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SEMICONTINUOUS pH-PARAMETRIC PUMP — EXPERIMENTAL STUDY

BY

HSIEN-CHIH LEE

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PRESENTED IN PARTIAL FULFILIMENT OF

THE REQUIREMENTS FOR THE DEGREE

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AT

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ABSTRACT

Voidage of SP Sephadex (C-50) ion exchanger at different aqueous buffer concentration and the pH have been estimated from draining the interstitial aqueous. Experimental results of the SP Sephadex (C-50) voidage at pH cycling zone are in quantitative agreement with previous test method, so the voidage estimated from draining the interstital aqueous can be used in pH-parametric pumping. The optional displacement volume in semicontinuous pH-parametric pump of different buffer concentration can be predicted from voidage. A semicontinuous pH-parametric pump for separating proteins has been experimentally investigated using the model system haemoglobin and albumin on a SP Sephadex (C-50) ion exchanger. The pump considered has a center feed between an enriching column and a stripping column, and is operated batchwise during upflow and continuously during downflow. It has been shown that parametric pump is capable of separating proteins with high separation factor. Displacement volume and the product flow rate of the product stream have been found to be important in determining the pumping performance. The cycle time and the location of the feed introduced affect separation in some condition. Separation can be improved with large displacement volume under the optional displacement volume. 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.

APPROVAL OF THESIS

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NEW JERSEY INSTITUTE OF TECHNOLOGY

BY

FACULTY COMMITTEE

APPROVED:_____

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NEWARK, NEW JERSEY

MAY, 1978

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— H. C. Lee

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CHAPTER I

INTRODUCTION

1. Parametric Pumping

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 (such as temperature, pressure, pH, electric field, etc.), and periodic changes in flow direction to separate the components of a fluid which flows past a solid adsorbent. Techniques commonly used in the separation of fluid mixtures, including adsorpation, extration, affinity chromatography, ion exchange chromatography, etc., might be adapted to parametric pumping. The adaption could be made in principle in those situations in which a reversible differential shift in the distribution of components between a mobile and an immobile phase could conveniently and practically be brought about by variation of an intensive variable.

There are four basic requirements for implementing the parametric pumping principle:

(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 perodically changing one or more of the intersive thermodynamic variables

that affect equilibrium.

In 1966 the late R. H. Wilhelm of princeton University applied parametric pumping to the separation process. Since the time of that invention, much experimental and theoretical work has been done on thermal and heatless parametric pumps. By contrast very little work has been done on pH-parametric pumping.

2. pH-Parametric Pumping Background

pH-parametric pumping is that pH alternations can cause equilibrium solute distributions in certain materials (e.g., ion exchange resins) to very, and so produce an alternating interphase solute flux. This flux, when coupled with an alternating axial solution flow, results in a separation. It has a direct and recuperative mode. To implement the direct pH mode, one needs a column whose walls are permeable to hydrogen ion. In this way a change in the pH of the liquid surrounding the column can influence the pH inside the bed. Such a column could be formed from a selective membrane, but transport through the membrane would significantly complicate matters. In the recuperative mode reservoirs at the column ends are maintained at two different pH values. As solution moves back and forth through the bed, ion exchange particles experience a lower pH when flow comes from the acidic end, and a higher pH when flow reverses.

Sabadell and Sweed (1970) used pH changes to remove K^+ and Na^+ from water. The separation utilized the recuperative mode of semibatch operation with low pH end being closed and the high pH end open. Aborosilicate glass column (30 X 1.1 cm i.d.) is packed with 30-50 mesh particles of IRC-84, a carboxylic polyacrylic resin (Rohm and Haas). The maximum separation factor obtained for total K^+ and Na^+ was 1.84.

Shaffer and Hamrin (1975) studied trypsin removal from α-chymotrypsin-trypsin mixtures by a parapumping system which is a batch unit. The parametric pumping runs were carried out using a 26 mm ID X 40 mm chromatographic column packed with Sepharose CVB CHOM. Separations were fitted to an equation of the form $\log_{10}(y_{B\ n}/y_0) = \alpha n$, where α is a constant. Separations were much less than those predicted from the equilibrium data. However, they do indicate that the pH parametric pumping has the viability for enzyme separations.

