separation of binary or multi-protein mixtures, and will provide necessary technical information for the design of full-scale parametric pumps with a sound engineering and economic basis.

APPROVAL OF THESIS

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FOR

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NEW JERSEY INSTITUTE OF TECHNOLOGY

BY

FACULTY COMMITTEE

APPROVED: ______________________

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NEWARK, NEW JERSEY
OCTOBER, 1976

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Parts of this thesis were presented at the 82nd A.I.Ch.E. national meeting, Atlantic City, New Jersey August, 1976.

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SCOPE

The name "parametric pumping" was applied to the separation process in 1966 by the inventor of the batch pump, the late R. H. Wilhelm of Princeton University. Since the time of that invention, many experimental and theoretical extensions have been done on separation by thermal and heatless (or pressure cycling) parametric pumping. They include Wilhelm et. al. (1966, 1968), Jenczewski and Meyors (1968, 1970), Wilhelm and Sweed (1968), Pigford et. al. (1968), Horn and Lin (1969), Rolke and Wilhelm (1969), Aris (1968), Gregory and Sweed (1970, 1971, 1972), Turnock and Kadlec (1971), Butts et. al. (1972), Kowler and Kadlec (1972), Shendelman and Michell (1972), Weaver and Hamrin (1974), and Chen et. al. (1971, 1972, 1973, 1974a, 1974b, 1974c, 1975, 1976) Comprehensive reviews of the subject have been made by Sweed (1971), Wankat (1974), and Chen (1976). However, much less studies have been done on the pH-parametric pumping. Sabadell and Sweed (1970) used pH changes to remove K^+ and Na^+ from H_2O. Shaffer and Hamrin (1975) studied trypsin removal from α-chymotrypsin-trypsin mixtures by affinity chromatography and parametric pumping. In this work a semi-continuous pH-parametric pump for separating proteins will be experimentally investigated. The pump considered here has a center feed between an enriching column and stripping column, and is operated batchwise during upflow and continuous during downflow.

Techniques commonly used for the separation of proteins include: gel filtration, affinity chromatography, ion exchange chromatography, etc. The proposed semi-continuous pH-parametric pumping has several advantages over the conventional separation methods.

1) Many proteins are often processed batchwise. Parametric pumping offers the possibility of continuous processing, thereby tending to minimize both processing time and degradation.
2) No regeneration chemicals are needed for the continuous process, and no regenerant can contaminate the product.
3) The continuous process can be achieved with very high separation factors, and the components removed can be concentrated to any desired practical level by setting the flow rate of the product stream containing these components at the required value.
4) Control problems for the continuous process may be simpler compared to those for competing batch processes.

CONCLUSIONS AND RECOMMENDATIONS

Semi-continuous pH-parametric pumping for separating hemoglobin and albumin was experimentally investigated. A sephadex ion exchange was used as an adsorbent. The feed is introduced between the enriching and stripping columns. The pump is operated batchwise during upflow and continuous during downflow. It has been shown that the pump has the capibility for separating protein mixtures.

It is recommended to try different ion exchanges and to extend the process to a true continuous one, that is with feed and product removal during both up and down flow. Also, the effect of the buffer's ionic strength on the separation should be studied or investigated.

EXPERIMENTAL

A. EQUIPMENT

The experimental apparatus used in this research is shown in figure 1. The equipment consists of two jacketed Pharmacia chromatographic columns (1.6 X 40 cm.) packed with a sephadex gel. The top column can be considered the stripping section, while the bottom is the enriching section. Feed is introduced between the enriching and stripping sections. Constant temperature of the column is maintained by the use of a Brinkmann Instrument unit. It circulates cooling water at 281° K, which prevents the proteins from denaturing. The reciprocating pumping of the reservoirs and the introduction of the feed into the system was accomplished by the use of two infusion-withdrawal syringe pumps, which were manufactured by Harvard Apparatus. The reservoir pumps are fitted with two 50-cm^3 glass syringes and the feed pump is fitted with one. The fluid is pumped through the system using capillary tubing (0.1 cm. id, 0.18 cm. od). To insure perfect mixing, small magnetic stirrers were employed in both reservoir syringes.

The change of pH was accomplished by the use of two dialysis cells. The particular cells used were made by Bio-Rad and were the bio-fiber 50 beaker model. The fibers are made of cellulose and have a total surface area of 900 cm^2. The nominal molecular weight cut off for this model is 5,000. The jacket volume is 100 cm^3. A buffer is circulated in the jacket part of the dialysis cell, while the solution which wishes to change its pH is passed through the fibers. The buffer employed is a mixture of monobasic sodium phosphate and dibasic sodium phosphate. For the low pH reservoir (pH=6.0), the proportion is 87.7% monobasic sodium phosphate and 12.3% dibasic sodium phosphate. For the high pH reservoir (pH=8.0), the proportion is 5.3% monobasic sodium phosphate and 94.7% dibasic sodium phosphate (Colowick & Kaplin, 1955). The concentration strength of both buffers is 0.1 M. A 2,000 cm^3 reservoir is used for the circulation of fresh buffer through each dialysis cell. A Bio-Rad peristaltic pump is used to circulate the buffer at 0.33 cm^3 per second. To eliminate stagnation of the buffer, magnetic stirrers are placed in the bottom of the dialysis cells.

Top and bottom product samples are collected with Gilmont micrometric capillary valves. These valves are used both to regulate and to impose a small back pressure on the flow of the fluid in the system. The samples are measured on a Beckmann DU spectrophotometer. A minimum of 2.6 cm^3 is needed for analysis.

Prior to each run, all the air is removed from the connecting tubing. This is done by filling all the lines with feed solution. Low pH feed is used for the tubing leading to and from the enriching section, while high pH feed is used for the lines leading to and from the stripping section.

The specific packing used is SP-sephadex (C-50). Preparation of the packing has been standardized in order to produce similar starting conditions for the runs. Initially, the packing was allowed to expand in 40 cm^3. of low pH buffer. After 24 hours, 10 cm^3. of the top liquid was decanted off and replaced with 100 cm^3. of low pH feed. After another 24 hours, 100 cm^3. of the liquid phase was decanted off, leaving the gel ready to be poured into the column. The pouring of the packing into the column has to be done in a careful manner. The technique employed is to pour the gel slowly down a glass rod, allowing the packing to settle without trapping any air. The remaining air in the tubing leading into the connectors of the column is blown out by compressing the packing slightly, replacing the air with some of the fluid phase. Then the column is sealed and the run is ready to start.

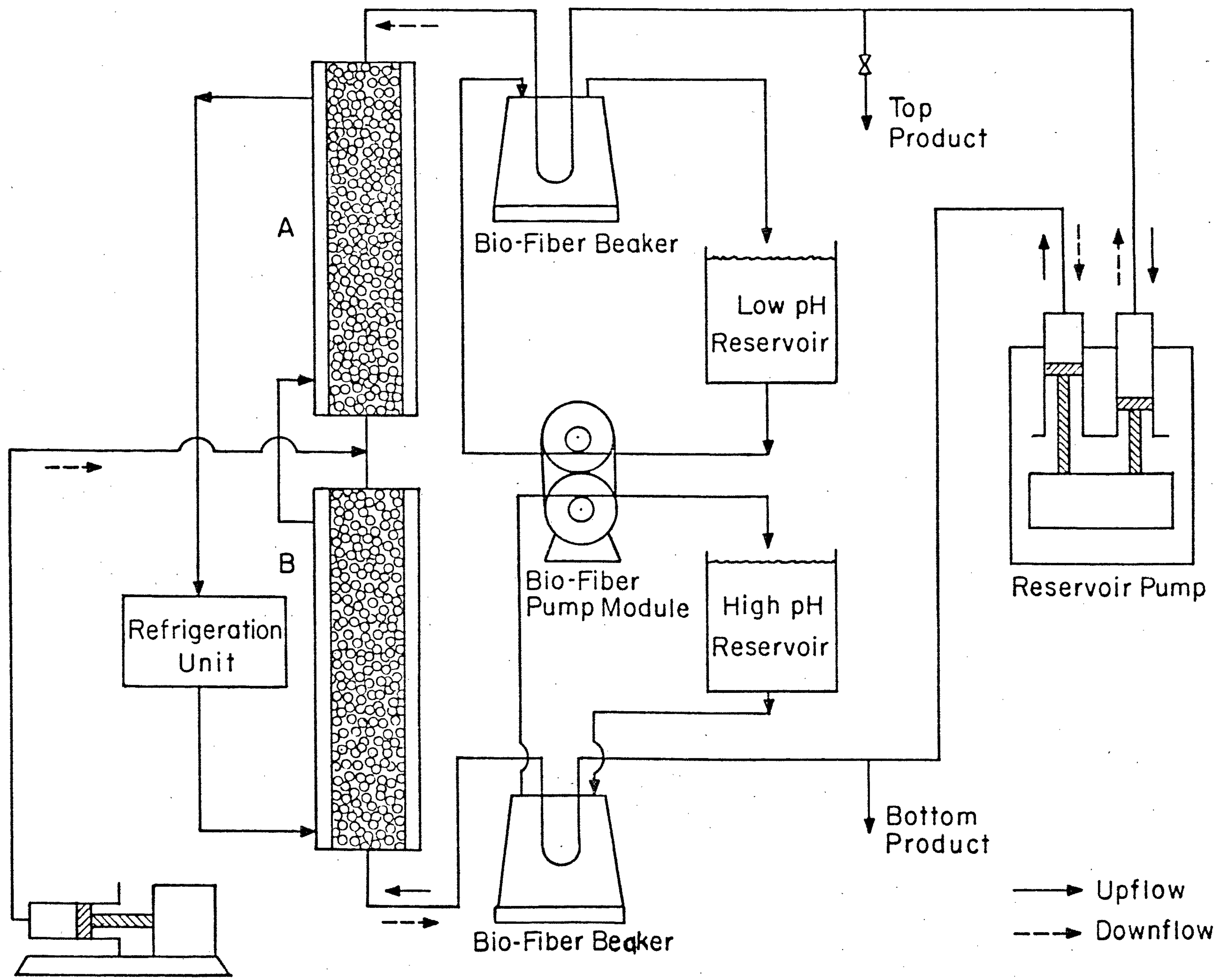
Top
Product
A
B
Bio-Fiber Beaker
Low pH
Reservoir
Reservoir Pump
Refrigeration
Unit
Bio-Fiber
Pump Module
High pH
Reservoir
Bottom
Product
Upflow
Downflow
Bio-Fiber Beaker
Feed Pump

FIGURE 1

B. MEASUREMENT

In order to determine the concentrations of the samples, three calibration curves must be prepared. Hemoglobin will absorb light at a wavelength of 403 mu and 280 mu, while albumin will absorb light only at 280 mu. A wavelength of 403 mu is in the visible spectrum, while 280 mu is in the ultra-violet range. For the hemoglobin, twenty known concentrations ranging from a weight percent of 0.001 to a weight percent of 0.010 in equal increments are measured at both wavelengths. Three sets of data points are made up for pH's of 6.0, 7.0, and 8.0. Initially, the four cells that are to be used for the measurements are filled with deionized water in order to calibrate them for any differences in transmission that they may have. The readings of the samples are then divided by this correction factor and multiplied by one hundred in order to get the actual transmission of the sample. When the readings for the samples are made, the first cell is the reference and is filled with the corresponding pH buffer as of the sample. The buffer is 0.05M and is of the same type that is actually used in the experiment. The remaining three cells are filled with the samples, and the readings are recorded in percent transmission. The absorbance is found by the simple relationship that it is equal to the common log of one hundred divided by the transmission A Log(100/T). Now absorbance is plotted against percent concentration on linear coordinates. A linear regression is performed on each set of data points, and the best straight line that passes through the origin is drawn. For 403 mu the slope, λ, at pH=6.0 is 59.82, at pH=7.0 λ=56.12, and at pH=8.0 λ=53.88. This calibration curve is depicted in figure 2. As the pH decreases the slope increases. For 280 mu λ at pH=6.0 is 16.49, at pH=7.0 λ=17.40, and at pH=8.0 λ=14.95. This calibration curve is shown in figure 3. At this wavelength, a maximum at a certain pH seems to occur. A rough working plot of slope verus pH is done at both frequencies (figures 5 & 6). Although these plots will not give exact slopes at a particular pH, they will give one within the accuracy of the spectrophotometer readings.

The albumin calibration curve at 280 mu (figure 4) is done in a similar fashion with the exception that the concentration range is increased because of the less sensitivy of the absorbance of albumin. There are twenty-two points ranging from a percent concentration of 0.005 to 0.100. The corresponding slopes for pH's of 6.0, 7.0, and 8.0 are 5.054, 5.013, and 4.995 respectively. The slopes seem to be relatively constant, indicating that pH has little affect on the readings of the samples. An average slope of 5.021 will be used in the calculations.

The samples for the runs are measured in the following manner. The first cell will contain the reference. For the top product, the reference will be the low pH buffer and for the bottom product it will be the high pH buffer. The remaining three cells will contain the samples. Readings will be taken at both 403 mu and 280 mu. The same procedure as before is used to calculate the absorbance of both the samples and the feed. Each sample is tested for pH and the appropriate slopes, which are obtained from figures 5 and 6, is used in the following calculations. For hemoglobin, the procedure for calculating the concentration is straight forward. The absorbance of the sample is divided by the slope of the calibration curve at 403 mu. The feed absorbance is also divided by its slope to determine the feed concentration. To normalize the results the concentration of the sample is divided by the concentration of the feed.

Since the concentrations that are used in the experiment are dilute, advantage can be taken of the additive property of absorbances for two components at a certain frequency in order to calculate the concentration of albumin. First the contribution of hemoglobin is found by multipling the concentration of hemoglobin, which was already found at 403 mu, by the corresponding slope of the calibration curve at 280 mu. This will give the absorbance that is contributed by the hemoglobin. This value is subtracted from the total absorbance at 280 mu, and this will give the contribution due to albumin. This value is then divided by the slope of the albumin calibration curve, which finally leads to the concentration of albumin. The same procedure is followed for the feed reading and the sample is normalized as before.