In this present work, a semicontinuous pH-parametric pump with a center feed for separating the mixture proteins of haemoglobin and albumin was experimentally investigated. Various factors affecting separations were examined. The solid phase was an ion exchange medium. Voidages of the ion exchange medium were to be estimated so that the optional displacement volume in semicontinuous pH-parametric pump could be predicted.

3. Properties of Semicontinuous pH-Parametric Pump

This study experiments pH-parametric pumping in recuperative mode for semicontinuous operation, to predict the conditions required for achieving the solute rich product. The pump system which we used shown in Figure 1. Flow is upward during a high pH half-cycle and downward during a low pH half-cycle. Flow to and from the reservoirs during upflow or downflow is at the rate Q. Each half-cycle time is $\frac{\tau\varepsilon}{w}$ and the displacement volume is $Q\frac{\tau\varepsilon}{w}$. The pump has the dead volumes V_T and V_B for top and bottom reservoirs, respectively. The flow rate within the column in upflow is identical to the reservoir displacement rate, Q, corresponding to batch operation. In downflow, both feed and product streams flow steadily into and out of the column. The feed is located between a stripping column (section 1) and an enriching column (section 2) with a feed flow rate equal to $(\phi_T + \phi_B)Q$. The Top and bottom flow rates are $\phi_T Q$ and $\phi_B Q$, respectively. Material balances show that the column flow rate is $(1-\phi_T)Q$ for the stripping section and $(1 + \phi_B)Q$ for the enriching section.

In the recuperative mode there exist two different pH region in the adsorbant bed for both upflow and down flow and the fluid phase concentration varies from one pH region to the another in each half-cycle of the pump operation. The protein is either adsorbed or desorbed depending upon pH of the region in a particular halfcycle of the pump operation. Periodic variation of the pH accompanied by synchronous changs in the flow direction induces a periodic

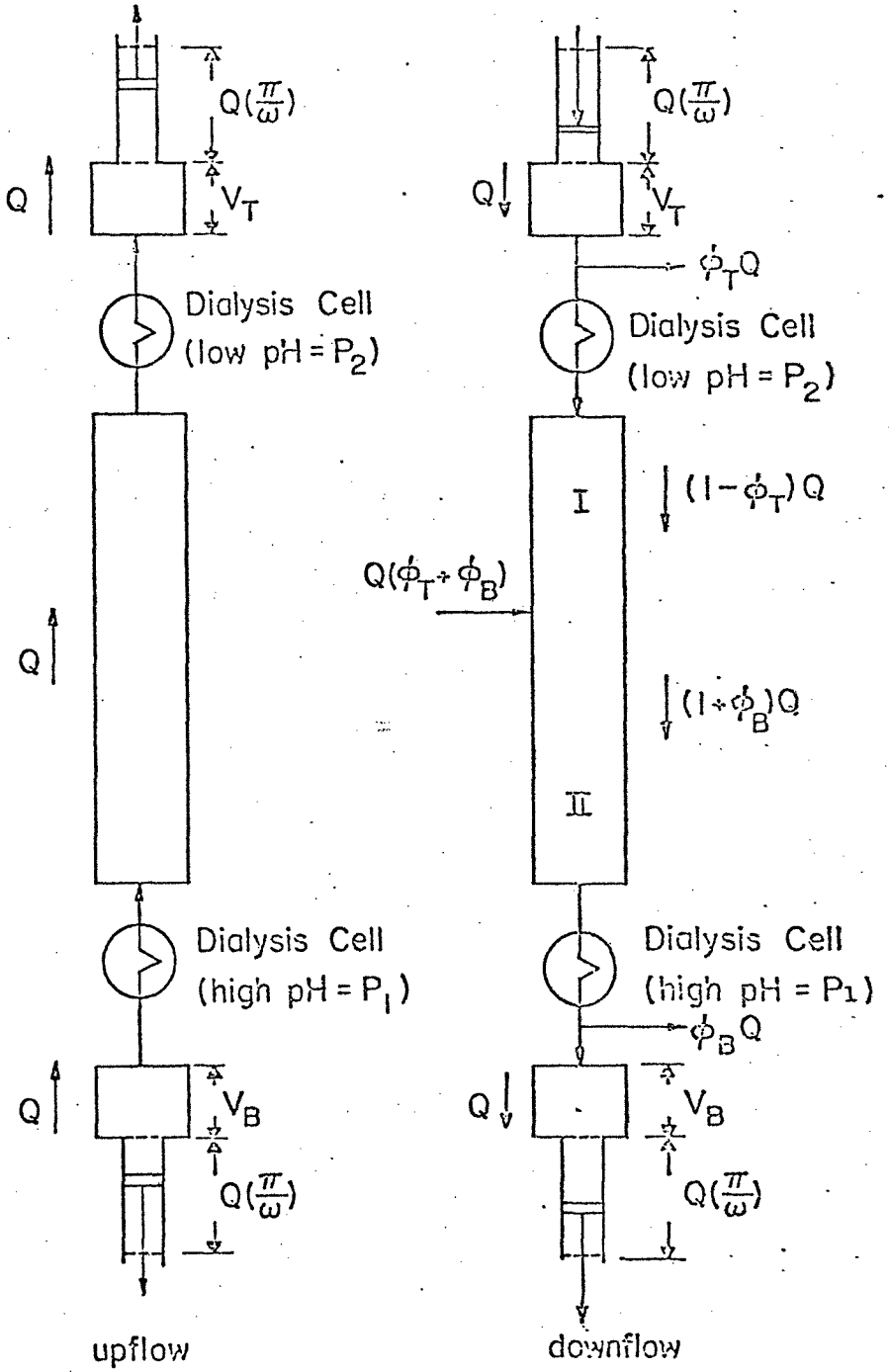


Fig. 1: Column of semicontinuous pH-parametric pump

variation in the composition of the flowing mixture, and gives rise to a net axial solute flux and hence a sustained separation.

Solid adsorbant which is chosen in this thesis experiments is one of the Sephadex ion exchange media manufactured by Pharmacia Fine Chemicals. The exchanger chosen was SP Sephadex (C-50). This is the sodium form of a relatively high porosity, strongly acidic, cation exchanger. The porosity is suitable for the molecular weight range 30 000 to 200 000. The particle size range is from 40 to 120 μm. The ion exchanger is relatively insensitive to pH over the range pH = 3 to 11. That is, swelling and shinking of ion exchanger is minized in this range. It is thus suitable for protein separations.

For separation by parametric pumping in recuperative mode the necessary condition is that the breakthrough of the pH waves must take place. Voidage is the fraction of the colume occupied by void volume. Voidage of ion exchanger can be defined as quotient void volume of ion exchanger and volume of packing column. It is observed that for separation to take place the displacement volume can be predicated by the voidage. Calculation of the voidage in this study is difficult, since the adsorbant used in this study has ion-exchang properties, the neutralization reaction must be included, and both buffers may be present simultaneously. Instead of unraveling these complexities, an empirical approach was used to determine ion exchanger voidage.

CHAPTER II

EXPERIMENT

Scope

This experimental work has been carried out in three parts:

(1) Draining the interstitial aqueous is study of SP Sephadex (C-50) voidage at different aqueous buffer concentration and pH to determine the displacement volume in semicontonuous pH-parametric pumping experiments.

(2) The voidage which estimated from draining the interstitial aqueous is checked by doing pH cycling zone experiments to show that results are in quantitative suit for pH-parametric pumping.

(3) The object of semicontinuous pH-parametric pumping experiment is to determine the effect of the displacement volume and product flow rate of the product stream on separation, and to find the other effect factors such as cycle time and the location of feed introduced.

1. Voidage Test of Draining the Interstitial Aqueous

A. Preparatory Method

The buffer employed is a mixture of monobasic sodium phosphate (NaH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4). For the low pH (pH 6), the proportion is 87.7% monobasic sodium phosphate and 12.3%

diabasic sodium phosphate. For the high pH (pH8), the proportion is 5.3% monobasic sodium phosphate and 94.7% diabasic sodium phosphate (Colowich & Kaplin, 1955).

The specific packing used is SP Sephadex (C-50). Preparation of the packing has been standardized in order to produce similar starting condition. Initially, measured the required amount of SP Sephadex in 100 c.c. beaker. Poured suitable buffer at the concentration and pH to be used in the experiment into SP Sephadex (C-50). Vigorous stirring should be avoid in order not to damage the ion exchanger beads. SP Sephadex (C-50) will be completely swollen, after 48 hours at room tempereture. Then, mixed the gel with starting buffer and checked the column it is mounted vertically. 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.