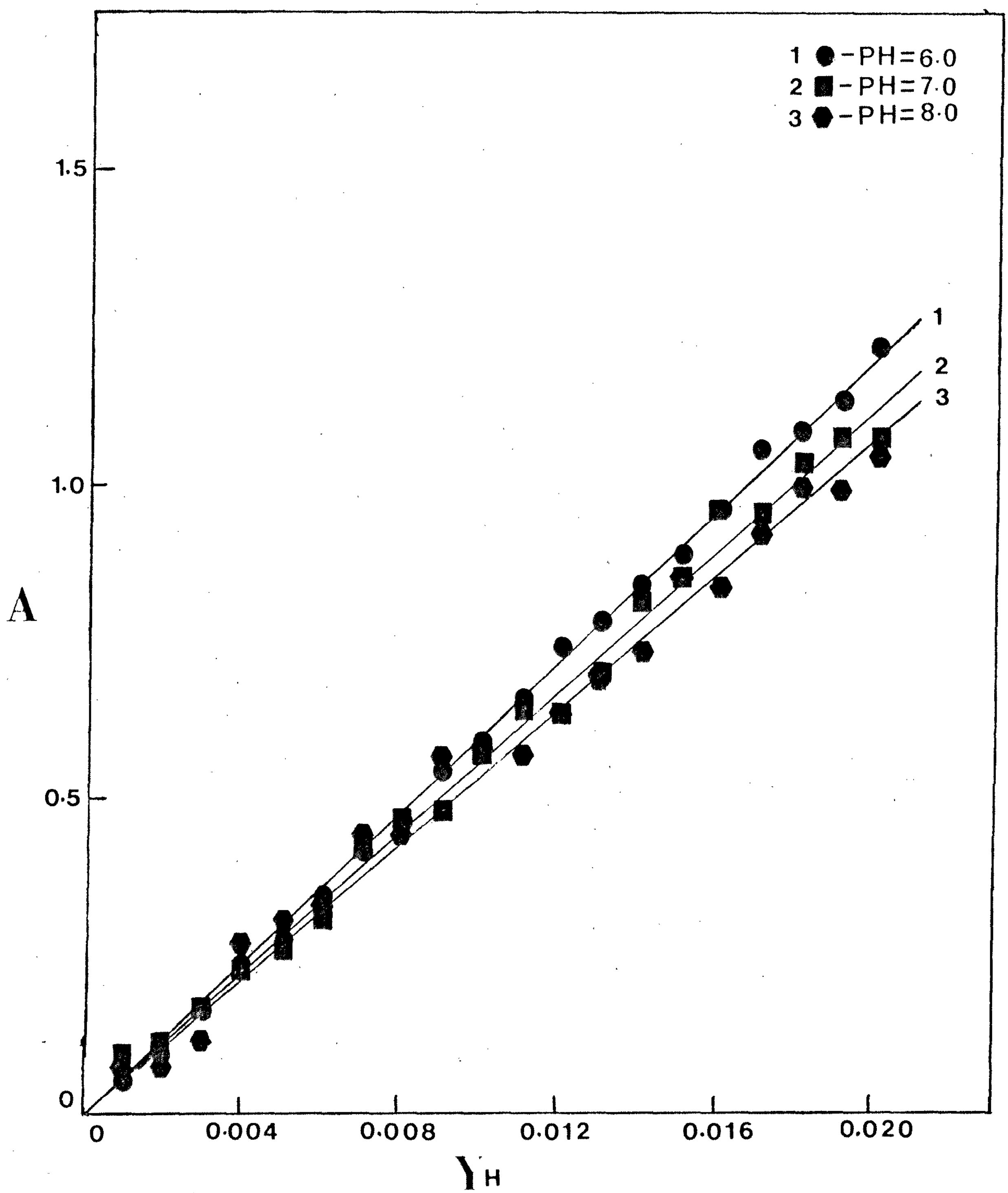

FIGURE 2

CALIBRATION CURVE
(HEMOGLOBIN-403 mu)

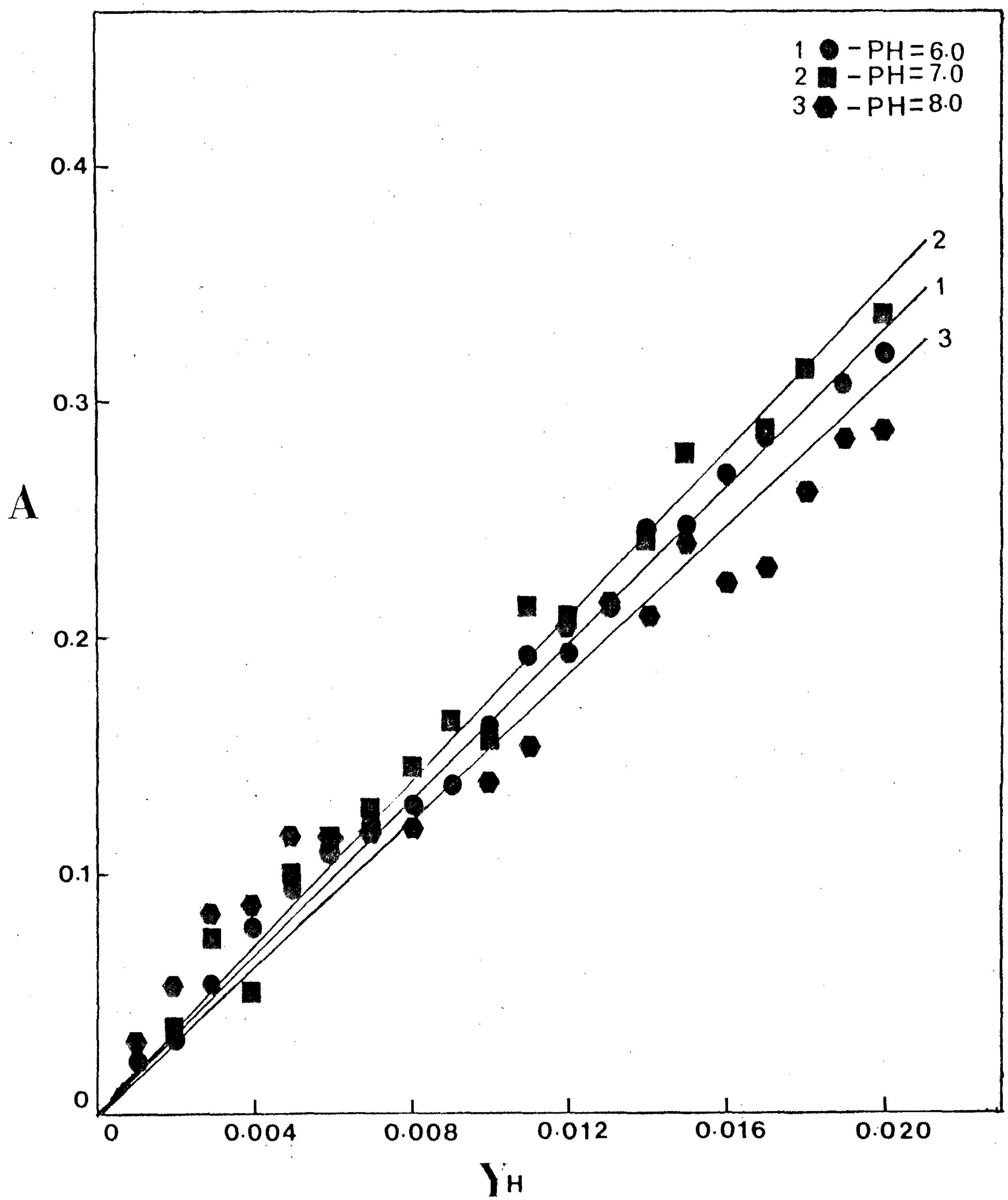

FIGURE 3

CALIBRATION CURVE
(HEMOGLOBIN - 280 mu)

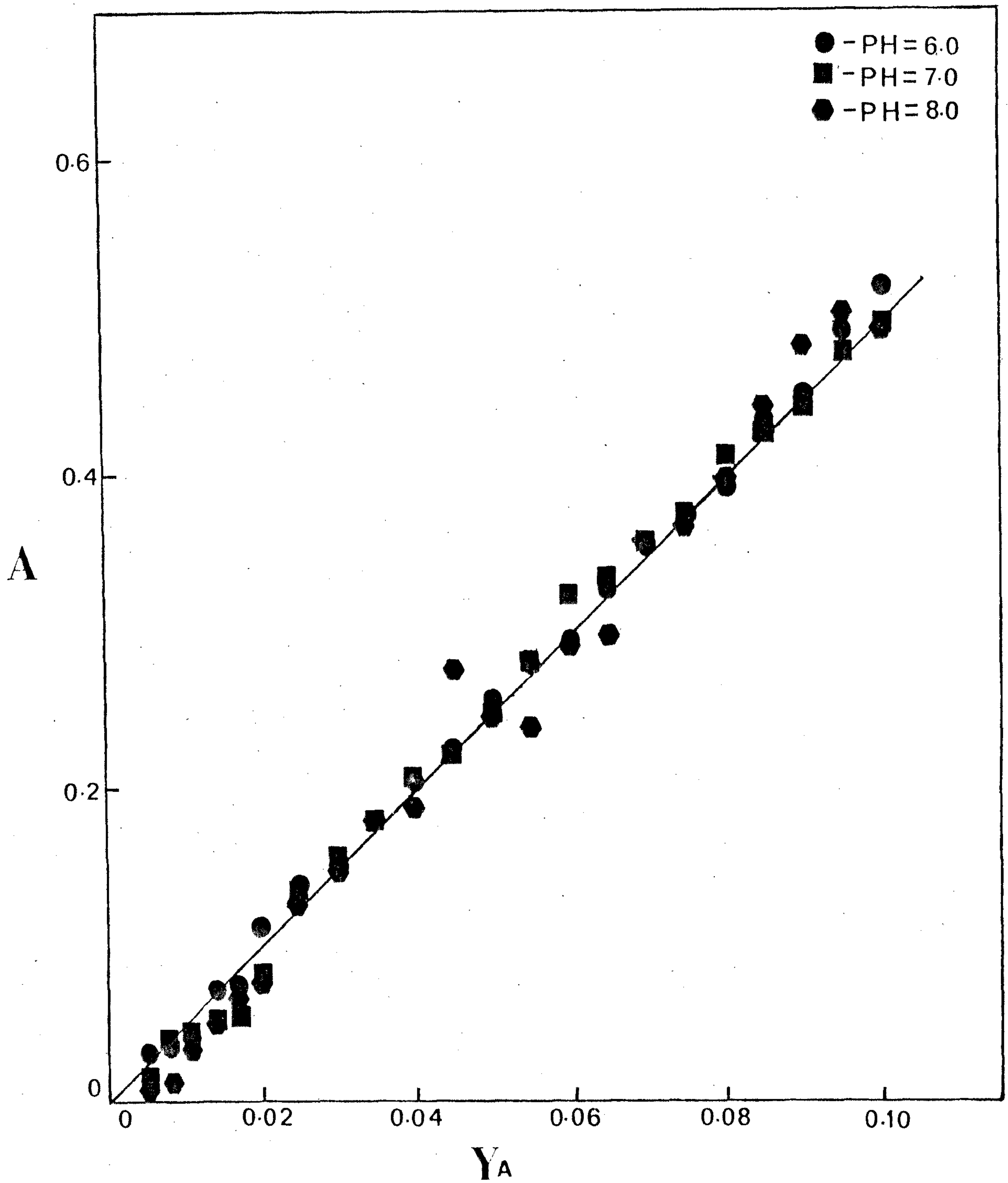

FIGURE 4

CALIBRATION CURVE
(ALBUMIN - 280 mu)

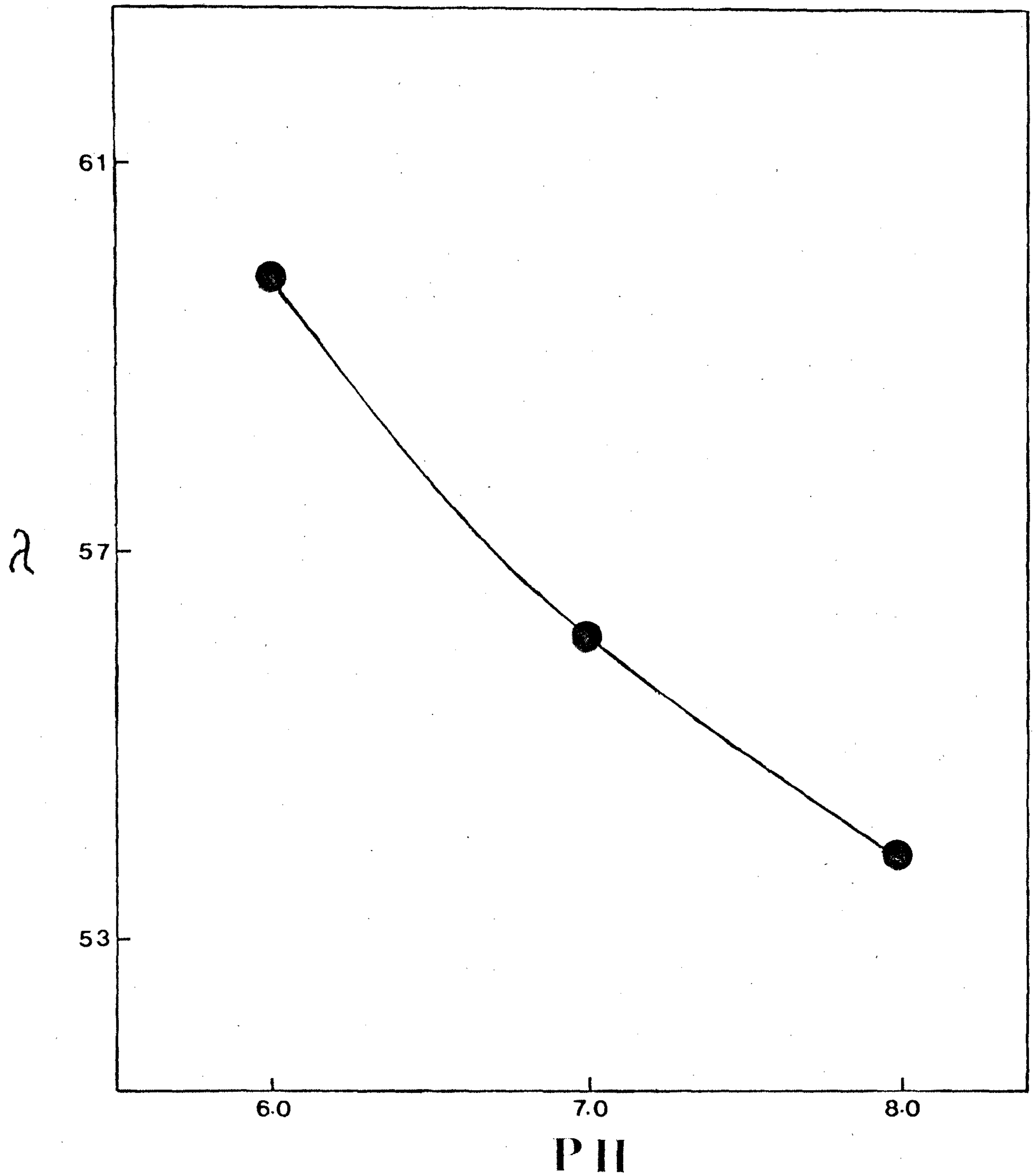

FIGURE 5

SLOPE VS. PH
(HEMOGLOBIN - 403 mu)