B. Experimental Procedure and pH Value Analysis

The operation of draining interstitial aqueous used a jacketed chromatogrphic column (0.016m. inside diameter and 0.4m. length,

manufactured by Pharmacia Fine Chemicals) packed with swelling SP Sephadex (C-50) gel which mixed with low pH buffer. The column was maintained at a constant temperature of 288°K by use of a refrigeration unit which circulates cooling water in the jacket. Then, the high pH buffer aqueous was introduced from top column line by an infusion pump with velocity 0.5c.c./m. and product which drained from bottom line was collected in 10c.c. graduated cylinder. The product was sampled at every 3 c.c. and measured pH by pH meter. The experimental apparatus used in this draining the interstitial aqueous is schematically in Figure 2.

We can draw pH verses voidage(@) on normal cartesian coordinates just like Figure 3. The abscissa represents voidage. It is quotient volume of collect product that have been effluent and volume. of packing column. pH begining change showed that interstitial aqueous nearly drained off. Initial voidage is the voidage of the outlet pH begining change. Final voidage is the outlet pH being equal to the feed pH. The outlet pH curve shows slope, this is character of system have mixture region of pH. Voidage and pH changing between initial and final voidage could be observed from this diagram curve.

C. Experimental Process of Voidage Test

All experimental parameter of voidage test were listed in Table 1. In order to determine the effect of buffer concentration and pH

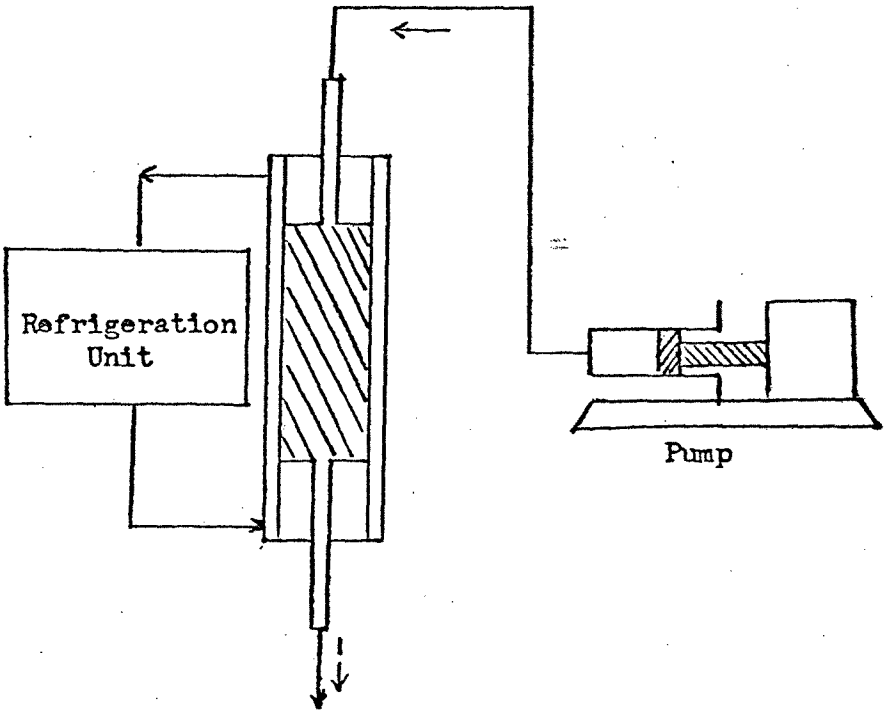


Fig. 2 Draining interstitial aqueous apparatus

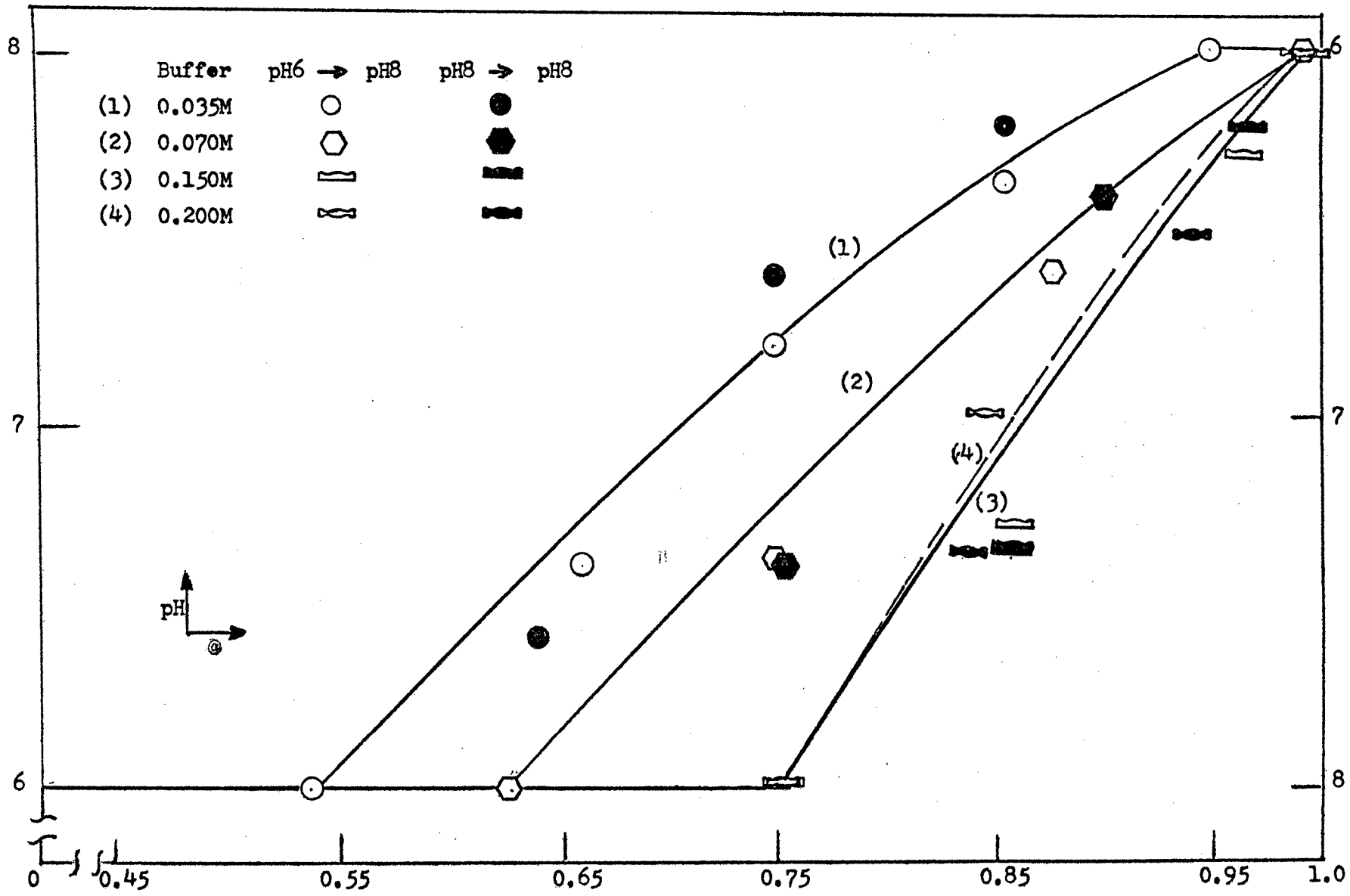


Fig. 3 Voidage at different buffer concentration and pH

on voidage, series of experiments were carried out using the previous method with different buffer concentration of content 0.035M, 0.070M, 0.15M and 0.20M at high, low pH buffer starting packed and infused reversely. These experiments data are shown in Table 2, 3, 4 and 5. In the next two experiments, the purpose was to study the effect of volume of packed colume and buffer which mixed with haemoglobin. The experimental data are showen in Table 6 and 7 respectively. These experiments were repeated at same method. From those experiments, SP Sephadex (C-50) gel voidage and swelling factor at different buffer concentration was established.