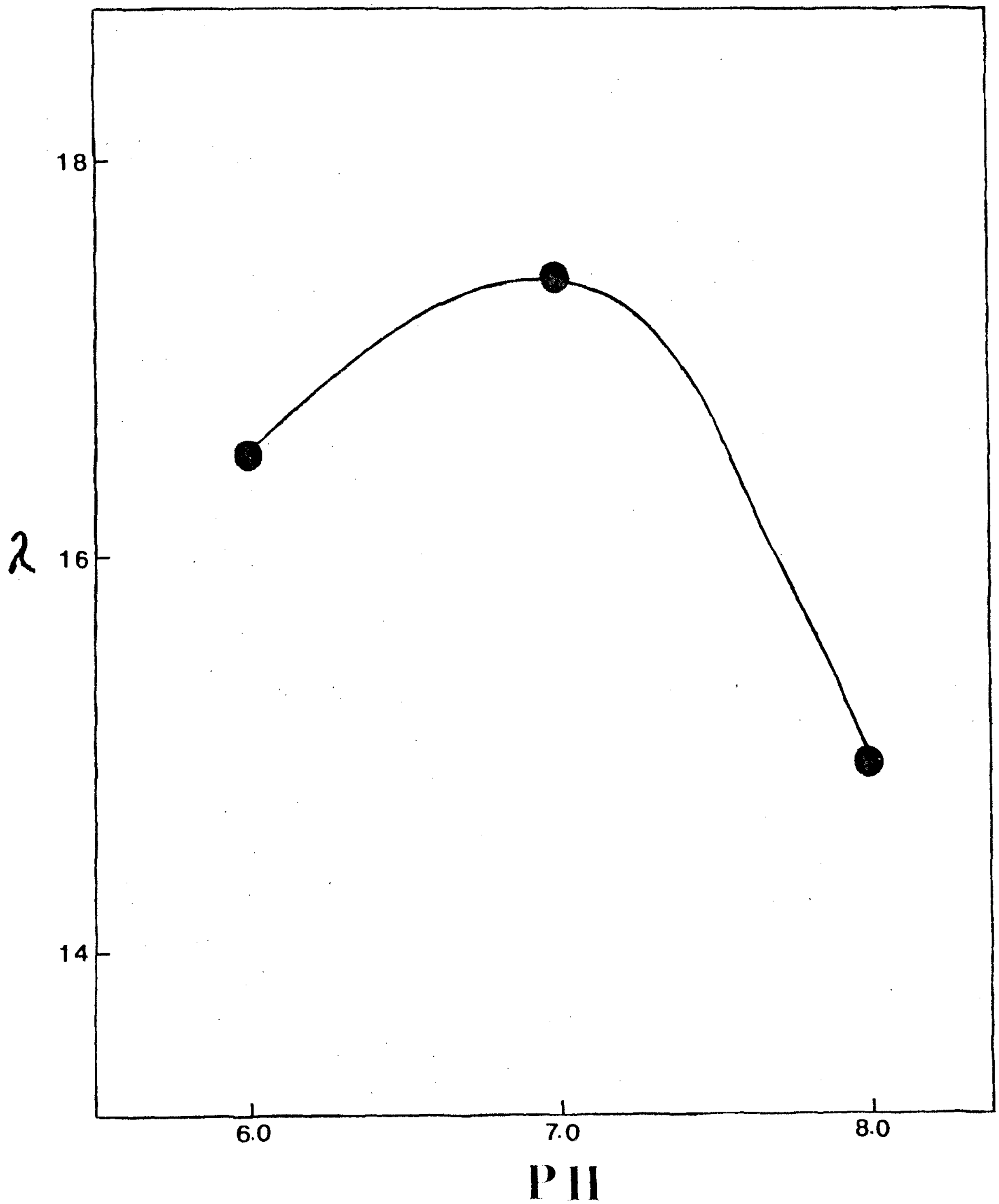

FIGURE 6

SLOPE VS. PH
(HEMOGLOBIN – 280 mu)

RESULTS AND DISCUSSION

Seven runs were carried out on the system, four were binaries and three were ternaries. Tables 4 and 5 lists the experimental conditions for all the batch and semi-continuous runs. Tables 6-10 contain the experimental data and results, and the final table, number 11, contains the separation factors for the runs. Comprising the binary runs were three batch operations and one of the semi-continuous mode done on the albumin-water system. The batch operation is a slight modification of figure 1. The exceptions are that it was performed with a single column and there is no feed or product removal. Initially, 15 cm^3. of feed solution was put in the top syringe and 5 cm^3. was put in the bottom syringe. The run started with a downward half-cycle and proceeded with its reciprocating motion for the required amount of cycles. The semi-continuous mode is one where the feed and the product removal is done on only one of the half-cycles. Run A4 was performed with a single column and both feed introduction and product removal was done on the downward half-cycle.

The batch runs indicate that there is very little difference in the concentrations at the top and bottom of the column. This can be expected because the column is operated between pH reservoirs of 6.0 and 8.0, and the isoelectric point of albumin is 4.9. The isoelectric point is the particular pH that a protein exhibits a net charge of zero. Above this point the protein will exhibit a net negative charge and below this point the net charge would be positive. The sephadex packing is negative and hence the albumin will be repelled, allowing it to flow through the column with little resistance. The concentration of albumin at the top of the column seems to be slightly higher than that of the bottom. This slight difference can be contributed to the fact that the pH of the top is 6.0 versus 8.0 at the bottom. At a pH of 6.0, albumin is a little bit less negative than at 8.0, and this difference will cause a slight attraction towards the top. The lower concentrations for run A3 is due to the added number of cycles. Ten cycles are not enough for the system to reach steady state. Eventually at steady state, the ratio of the concentrations in the reservoirs to that of the feed will approach unity. The results of the batch runs are listed in table 6.

The semi-continuous binary run for albumin seems to follow the batch results. Initially, there is a transient period with a lot of scattering and then both the top and bottom product concentrations approach unity. Figure 7 is a plot of sample concentration divided by feed concentration versus cycles (Y_{AS}/Y_{AF} VS. n) for run A4. Another plot for run A4 is separation factor versus cycles and this is shown in figure 14. Separation factor is defined as the concentration of the bottom product divided by the concentration for the top product.

The three ternary runs consist of two done with the single column, similar to run A4, and the last run performed with the double column, run T3. The experimental conditions for runs T1 and T2 where similar with the exception that in run T2, 0.05M NaCl was added to both the feed and the packing. Salt was added to try to break up the attraction albumin and hemoglobin would have for each other due to opposite charges. The results indicated that the salt had little or no effect and this can be seen by the similarity between figures 8 and 10. For the bottom hemoglobin product, both curves show an initial peak followed by a steady decline and then a leveling out to a value of a little bit under the feed. The top products seem to exhibit an initial transient period and then the concentrations level off. The separation factor for the hemoglobin for both runs is about 1.2. The plots for the separation factor versus cycles are also similar. The curves can be compared to the characteristic shape of the bottom products, an initial peak followed by a decline and leveling off. These plots are depicted in figures 15 and 16.

The plots for the albumin, figures 9 and 11, have a great deal of scattering in them, but the general trend seems to agree with the binary results. Eventually after an initial transient period the concentration at the top and the bottom of the column will level off to unity. The scattering in the curves can be contributed to two main factors. The first is the sensitivity of the albumin calibration curve compared with the hemoglobin calibration curve. Albumin is much less sensitive to ultra-violet light than hemoglobin. Through the manipulations that have to be performed to get a final albumin concentration, any error or fluctuation in the initial reading would lead into a considerable difference in the final results. It is recommended that further study should be made in albumin measuring technique. The second factor is that the semi-continuous runs were performed on two separate days, with a stoppage in operation between days. This stoppage can be seen in the sharp rise in the concentration of the albumin. Some of these points were eliminated from the graphs for this reason. The concentration rise is due to the mass transfer of the albumin. Since albumin and the packing have the same charge, the albumin wants to escape from the packing and go into the reservoirs.

For runs A4, T1, and T2 the top product was combined for each three consecutive cycles. The products were combined in order to get enough sample for measurement. The concentration for these samples were reported for the middle cycle.

The final run performed on the system was T3, which used the apparatus pictured in figure 1. By using stripping and enriching sections with feed introduced between sections, the separation was vastly improved. The hemoglobin can now be trapped in the bottom or enriching section, while the top or stripping section can be relatively free of hemoglobin. Figure 12 helps point out these results, by showing the concentrations of the top and bottom products for hemoglobin. Figure 13 is a plot for albumin and follows the preceding albumin results. The scattering can also be explained by the two preceding reasons. Separation factor versus cycles has been plotted in figure 17. The final separation factor is around 4 which is far superior to runs T1 and T2 which had separation factors around 1.2. A final plot, figure 18, is a comparison of the top hemoglobin products for runs H14(Falcon, 1976), T1, and T3. The plot exemlifies two points. The first is the superiority of the double column over the single one. The second is the lowering of the efficiency of the column by the attraction between the hemoglobin and albumin. Runs H14 and T1 were performed under the same conditions except for the fact that T1 had the additional protein albumin. Even with this lowering of the efficiency, the double column produced a low concentration of hemoglobin in the top or stripping section.

No attempt at optimizing the process was tried in this thesis. The primary objective was to show the future for pH-parametric pumping. The results from run T3 are good enough to indicate the viability of pH-parametric pumping as a separation process for human or natural proteins.

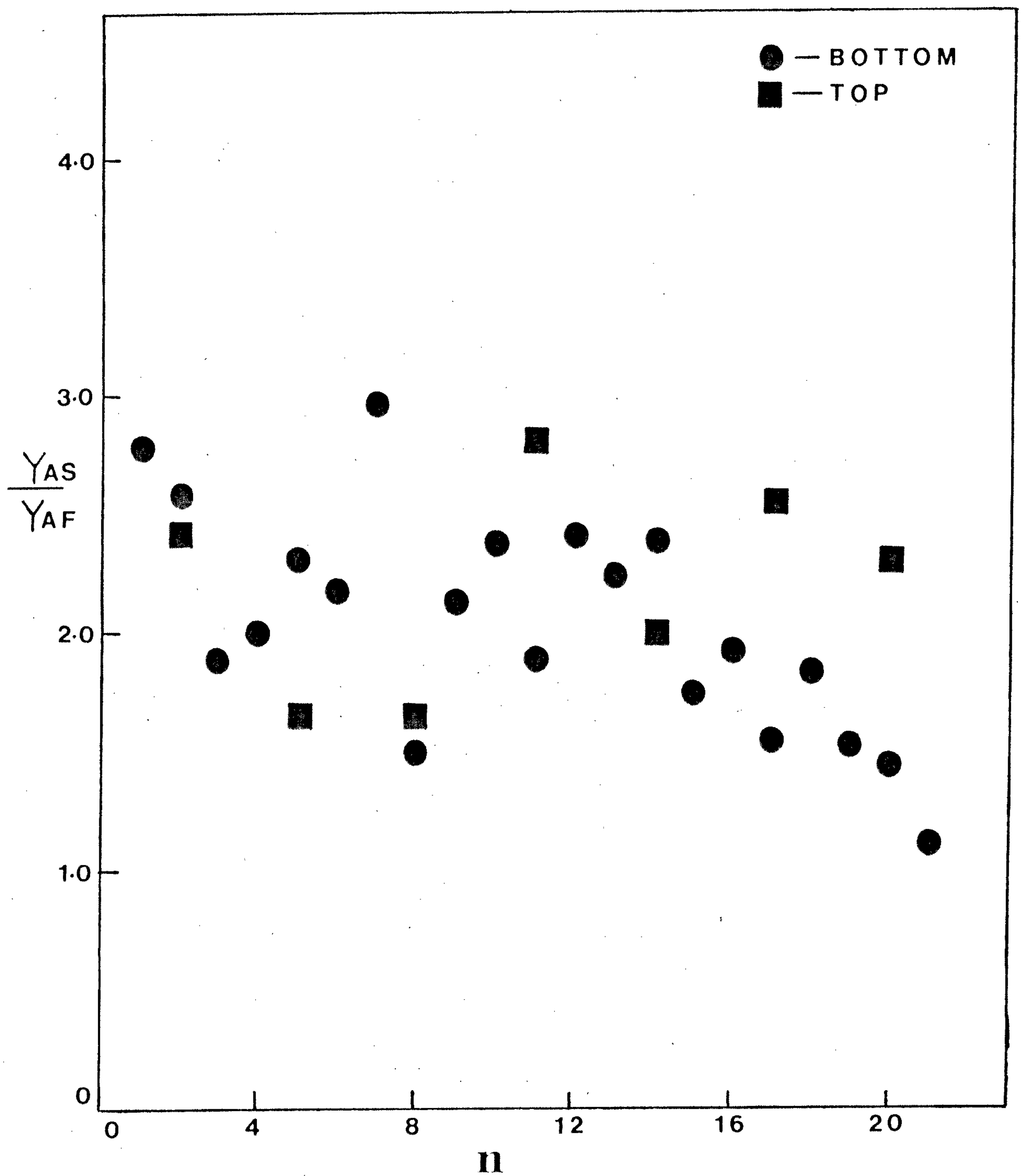

FIGURE 7 (RUN A4)

CONCENTRATION TRANSIENT
(ALBUMIN)

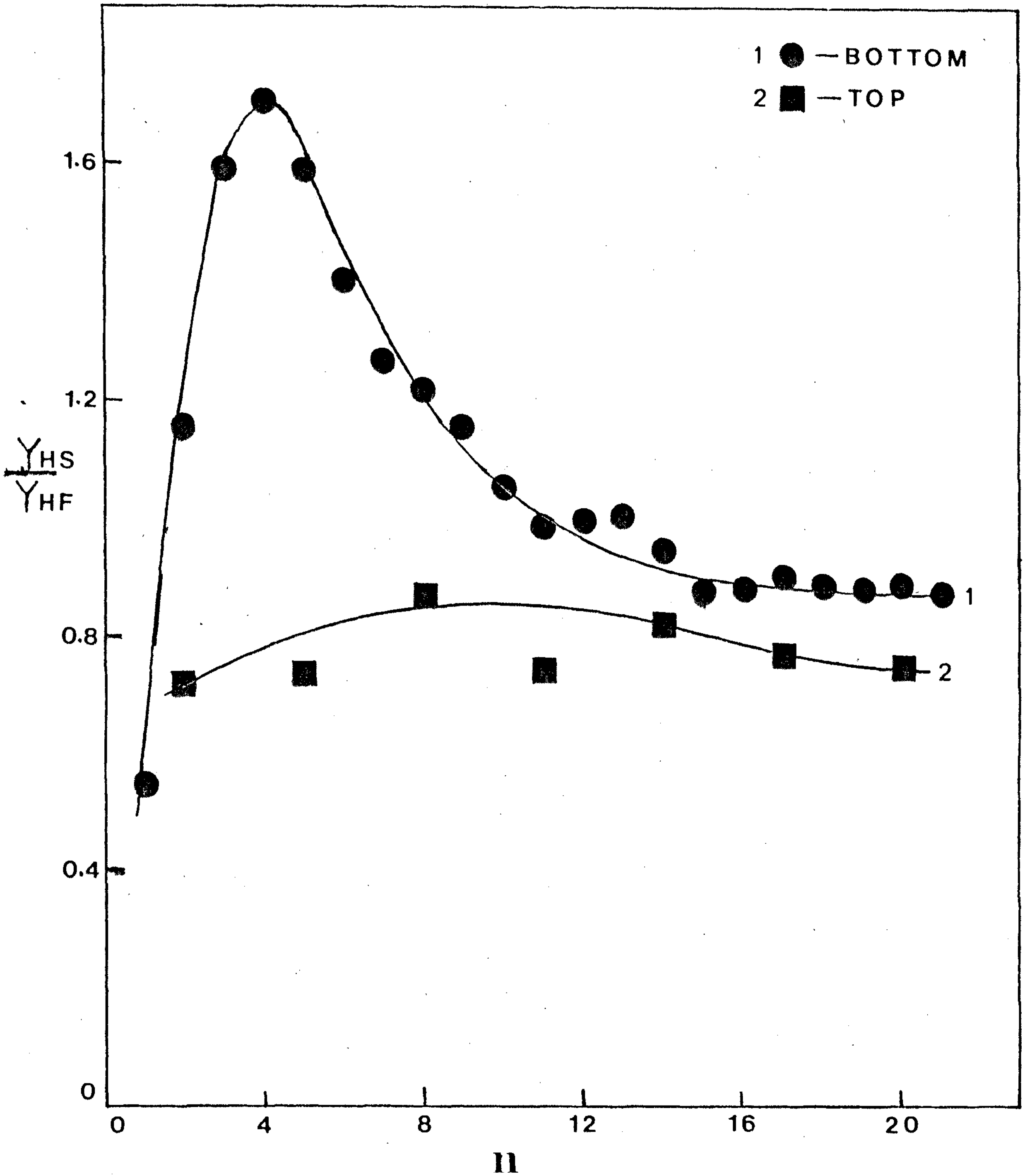

FIGURE 8 (RUN T1)

CONCENTRATION TRANSIENT (HEMOGLOBIN)

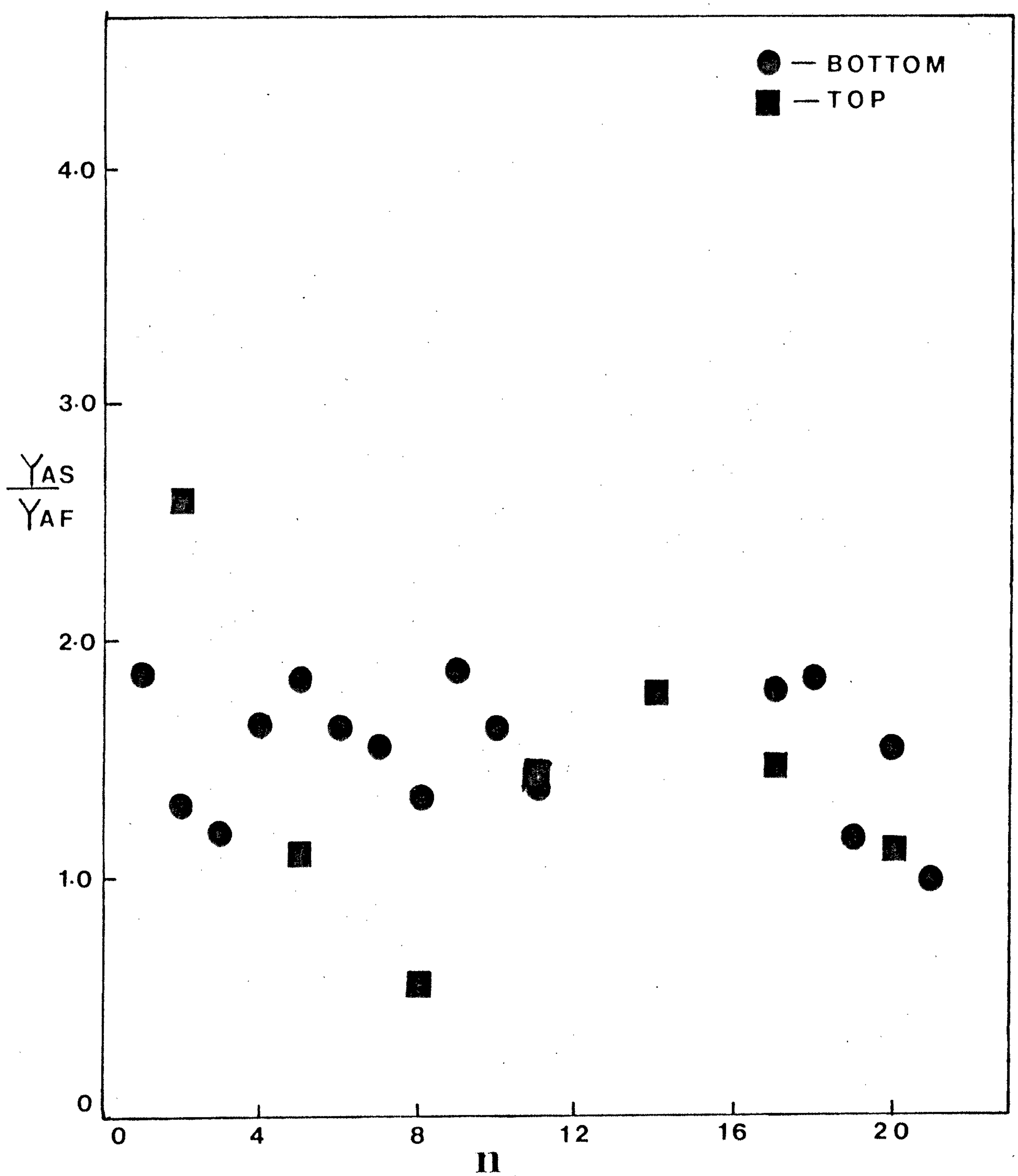

FIGURE 9 (RUN T1)

CONCENTRATION TRANSIENT
(ALBUMIN)

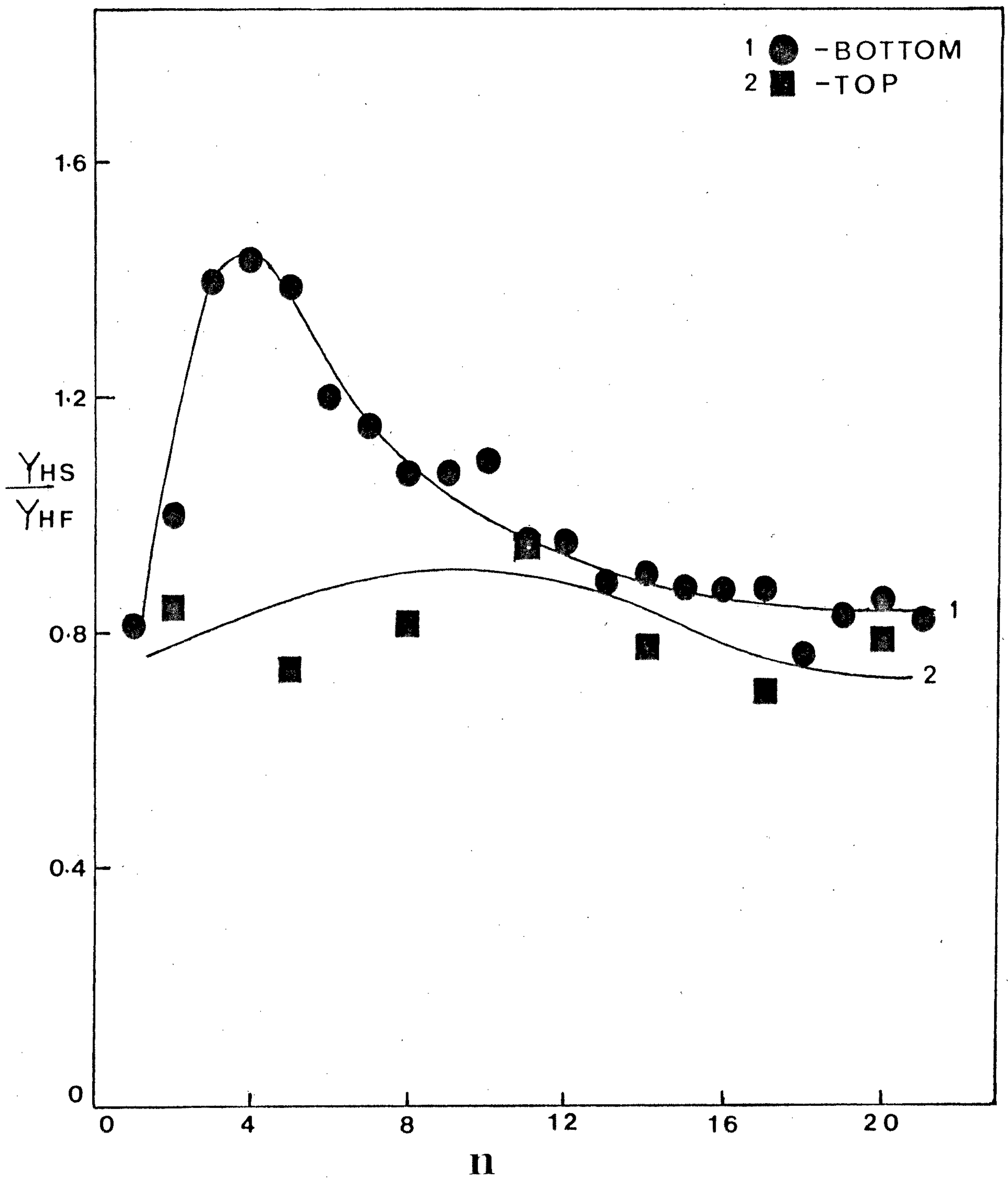

FIGURE 10 (RUN T2)

CONCENTRATION TRANSIENT
(HEMOGLOBIN)

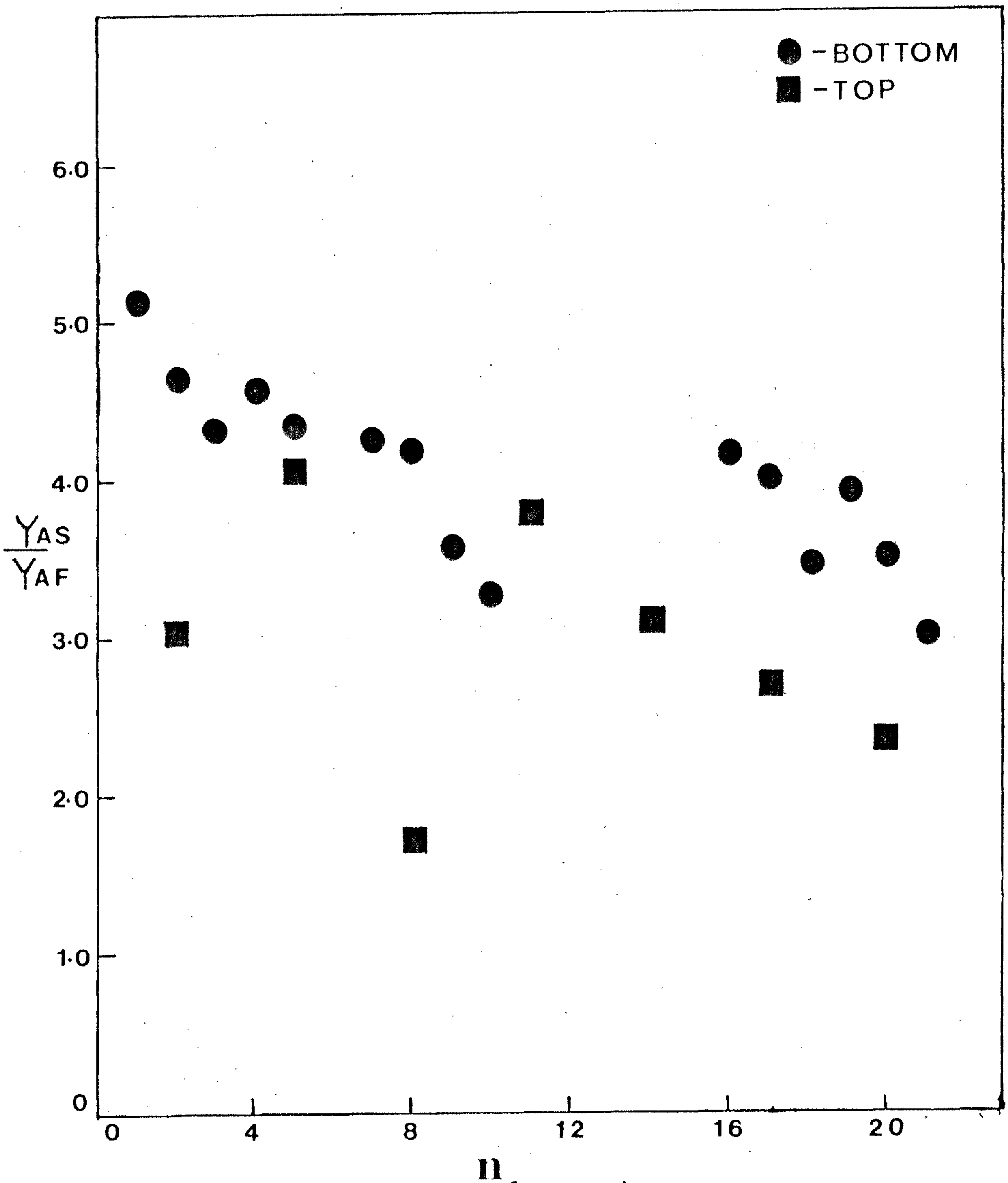

FIGURE 11 (RUN T2)

CONCENTRATION TRANSIENT
(ALBUMIN)

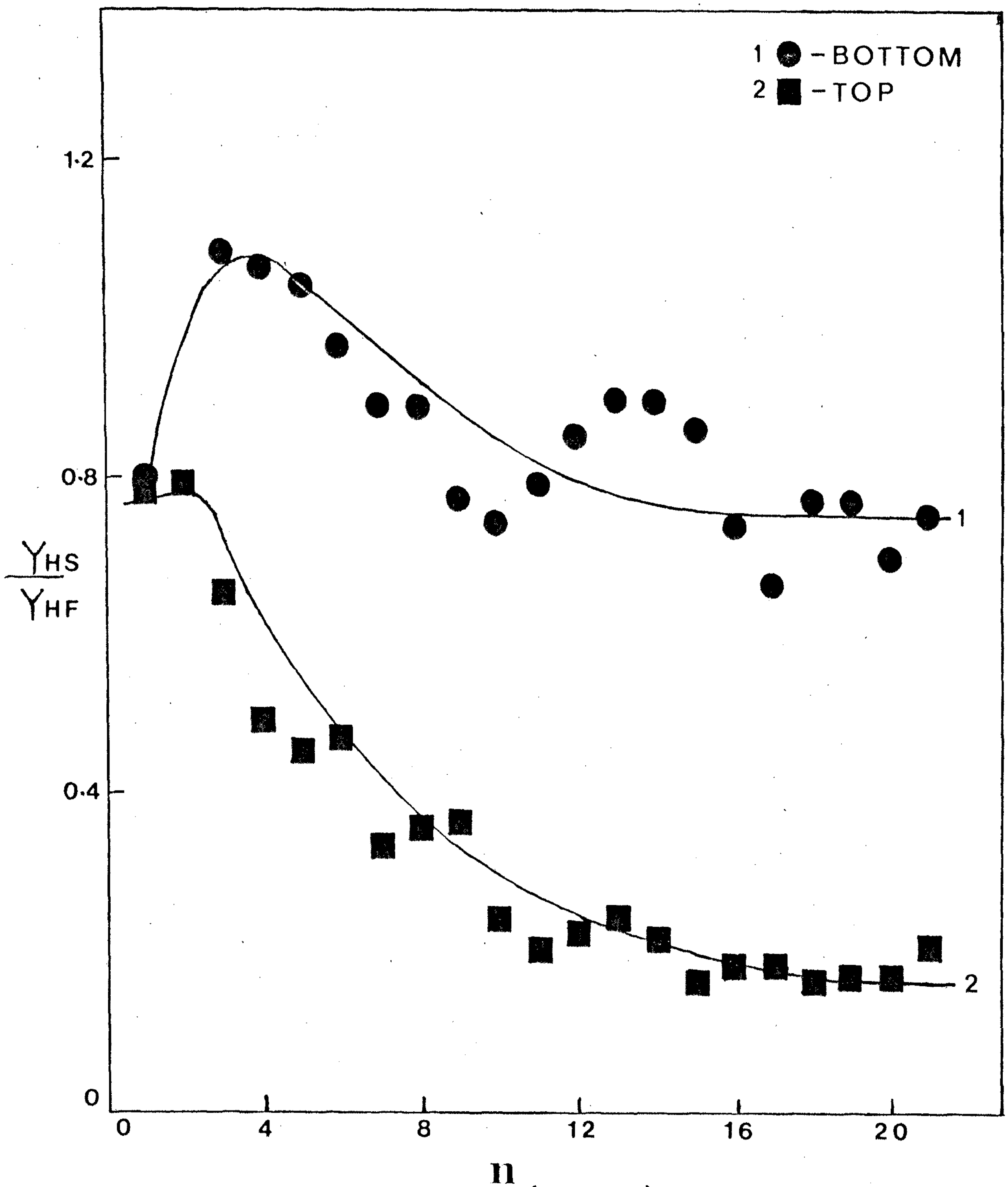

FIGURE 12 (RUN T3)

CONCENTRATION TRANSIENT
(HEMOGLOBIN)

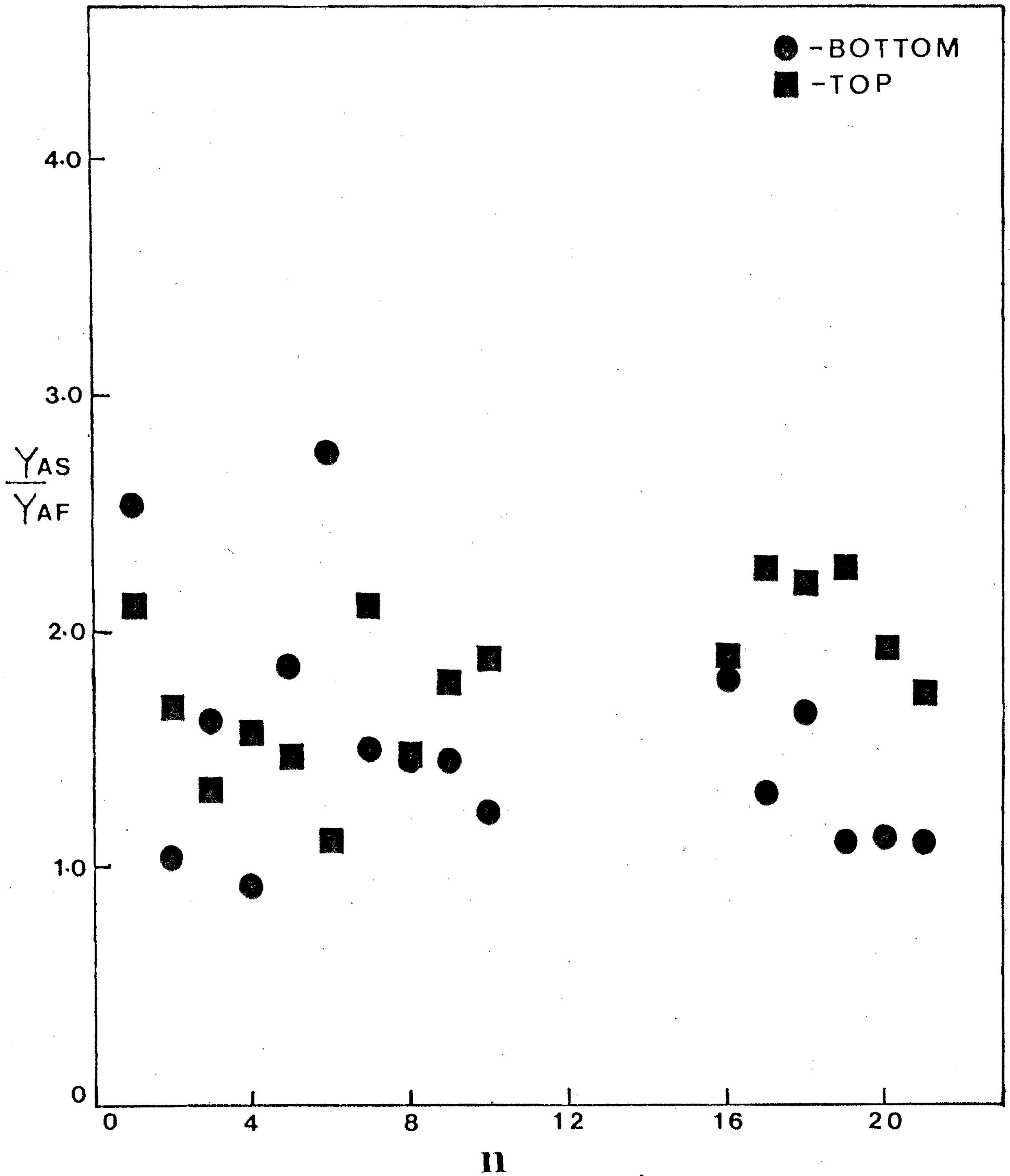

FIGURE 13 (RUN T3)

CONCENTRATION TRANSIENT
(ALBUMIN)

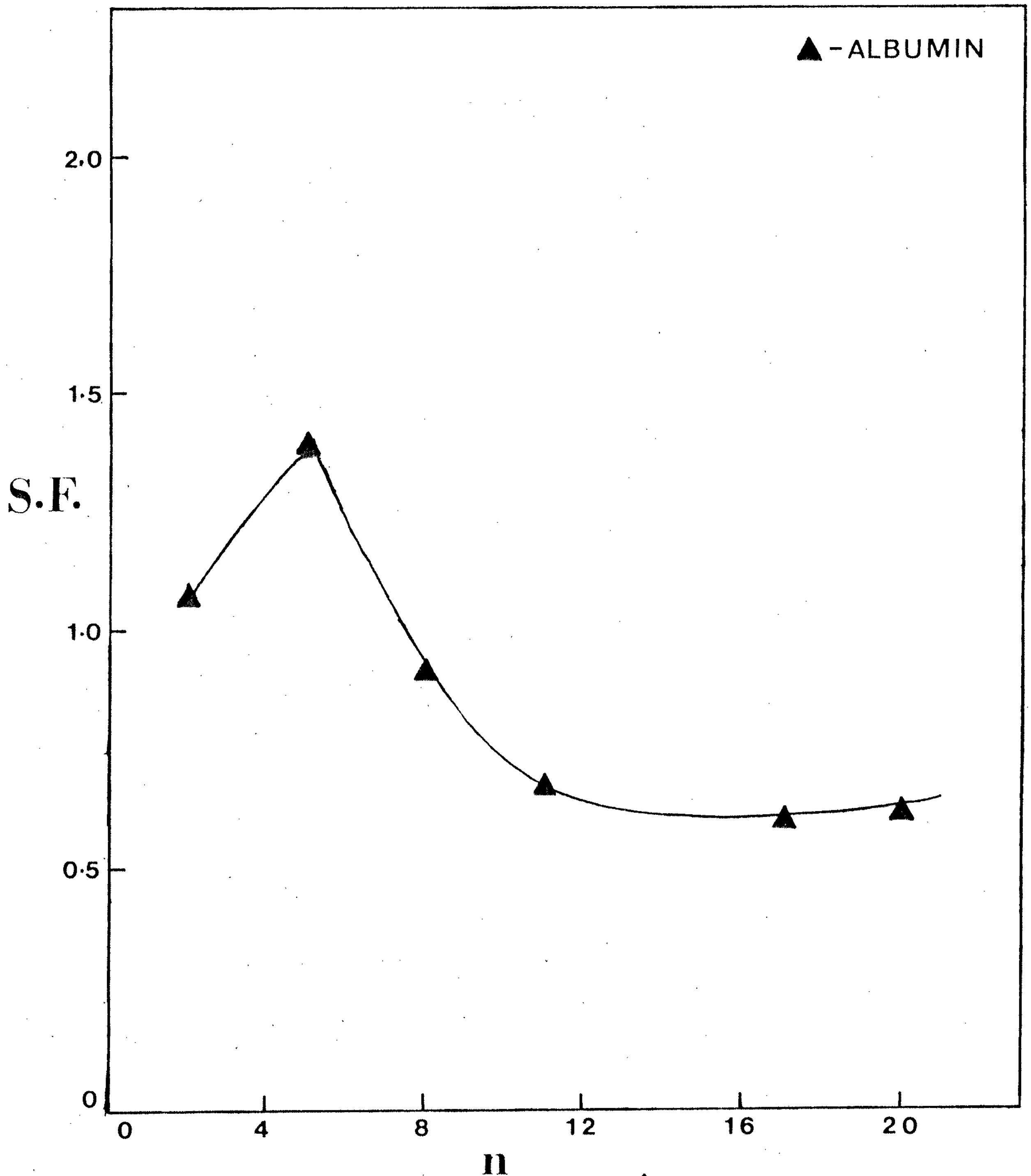

FIGURE 14 (RUN A4)

SEPARATION FACTOR VS. CYCLES

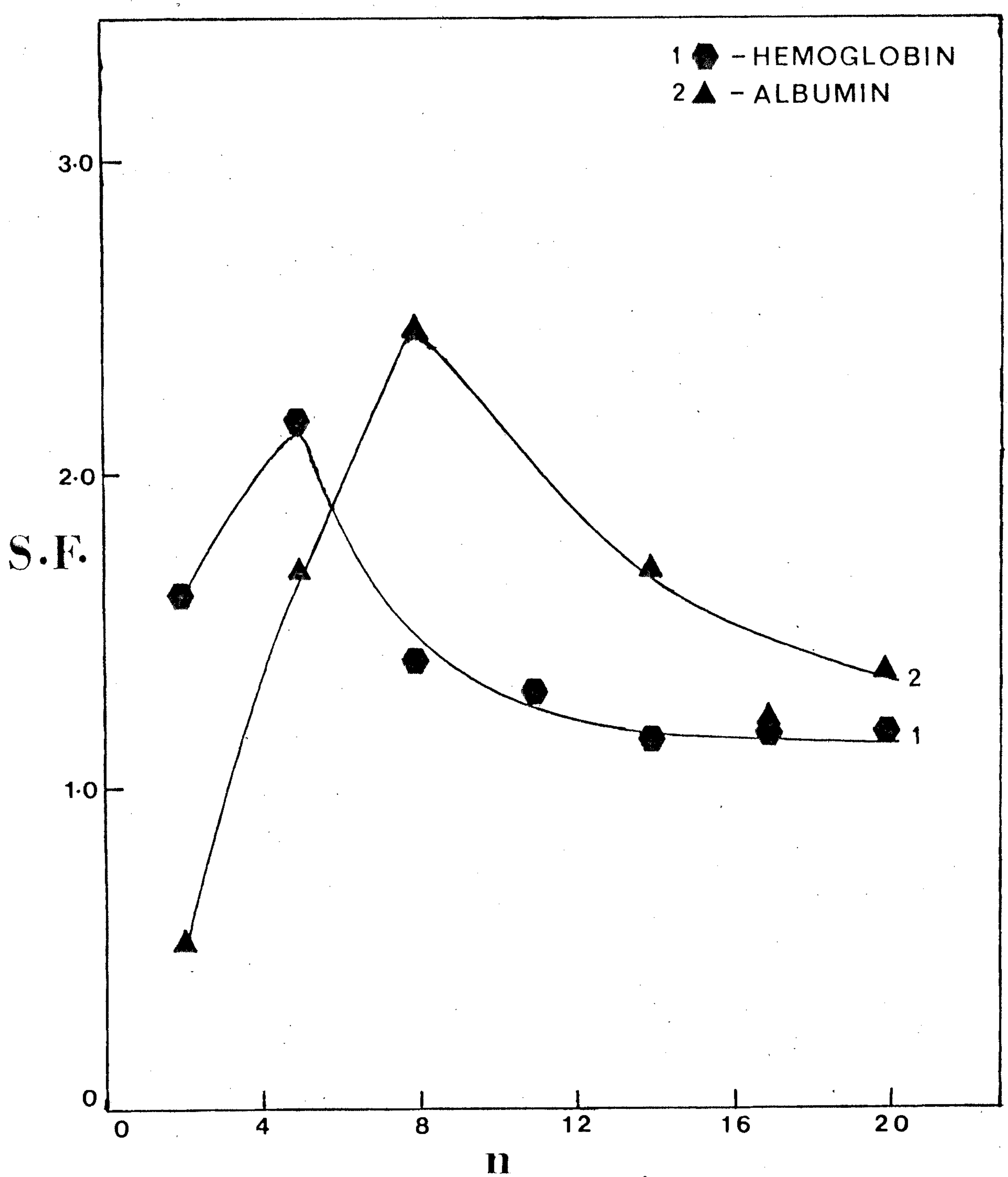

FIGURE 15 (RUN T1)

SEPARATION FACTOR VS. CYCLES

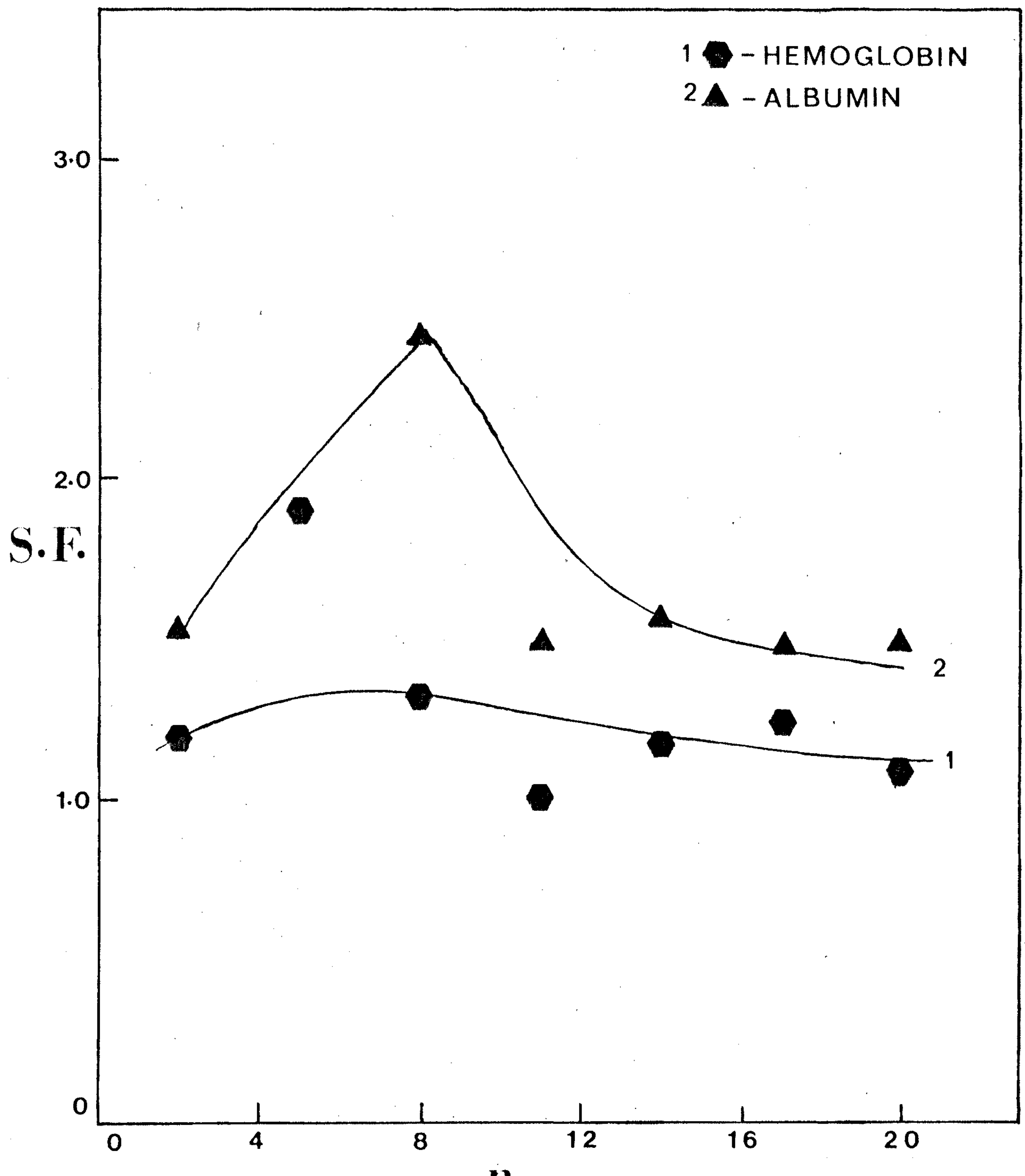

FIGURE 16 (RUN T2)

SEPARATION FACTOR VS. CYCLES

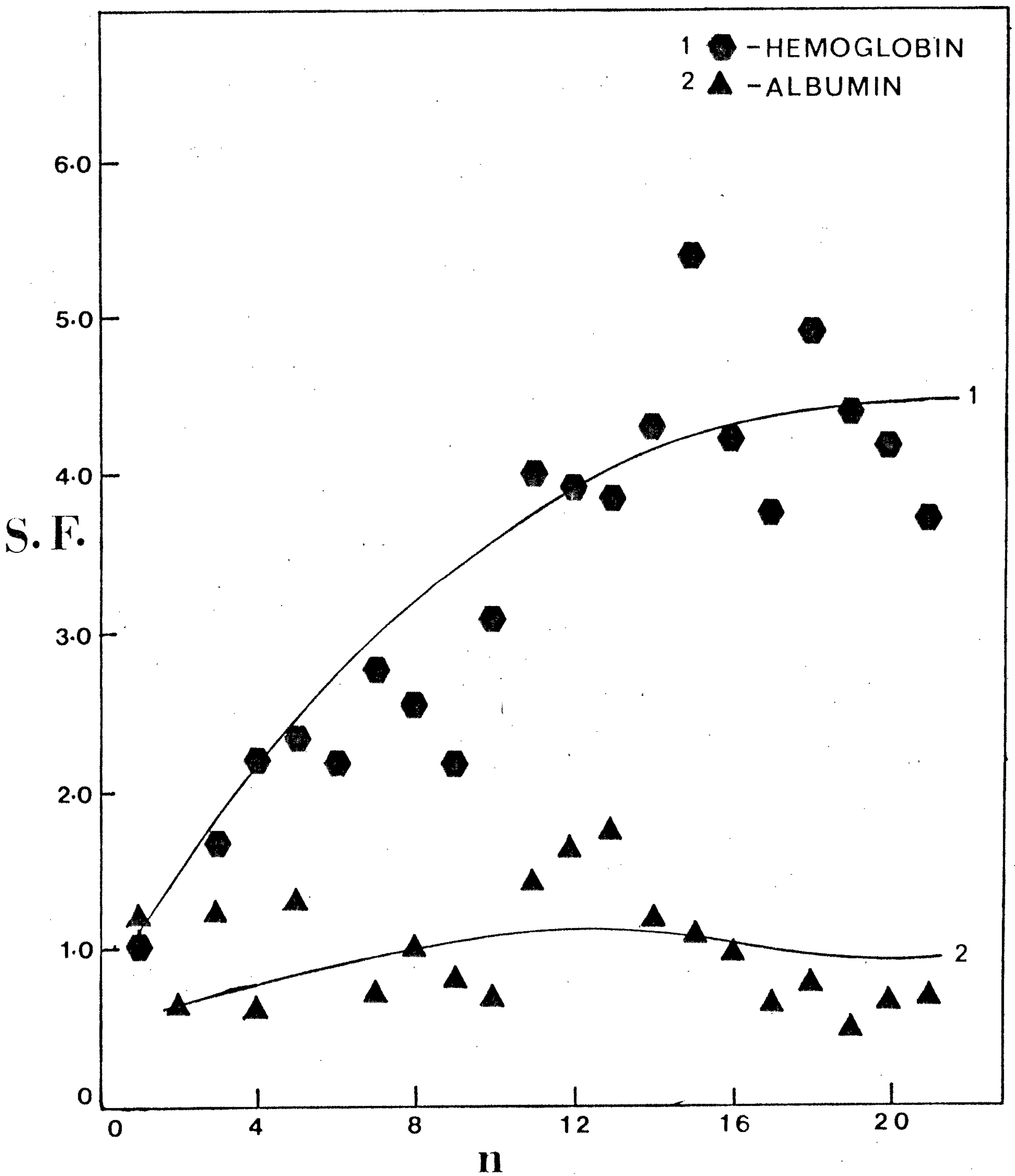

FIGURE 17 (RUN T3)

SEPARATION FACTOR VS. CYCLES

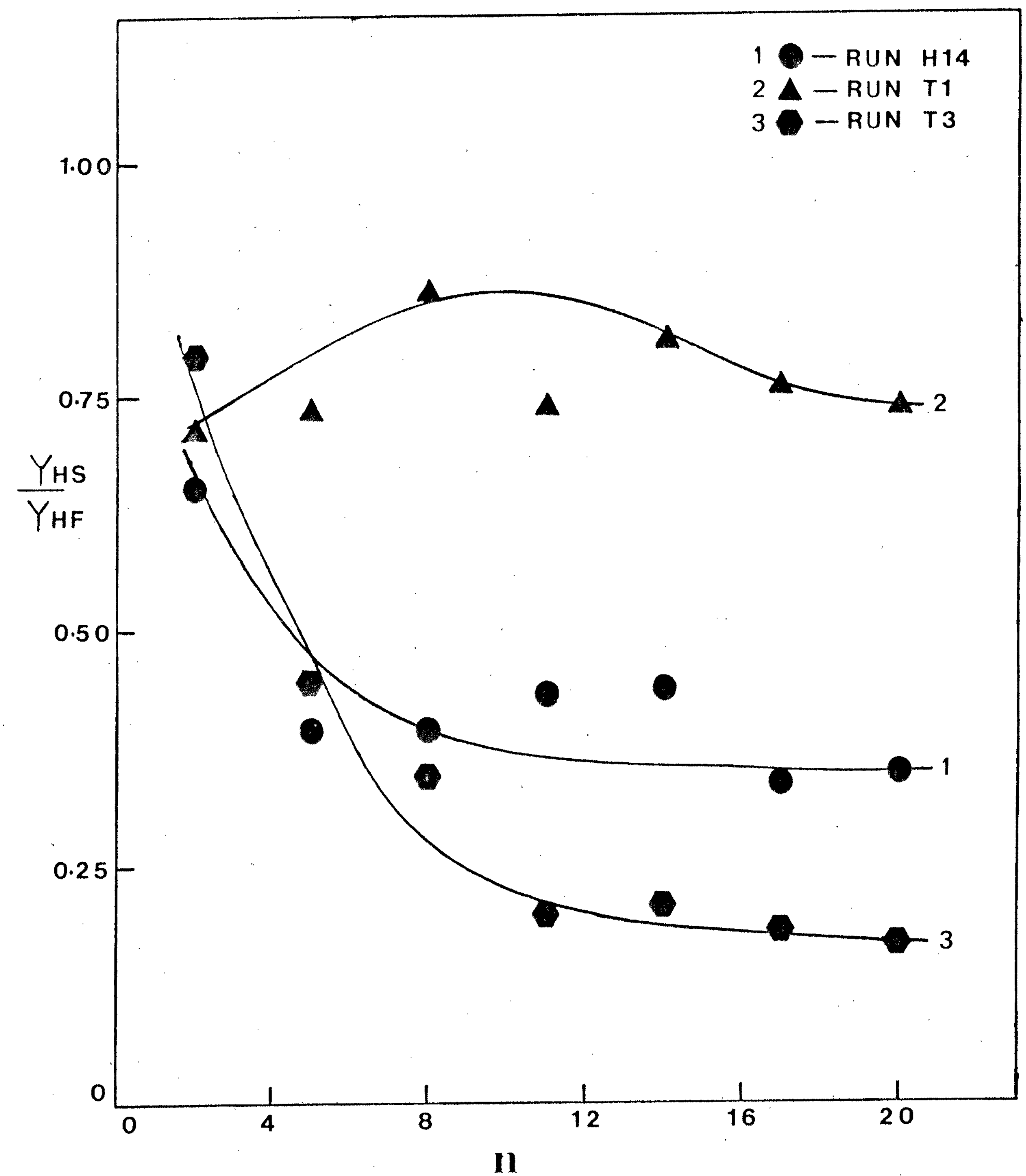

FIGURE 18

HEMOGLOBIN TOP PRODUCTS

NOTATION

A - Absorbance

n - number of cycles

S.F. - Separation Factor

T - Transmission

Y - Concentration

Y_{HS} - Hemoglobin concentration of the sample

Y_{HF} - Hemoglobin concentration of the feed

Y_{AS} - Albumin concentration of the sample

Y_{AF} - Albumin concentration of the feed

λ - Slope

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APPENDIX

TABLES

TABLE 1: CALIBRATION CURVE - HEMOGLOBIN 403mu

CONCENTRATION	(PH=6.0) ABSORBANCE	(PH=7.0) ABSORBANCE	(PH=8.0) ABSORBANCE
0.001	0.047	0.091	0.056
0.002	0.090	0.101	0.076
0.003	0.161	0.162	0.107
0.004	0.227	0.217	0.262
0.005	0.276	0.261	0.302
0.006	0.342	0.303	0.326
0.007	0.409	0.410	0.428
0.008	0.465	0.467	0.440
0.009	0.538	0.479	0.561
0.010	0.590	0.573	0.578
0.011	0.652	0.640	0.572
0.012	0.730	0.629	0.629
0.013	0.783	0.699	0.697
0.014	0.830	0.807	0.728
0.015	0.893	0.848	0.848
0.016	0.955	0.955	0.827
0.017	1.051	0.951	0.914
0.018	1.086	1.032	0.996
0.019	1.125	1.076	0.991
0.020	1.208	1.076	1.046

$A = 59.82C$ (PH=6.0)

$A = 56.12C$ (PH=7.0)

$A = 53.88C$ (PH=8.0)

TABLE 2: CALIBRATION CURVE - HEMOGLOBIN 280mu

CONCENTRATION	(PH=6.0) ABSORBANCE	(PH=7.0) ABSORBANCE	(PH=8.0) ABSORBANCE
0.001	0.021	-----	0.028
0.002	0.030	0.034	0.052
0.003	0.052	0.071	0.082
0.004	0.077	0.048	0.085
0.005	0.093	0.099	0.116
0.006	0.107	0.114	0.114
0.007	0.120	0.126	0.115
0.008	0.128	0.144	0.118
0.009	0.137	0.164	-----
0.010	0.162	0.156	0.139
0.011	0.192	0.212	0.153
0.012	0.193	0.207	0.203
0.013	0.213	-----	0.214
0.014	0.244	0.240	0.207
0.015	0.245	0.278	0.239
0.016	0.268	-----	0.222
0.017	0.283	0.285	0.228
0.018	-----	0.313	0.261
0.019	0.305	-----	0.282
0.020	0.322	0.335	0.286

A=16.49C (PH=6.0)
A=17.40C (PH=7.0)
A=14.95C (PH=8.0)

TABLE 3: CALIBRATION CURVE - ALBUMIN 280mu

CONCENTRATION	(PH=6.0) ABSORBANCE	(PH=7.0) ABSORBANCE	(PH=8.0) ABSORBANCE
0.005	0.028	0.011	0.004
0.008	0.032	0.036	0.008
0.011	0.038	0.042	0.029
0.014	0.068	0.049	0.047
0.017	0.072	0.052	0.062
0.020	0.109	0.080	0.073
0.025	0.138	0.135	0.122
0.030	0.149	0.154	0.146
0.035	0.178	0.178	0.178
0.040	0.204	0.206	0.184
0.045	0.224	0.221	0.277
0.050	0.257	0.246	0.244
0.055	0.280	0.281	0.235
0.060	0.295	0.321	0.289
0.065	0.323	0.331	0.298
0.070	0.353	0.354	0.354
0.075	0.373	0.373	0.363
0.080	0.389	0.410	0.393
0.085	0.434	0.424	0.441
0.090	0.450	0.441	0.481
0.095	0.491	0.478	0.502
0.100	0.520	0.498	0.493

$A = 5.054C$ (PH=6.0)

$A = 5.013C$ (PH=7.0)

$A = 4.995C$ (PH=8.0)

Average Slope = 5.021

TABLE 4: EXPERIMENTAL CONDITIONS FOR BATCH RUNS

Packing Height

A1 - 9.6 Cm.
A2 - 10.1 Cm.
A3 - 11.3 Cm.

Displacement

10 Cm^3. for all runs

Dead Volumn

5 Cm^3. for all runs

Flow Rate for Syringe Reservoirs

0.00833 Cm^3./Sec.

High PH Buffer

PH-8.0 (.05M) for all runs

Low PH Buffer

PH-6.0 (.05M) for all runs

Initial Reservoir Concentration

.05% Albumin for all runs

Initial PH of Packing

A1 - 6.0
A2 - 8.0
A3 - 6.0

Number of Cycles for the Run

A1 - 10
A2 - 10
A3 - 17

TABLE 5: EXPERIMENTAL CONDITIONS FOR SEMI-CONTINUOUS RUNS

Type of Run

A4 - binary for Albumin performed with single column

T1 - ternary performed with single column

T2 - ternary with .05M NaCl added to feed and packing performed with single column

T3 - ternary performed with double column with feed between columns

Packing Height

A4 - 9.4 Cm.
T1 - 9.1 Cm.
T2 - 8.3 Cm.
T3 - 8.6 Cm. (bottom), 8.5 Cm. (top)

Displacement

12 $Cm.^3$ for all runs

Dead Volumn

5 $Cm.^3$ for all runs

Flow Rate for Syringe Reservoirs

0.00833 $Cm.^3$/Sec.

Flow Rate for Feed

0.00283 $Cm.^3$/Sec. for A4,T1,T2
0.00417 $Cm.^3$/Sec. for T3

High PH Buffer

PH-8.0 (.10M) for all runs

Low PH Buffer

PH-6.0 (.10M) for all runs

Feed Concentration

A4 - .01% Albumin (High PH)
T1 - .01% Albumin, .01% Hemoglobin (High PH)
T2 - .01% Albumin, .01% Hemoglobin, .05M NaCl (high PH)
T3 - .01% albumin, .01% hemoglobin (Low PH)

TABLE 5 - Continued

Initial PH of Packing

6.0 for all runs

Number of Cycles for the Run

21 for all runs

TABLE 6: BATCH RUNS (A1, A2, A3)

RUN	SAMPLE	(280) TRANSMISSION	(280) ABSORBANCE	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$	SEPARATION FACTOR
	RB	40.9	0.388	0.07728	1.61	
A1	RT	37.2	0.429	0.08544	1.78	0.90
	FEED	57.4	0.241	0.04800	----	
	RB	37.2	0.429	0.08544	1.65	
A2	RT	35.6	0.449	0.08942	1.73	0.96
	FEED	54.9	0.260	0.05178	----	
	RB	44.9	0.348	0.06931	1.35	
A3	RT	41.7	0.380	0.07568	1.47	0.92
	FEED	55.2	0.258	0.05138	----	

TABLE 7: RUN A4

SAMPLE	AMOUNT(CM)^3 COLLECTED	PH	(280) TRANSMISSION	(280) ABSORBANCE	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
1B	2.6	7.25	62.2	0.206	0.04103	2.78
1T	1.1	----	----	-----	-------	----
2B	2.7	7.40	64.4	0.191	0.03804	2.58
2T	1.0	6.05	66.4	0.178	0.03545	2.41
3B	2.7	7.65	72.6	0.139	0.02768	1.88
3T	1.0	----	----	-----	-------	----
4B	2.8	7.80	71.4	0.147	0.02928	1.99
4T	1.2	----	----	-----	-------	----
5B	2.7	7.80	67.5	0.170	0.03386	2.30
5T	0.9	6.00	75.6	0.122	0.02430	1.64
6B	2.9	7.85	69.2	0.160	0.03187	2.16
6T	1.1	----	----	-----	-------	----
7B	2.9	7.90	60.3	0.220	0.04382	2.97
7T	1.1	----	----	-----	-------	----
8B	2.9	7.95	77.7	0.110	0.02191	1.49
8T	1.0	6.05	75.7	0.121	0.02410	1.64
9B	2.9	8.00	69.7	0.157	0.03127	2.12
9T	1.1	----	----	-----	-------	----
10B	2.7	7.90	66.5	0.177	0.03525	2.39
10T	1.6	----	----	-----	-------	----
11B	2.8	8.00	72.5	0.140	0.02788	1.89
11T	0.8	6.10	61.9	0.208	0.04143	2.81
12B	2.8	7.95	66.4	0.178	0.03545	2.41
12T	0.9	----	----	-----	-------	----
13B	2.7	7.90	68.4	0.165	0.03286	2.23
13T	1.2	----	----	-----	-------	----

TABLE 7 - Continued

SAMPLE	AMOUNT(CM)^3 COLLECTED	PH	(280) TRANSMISSION	(280) ABSORBANCE	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
14B	3.0	8.00	66.5	0.177	0.03525	2.39
14T	1.0	6.05	71.2	0.148	0.02948	2.00
15B	2.8	8.00	74.0	0.131	0.02609	1.77
15T	1.0	----	----	-----	-------	----
16B	3.0	7.95	72.1	0.142	0.02828	1.92
16T	1.0	----	----	-----	-------	----
17B	3.0	8.00	76.7	0.115	0.02290	1.55
17T	1.1	6.10	64.5	0.190	0.03784	2.57
18B	3.0	8.00	73.1	0.136	0.02709	1.84
18T	0.9	----	----	-----	-------	----
19B	2.6	8.00	77.1	0.113	0.02251	1.53
19T	0.7	----	----	-----	-------	----
20B	2.9	8.00	78.3	0.106	0.02111	1.43
20T	2.3	6.15	67.6	0.170	0.03386	2.30
21B	3.2	8.00	82.7	0.082	0.01633	1.11
21T	1.1	----	----	-----	-------	----
RB	---	7.90	81.0	0.092	0.01832	1.24
RT	---	6.10	64.2	0.192	0.03824	2.59
FEED	---	8.00	84.4	0.074	0.01474	----

TABLE 8: RUN T1

SAMPLE	AMOUNT(CM)^3 COLLECTED	PH	(403) TRANSMISSION	(280) TRANSMISSION	(403) ABSORBANCE
1B	3.0	6.95	52.7	58.4	0.278
1T	1.0	----	----	----	-----
2B	3.3	7.20	26.1	51.8	0.583
2T	0.9	6.05	41.5	48.6	0.382
3B	3.0	7.30	15.8	45.7	0.801
3T	1.1	----	----	----	-----
4B	3.0	7.55	14.1	41.1	0.851
4T	1.0	----	----	----	-----
5B	3.2	7.70	16.2	41.8	0.790
5T	0.8	6.00	39.8	63.6	0.400
6B	2.9	7.80	20.4	46.8	0.690
6T	1.3	----	----	----	-----
7B	3.0	7.80	23.9	49.7	0.622
7T	1.1	----	----	----	-----
8B	2.8	7.90	25.4	53.0	0.596
8T	1.3	6.10	34.2	66.9	0.466
9B	3.0	7.90	27.0	48.8	0.568
9T	0.9	----	----	----	-----
10B	3.0	7.95	30.4	53.1	0.517
10T	1.0	----	----	----	-----
11B	3.0	7.95	32.8	56.8	0.484
11T	1.0	6.10	39.8	59.4	0.400
12B	3.1	7.90	32.5	35.4	0.488
12T	1.1	----	----	----	-----
13B	2.9	8.00	32.3	34.6	0.491
13T	1.0	----	----	----	-----

TABLE 8 - Continued

SAMPLE	AMOUNT(CM)^3 COLLECTED	PH	(403) TRANSMISSION	(280) TRANSMISSION	(403) ABSORBANCE
14B	3.0	7.95	34.7	42.3	0.460
14T	1.1	6.10	36.7	54.5	0.435
15B	3.0	7.90	37.6	49.4	0.424
15T	1.0	----	----	----	-----
16B	3.0	7.95	37.3	47.0	0.428
16T	1.0	----	----	----	-----
17B	3.0	8.10	36.3	54.4	0.440
17T	1.2	6.15	39.4	58.7	0.405
18B	3.1	8.00	37.1	54.2	0.430
18T	1.1	----	----	----	-----
19B	3.1	7.95	37.1	61.0	0.430
19T	1.0	----	----	----	-----
20B	3.0	8.00	36.9	57.1	0.433
20T	1.4	6.10	40.1	63.1	0.397
21B	3.1	7.95	37.9	63.6	0.421
21T	0.9	----	----	----	-----
RB	---	7.95	37.6	61.7	0.424
RT	---	6.15	35.4	62.6	0.451
FEED	---	8.00	32.3	60.8	0.491

TABLE 8 - Continued

SAMPLE	(280) ABSORBANCE	HEMOGLOBIN (280) CONTRIBUTION	ALBUMIN (280) CONTRIBUTION	λH403	λH280
1B	0.235	0.086	0.149	56.23	17.36
1T	-----	-----	-----	-----	-----
2B	0.285	0.180	0.105	55.62	17.14
2T	0.313	0.106	0.207	59.45	16.55
3B	0.340	0.245	0.095	55.37	16.95
3T	-----	-----	-----	-----	-----
4B	0.386	0.254	0.132	54.78	16.35
4T	-----	-----	-----	-----	-----
5B	0.379	0.231	0.148	54.45	15.93
5T	0.197	0.110	0.087	59.82	16.49
6B	0.330	0.199	0.131	54.25	15.63
6T	-----	-----	-----	-----	-----
7B	0.304	0.179	0.125	54.25	15.63
7T	-----	-----	-----	-----	-----
8B	0.276	0.169	0.107	54.05	15.29
8T	0.174	0.131	0.043	59.15	16.60
9B	0.311	0.161	0.150	54.05	15.29
9T	-----	-----	-----	-----	-----
10B	0.275	0.145	0.130	53.96	15.12
10T	-----	-----	-----	-----	-----
11B	0.246	0.136	0.110	53.96	15.12
11T	0.226	0.112	0.114	59.15	16.60
12B	0.451	0.138	0.313	54.05	15.29
12T	-----	-----	-----	-----	-----
13B	0.460	0.136	0.324	53.88	14.95
13T	-----	-----	-----	-----	-----

TABLE 8 - Continued

SAMPLE	(280) ABSORBANCE	HEMOGLOBIN(280) CONTRIBUTION	ALBUMIN(280) CONTRIBUTION	λH403	λH280
14B	0.373	0.129	0.244	53.96	15.12
14T	0.264	0.122	0.142	59.15	16.60
15B	0.306	0.120	0.186	54.05	15.29
15T	-----	-----	-----	-----	-----
16B	0.328	0.120	0.208	53.96	15.12
16T	-----	-----	-----	-----	-----
17B	0.265	0.122	0.143	53.88	14.95
17T	0.232	0.115	0.117	58.88	16.95
18B	0.266	0.119	0.147	53.88	14.95
18T	-----	-----	-----	-----	-----
19B	0.214	0.121	0.093	53.96	15.12
19T	-----	-----	-----	-----	-----
20B	0.243	0.120	0.123	53.88	14.95
20T	0.200	0.111	0.089	59.15	16.60
21B	0.197	0.118	0.079	53.96	15.12
21T	-----	-----	-----	-----	-----
RB	0.210	0.119	0.091	53.96	15.12
RT	0.203	0.128	0.075	58.88	16.65
FEED	0.216	0.136	0.080	53.88	14.95

TABLE 8 - Continued

SAMPLE	HEMOGLOBIN CONCENTRATION	$\frac{Y_{HS}}{Y_{HF}}$	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
1B	0.00494	0.54	0.02968	1.86
1T	-------	----	-------	----
2B	0.01048	1.15	0.02091	1.31
2T	0.00643	0.71	0.04123	2.59
3B	0.01447	1.59	0.01892	1.19
3T	-------	----	-------	----
4B	0.01553	1.70	0.02629	1.65
4T	-------	----	-------	----
5B	0.01451	1.59	0.02948	1.85
5T	0.00669	0.73	0.01733	1.09
6B	0.01272	1.40	0.02609	1.64
6T	-------	----	-------	----
7B	0.01147	1.26	0.02490	1.56
7T	-------	----	-------	----
8B	0.01103	1.21	0.02131	1.34
8T	0.00788	0.86	0.00856	0.54
9B	0.01051	1.15	0.02987	1.88
9T	-------	----	-------	----
10B	0.00958	1.05	0.02589	1.63
10T	-------	----	-------	----
11B	0.00897	0.98	0.02191	1.38
11T	0.00676	0.74	0.02270	1.42
12B	0.00903	0.99	0.06234	3.91
12T	-------	----	-------	----
13B	0.00908	1.00	0.06453	4.05
13T	-------	----	-------	----

TABLE 8 - Continued

SAMPLE	HEMOGLOBIN CONCENTRATION	$\frac{Y_{HS}}{Y_{HF}}$	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
14B	0.00852	0.94	0.04860	3.05
14T	0.00735	0.81	0.02828	1.78
15B	0.00784	0.86	0.03704	2.33
15T	--------	----	--------	----
16B	0.00793	0.87	0.04143	2.60
16T	--------	----	--------	----
17B	0.00817	0.90	0.02848	1.79
17T	0.00688	0.76	0.02330	1.46
18B	0.00798	0.88	0.02928	1.84
18T	--------	----	--------	----
19B	0.00797	0.87	0.01852	1.16
19T	--------	----	--------	----
20B	0.00804	0.88	0.02450	1.54
20T	0.00671	0.74	0.01773	1.11
21B	0.00780	0.86	0.01573	0.99
21T	--------	----	--------	----
RB	0.00786	0.86	0.01812	1.14
RT	0.00766	0.84	0.01494	0.94
FEED	0.00911	----	0.01593	----

TABLE 9: RUN T2

SAMPLE	AMOUNT(CM)^3 COLLECTED	PH	(403) TRANSMISSION	(280) TRANSMISSION	(403) ABSORBANCE
1B	3.0	7.15	40.2	38.2	0.395
1T	1.0	----	----	----	-----
2B	3.1	7.25	32.5	38.2	0.488
2T	1.0	6.30	37.3	50.4	0.428
3B	3.0	7.40	21.2	35.3	0.673
3T	0.9	----	----	----	-----
4B	3.0	7.55	20.6	34.0	0.687
4T	1.4	----	----	----	-----
5B	3.0	7.75	21.8	36.1	0.662
5T	0.9	6.40	42.7	45.5	0.369
6B	2.8	7.80	26.7	33.3	0.573
6T	1.0	----	----	----	-----
7B	2.9	7.85	28.4	40.3	0.546
7T	1.0	----	----	----	-----
8B	2.8	7.90	31.0	41.3	0.509
8T	1.0	6.35	38.4	60.6	0.415
9B	3.0	8.00	31.2	45.2	0.505
9T	1.1	----	----	----	-----
10B	2.9	8.00	30.5	46.7	0.516
10T	1.9	----	----	----	-----
11B	3.1	7.90	35.2	35.1	0.454
11T	1.0	6.55	33.4	43.8	0.476
12B	2.9	7.95	35.5	33.0	0.450
12T	0.9	----	----	----	-----
13B	2.9	8.00	38.3	38.6	0.417
13T	1.0	----	----	----	-----

TABLE 9 - Continued

SAMPLE	AMOUNT(CM)^3 COLLECTED	PH	(403) TRANSMISSION	(280) TRANSMISSION	(403) ABSORBANCE
14B	2.8	8.00	37.7	40.3	0.424
14T	0.9	6.25	40.3	51.3	0.394
15B	3.1	8.10	38.8	43.2	0.411
15T	1.1	----	----	----	-----
16B	3.0	7.95	38.6	44.1	0.414
16T	1.1	----	----	----	-----
17B	3.1	8.00	38.8	45.3	0.411
17T	0.9	6.25	43.6	55.2	0.360
18B	3.0	8.10	40.8	50.4	0.390
18T	1.0	----	----	----	-----
19B	3.1	8.00	39.5	46.5	0.403
19T	1.5	----	----	----	-----
20B	3.0	8.00	39.4	48.7	0.405
20T	1.0	6.10	42.5	56.5	0.371
21B	3.0	7.95	41.3	52.2	0.385
21T	0.8	----	----	----	-----
RB	---	7.90	38.1	50.7	0.419
RT	---	6.10	36.1	54.0	0.443
FEED	---	8.00	33.6	64.6	0.473

TABLE 9 - Continued

SAMPLE	(280) ABSORBANCE	HEMOGLOBIN(280) CONTRIBUTION	ALBUMIN(280) CONTRIBUTION	∂H403	∂H280
1B	0.418	0.122	0.296	55.75	17.23
1T	-----	-----	-----	-----	-----
2B	0.418	0.150	0.268	55.50	17.05
2T	0.298	0.123	0.175	58.22	16.80
3B	0.452	0.204	0.248	55.14	16.73
3T	-----	-----	-----	-----	-----
4B	0.469	0.205	0.264	54.78	16.35
4T	-----	-----	-----	-----	-----
5B	0.442	0.192	0.250	54.35	15.78
5T	0.342	0.108	0.234	57.87	16.90
6B	0.478	0.165	0.313	54.25	15.63
6T	-----	-----	-----	-----	-----
7B	0.395	0.151	0.244	54.15	14.96
7T	-----	-----	-----	-----	-----
8B	0.384	0.144	0.240	54.05	15.29
8T	0.218	0.120	0.098	58.05	16.85
9B	0.345	0.140	0.205	53.88	14.95
9T	-----	-----	-----	-----	-----
10B	0.330	0.143	0.187	53.88	14.95
10T	-----	-----	-----	-----	-----
11B	0.454	0.128	0.326	54.05	15.29
11T	0.359	0.142	0.217	57.36	17.05
12B	0.482	0.126	0.356	53.96	15.12
12T	-----	-----	-----	-----	-----
13B	0.414	0.116	0.298	53.88	14.95
13T	-----	-----	-----	-----	-----

TABLE 9 - Continued

SAMPLE	(280) ABSORBANCE	HEMOGLOBIN(280) CONTRIBUTION	ALBUMIN(280) CONTRIBUTION	λH403	λH280
14B	0.395	0.118	0.277	53.88	14.95
14T	0.290	0.113	0.177	58.43	16.75
15B	0.365	0.114	0.251	53.88	14.95
15T	-----	-----	-----	-----	-----
16B	0.355	0.116	0.239	53.96	15.12
16T	-----	-----	-----	-----	-----
17B	0.344	0.114	0.230	53.88	14.95
17T	0.258	0.103	0.155	58.43	16.75
18B	0.298	0.100	0.198	53.88	14.95
18T	-----	-----	-----	-----	-----
19B	0.333	0.108	0.225	53.88	14.95
19T	-----	-----	-----	-----	-----
20B	0.312	0.112	0.200	53.88	14.95
20T	0.248	0.114	0.134	59.15	16.60
21B	0.282	0.108	0.174	53.96	15.12
21T	-----	-----	-----	-----	-----
RB	0.295	0.118	0.177	54.05	15.29
RT	0.267	0.124	0.143	59.15	16.60
FEED	0.190	0.132	0.058	53.88	14.95

TABLE 9 - Continued

SAMPLE	HEMOGLOBIN CONCENTRATION	$\frac{Y_{HS}}{Y_{HF}}$	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
1B	0.00709	0.81	0.05595	5.10
1T	--------	----	--------	----
2B	0.00879	1.00	0.05338	4.62
2T	0.00735	0.84	0.03485	3.02
3B	0.01221	1.39	0.04939	4.28
3T	--------	----	--------	----
4B	0.01254	1.43	0.05258	4.55
4T	--------	----	--------	----
5B	0.01218	1.38	0.04979	4.31
5T	0.00638	0.73	0.04660	4.03
6B	0.01056	1.20	0.06234	5.40
6T	--------	----	--------	----
7B	0.01008	1.15	0.04860	4.21
7T	--------	----	--------	----
8B	0.00942	1.07	0.04780	4.14
8T	0.00715	0.81	0.01952	1.69
9B	0.00937	1.07	0.04083	3.53
9T	--------	----	--------	----
10B	0.00958	1.09	0.03724	3.22
10T	--------	----	--------	----
11B	0.00840	0.95	0.06493	5.62
11T	0.00830	0.94	0.04322	3.74
12B	0.00834	0.95	0.07090	6.14
12T	--------	----	--------	----
13B	0.00774	0.88	0.05935	5.14
13T	--------	----	--------	----

TABLE 9 - Continued

SAMPLE	HEMOGLOBIN CONCENTRATION	$\frac{Y_{HS}}{Y_{HF}}$	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
14B	0.00787	0.89	0.05517	4.78
14T	0.00674	0.77	0.03525	3.05
15B	0.00763	0.87	0.04999	4.33
15T	-------	----	-------	----
16B	0.00767	0.87	0.04760	4.12
16T	-------	----	-------	----
17B	0.00763	0.87	0.04581	3.97
17T	0.00616	0.70	0.03087	2.67
18B	0.00668	0.76	0.03943	3.41
18T	-------	----	-------	----
19B	0.00724	0.82	0.04481	3.88
19T	-------	----	-------	----
20B	0.00748	0.85	0.03983	3.45
20T	0.00685	0.79	0.02669	2.31
21B	0.00713	0.81	0.03465	3.00
21T	-------	----	-------	----
RB	0.00775	0.88	0.03525	3.05
RT	0.00749	0.85	0.02848	2.47
FEED	0.00880	----	0.01155	----

TABLE 10: RUN T3

SAMPLE	AMOUNT(CM)^3 COLLECTED	PH	(403) TRANSMISSION	(280) TRANSMISSION	(403) ABSORBANCE
1B	3.0	7.20	40.4	53.7	0.394
1T	3.0	6.00	38.4	57.8	0.416
2B	3.0	6.85	55.2	72.6	0.258
2T	3.1	6.00	38.2	61.2	0.418
3B	2.9	7.20	29.4	55.1	0.532
3T	3.0	6.00	45.0	66.9	0.347
4B	2.9	7.20	29.9	61.0	0.524
4T	3.0	6.00	55.1	68.8	0.259
5B	3.0	7.35	31.1	54.4	0.508
5T	3.0	6.00	57.7	70.5	0.239
6B	2.9	7.40	34.1	49.6	0.467
6T	3.0	6.00	56.1	73.5	0.251
7B	2.9	7.50	36.8	60.6	0.434
7T	3.0	6.00	67.1	67.3	0.173
8B	3.0	7.65	37.1	61.2	0.430
8T	3.0	6.00	65.2	72.8	0.186
9B	3.0	7.55	42.3	63.6	0.374
9T	3.0	6.00	64.3	69.5	0.192
10B	3.0	7.50	43.6	66.1	0.360
10T	3.1	6.00	74.4	71.3	0.129
11B	3.0	7.60	41.2	43.4	0.385
11T	3.1	6.00	78.5	62.2	0.105
12B	3.0	7.60	38.9	44.0	0.410
12T	3.1	6.05	76.8	66.7	0.114
13B	2.8	7.50	36.5	43.6	0.438
13T	3.0	6.00	74.9	68.0	0.125

TABLE 10 - Continued

SAMPLE	AMOUNT (CM)^3 COLLECTED	PH	(403) TRANSMISSION	(280) TRANSMISSION	(403) ABSORBANCE
14B	3.0	7.60	36.5	48.6	0.437
14T	3.0	6.00	77.3	64.7	0.112
15B	3.0	7.50	38.0	54.1	0.420
15T	3.0	6.00	82.3	69.6	0.085
16B	2.8	7.50	43.4	60.9	0.362
16T	3.0	6.00	80.3	73.0	0.095
17B	3.0	7.50	47.1	66.8	0.327
17T	3.1	6.00	80.2	69.3	0.096
18B	2.9	7.45	42.1	61.6	0.376
18T	3.1	6.00	82.6	70.3	0.083
19B	3.1	7.50	42.4	66.7	0.373
19T	3.1	6.00	81.0	69.5	0.092
20B	3.1	7.45	45.7	68.0	0.340
20T	3.2	6.00	81.3	72.6	0.090
21B	2.9	7.40	43.1	66.8	0.366
21T	3.1	6.00	77.9	73.4	0.109
RB	---	7.40	47.7	79.4	0.321
RT	---	6.00	80.2	71.5	0.096
FEED	---	6.00	29.5	62.4	0.530

TABLE 10 - Continued

SAMPLE	(280) ABSORBANCE	HEMOGLOBIN (280) CONTRIBUTION	ALBUMIN (280) CONTRIBUTION	$\lambda H403$	$\lambda H280$
1B	0.270	0.121	0.149	55.62	17.14
1T	0.238	0.115	0.123	59.82	16.49
2B	0.139	0.079	0.060	56.48	17.29
2T	0.213	0.115	0.098	59.82	16.49
3B	0.259	0.164	0.095	55.62	17.14
3T	0.174	0.096	0.078	59.82	16.49
4B	0.214	0.161	0.053	55.62	17.14
4T	0.163	0.071	0.092	59.82	16.49
5B	0.264	0.155	0.109	55.25	16.85
5T	0.152	0.066	0.086	59.82	16.49
6B	0.304	0.142	0.162	55.14	16.73
6T	0.134	0.069	0.065	59.82	16.49
7B	0.218	0.130	0.088	54.90	16.48
7T	0.172	0.048	0.124	59.82	16.49
8B	0.213	0.127	0.086	54.56	16.08
8T	0.138	0.051	0.087	59.82	16.49
9B	0.197	0.112	0.085	54.78	16.35
9T	0.158	0.053	0.105	59.82	16.49
10B	0.180	0.108	0.072	54.90	16.48
10T	0.147	0.036	0.111	59.82	16.49
11B	0.363	0.114	0.249	54.67	16.22
11T	0.206	0.029	0.177	59.82	16.49
12B	0.356	0.122	0.234	54.67	16.22
12T	0.176	0.032	0.144	59.45	16.55
13B	0.361	0.132	0.229	54.90	16.48
13T	0.167	0.034	0.133	59.82	16.49

TABLE 10 - Continued

SAMPLE	(280) ABSORBANCE	HEMOGLOBIN (280) CONTRIBUTION	ALBUMIN (280) CONTRIBUTION	λH403	λH280
14B	0.313	0.130	0.183	54.67	16.22
14T	0.189	0.031	0.158	59.82	16.49
15B	0.267	0.126	0.141	54.90	16.48
15T	0.157	0.023	0.134	59.82	16.49
16B	0.215	0.109	0.106	54.90	16.48
16T	0.137	0.026	0.111	59.82	16.49
17B	0.175	0.098	0.077	54.90	16.48
17T	0.159	0.026	0.133	59.82	16.49
18B	0.210	0.114	0.096	55.00	16.60
18T	0.153	0.023	0.130	59.82	16.49
19B	0.176	0.112	0.064	54.90	16.48
19T	0.158	0.025	0.133	59.82	16.49
20B	0.168	0.102	0.066	55.00	16.60
20T	0.139	0.025	0.114	59.82	16.49
21B	0.175	0.111	0.064	55.14	16.73
21T	0.134	0.030	0.104	59.82	16.49
RB	0.100	0.097	0.003	55.14	16.73
RT	0.146	0.026	0.120	59.82	16.49
FEED	0.205	0.146	0.059	59.82	16.49

TABLE 10 - Continued

SAMPLE	HEMOGLOBIN CONCENTRATION	$\frac{Y_{HS}}{Y_{HF}}$	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
1B	0.00708	0.80	0.02968	2.53
1T	0.00695	0.78	0.02450	2.09
2B	0.00457	0.52	0.01195	1.02
2T	0.00699	0.79	0.01952	1.66
3B	0.00956	1.08	0.01852	1.61
3T	0.00580	0.65	0.01553	1.32
4B	0.00942	1.06	0.01056	0.90
4T	0.00433	0.49	0.01852	1.56
5B	0.00919	1.04	0.02171	1.85
5T	0.00400	0.45	0.01713	1.46
6B	0.00847	0.96	0.03226	2.75
6T	0.00420	0.47	0.01295	1.10
7B	0.00791	0.89	0.01753	1.49
7T	0.00289	0.33	0.02470	2.10
8B	0.00788	0.89	0.01713	1.46
8T	0.00311	0.35	0.01733	1.47
9B	0.00683	0.77	0.01693	1.44
9T	0.00321	0.36	0.02091	1.78
10B	0.00656	0.74	0.01434	1.22
10T	0.00216	0.24	0.02211	1.88
11B	0.00704	0.79	0.04959	4.22
11T	0.00176	0.20	0.03525	3.00
12B	0.00750	0.85	0.04660	3.97
12T	0.00192	0.22	0.02868	2.44
13B	0.00798	0.90	0.04561	3.88
13T	0.00209	0.24	0.02649	2.25

TABLE 10 - Continued

SAMPLE	HEMOGLOBIN CONCENTRATION	$\frac{Y_{HS}}{Y_{HF}}$	ALBUMIN CONCENTRATION	$\frac{Y_{AS}}{Y_{AF}}$
14B	0.00799	0.90	0.03645	3.10
14T	0.00187	0.21	0.03147	2.68
15B	0.00765	0.86	0.02808	2.39
15T	0.00142	0.16	0.02669	2.27
16B	0.00659	0.74	0.02111	1.80
16T	0.00159	0.18	0.02211	1.88
17B	0.00596	0.67	0.01534	1.31
17T	0.00160	0.18	0.02649	2.25
18B	0.00684	0.77	0.01912	1.63
18T	0.00139	0.16	0.02589	2.20
19B	0.00679	0.77	0.01275	1.09
19T	0.00154	0.17	0.02649	2.25
20B	0.00618	0.70	0.01314	1.12
20T	0.00150	0.17	0.02270	1.93
21B	0.00664	0.75	0.01275	1.09
21T	0.00182	0.21	0.02071	1.76
RB	0.00582	0.66	0.00060	0.05
RT	0.00160	0.18	0.02390	2.03
FEED	0.00886	----	0.01175	----

TABLE 11: SEPARATION FACTORS

CYCLE	A4 (Alb.)	T1 (Hem.)	T1 (Alb.)	T2 (Hem.)	T2 (Alb.)	T3 (Hem.)	T3 (Alb.)
1	----	----	----	----	----	1.02	1.21
2	1.07	1.62	0.51	1.20	1.53	0.65	0.61
3	----	----	----	----	----	1.65	1.22
4	----	----	----	----	----	2.18	0.58
5	1.39	2.18	1.70	1.91	1.07	2.30	1.27
6	----	----	----	----	----	2.17	2.49
7	----	----	----	----	----	2.74	0.71
8	0.91	1.41	2.48	1.32	2.45	2.53	0.99
9	----	----	----	----	----	2.13	0.81
10	----	----	----	----	----	3.04	0.65
11	0.67	1.32	0.97	1.01	1.50	4.00	1.41
12	----	----	----	----	----	3.91	1.62
13	----	----	----	----	----	3.82	1.72
14	1.20	1.16	1.71	1.17	1.57	4.27	1.16
15	----	----	----	----	----	5.39	1.05
16	----	----	----	----	----	4.14	0.95
17	0.60	1.18	1.23	1.24	1.48	3.72	0.58
18	----	----	----	----	----	4.92	0.74
19	----	----	----	----	----	4.41	0.48
20	0.62	1.19	1.39	1.09	1.49	4.12	0.58
21	----	----	----	----	----	3.65	0.62
R	0.49	1.02	1.21	1.03	1.24	3.64	0.03