TABLE 1

A List of Ion Exchanger Voidage Test Parameters for All Run

Test	Buffer Concentration M	Dry Ion Exchanger Weight g	Starting PH	Infusion PH	Column Height cm	Column Volume cc	Infusion Rate cc/m.
T1-A	0.035	1.12	6	8	14	28	0.5
T1-B	0.035	1.12	8	6	14	28	0.5
T2-A	0.070	1.20	6	8	12	24	0.5
T2-B	0.070	1.20	8	6	10	20	0.5
T3-A	0.150	1.60	6	8	14	28	0.5
T3-B	0.150	1.60	8	6	14	28	0.5
T4-A	0.200	2.00	6	8	16	32	0.5
T4-B	0.200	2.00	8	6	16	32	0.5
T5	0.150	0.80	6	8	8	16	0.5
T6	0.150	1.60	6*	8*	14	28	0.5

* T6 starting and infusion buffer aqueous mixed with 0.01% haemoglobin.

TABLE 2

Voidage of Ion Exchanger at 0.035 M Buffer

Buffer Concentration : 0.035 M

Weight of dry SP Sephadex (C 50) : 1.12 g

Packing column Volume : 28 cc

No. of Test	T1-A PH6--->PH8				T1B PH8--->PH6			
	PH	Volume cc	Total Volume	Voidage	PH	Volume cc	Total Volume	Voidage
1	6	3	3	0.11	8	3	3	0.11
2	6	3	6	0.21	8	3	6	0.21
3	6	3	9	0.32	8	3	9	0.32
4	6	3	12	0.43	8	3	12	0143
5	6	3	15	0.54	8	3	15	0.54
6	6.6	3.8	18	0.67	7.6	3	18	0.64
7	7.2	2.2	21	0.75	6.6	3	21	0.75
8	7.65	3	24	0.86	6.2	3	24	0.86
9	8	3	27	0.96	6	3	27	0.96
10	8	3	30	1.07	6	3	30	1.07
11	8	3	33	1.18	6	3	33	1.18
12	8	3	36	1.29	6	3	36	1.29

TABLE 3

Voidage of Ion Exchanger at 0.070 M Buffer

Buffer concentration : 0.070 M

Weight of dry SP Sephadex (C 50) : 1.2 g

Packing column Volume : T2-A:24 cc ; T2-B:20cc

No. of Test	T2-A PH6-->PH8				T2-B PH8-->PH6			
	pH	Volume cc	Total Volume	Voidage	pH	Volume cc	Total Volume	Voidage
1	6	3	3	0.12	8	3	3	0.12
2	6	3	6	0.25	8	3	6	0.25
3	6	3	9	0.75	8	3	9	0.25
4	6	3	12	0.50	8	3	12	0.50
5	6	3	15	0.62	7.4	3	15	0.62
6	6.6	3	18	0.75	6.4	3	18	0.75
7	7.4	3	21	0.87	6	3	21	0.87
8	8	3	24	1.0	6	3	24	1.0

TABLE 4

Voidage of Ion Exchanger at 0.15 M Buffer

Buffer Concentration : 0.15 M

Weight of dry SP Sephadex (C-50) : 1.60 g

Packing colume volume : 28 cc

No. of Test	T3-A PH6→PH8				T3-B PH8→PH6			
	PH	Volume cc	Total Volume	Voidage	PH	Volume cc	Total Volume	Voidage
1	6	3	3	0.11	8.2	3	3	0.11
2	6	3	6	0.21	8.2	3	6	0.21
3	6	3	9	0.32	8.2	3	9	0.32
4	6	3	12	0.43	8.2	3	12	0.43
5	6	3	15	0.54	8.2	3	15	0.54
6	6	3	18	0.64	8.2	3	18	0.64
7	6	3	21	0.75	8.2	3	21	0.75
8	6.7	3	24	0.86	7.2	3	24	0.86
9	7.5	3	27	0.96	6.1	3	27	0.96
10	8.2	3	30	1.07	6	3	30	1.07
11	8.2	3	33	1.18	6	3	33	1.18
12	8.2	3	36	1.28	6	3	36	1.28

TABLE 5

Voidage of Ion Exchanger at 0.200 M Buffer

Buffer Concentration : 0.200 M

Weight of dry SP Sephadex (C-50) : 2.00 g

Packing column Volume : 32 cc

No. of Test	T4-A PH6→PH8				T4-B PH8→PH6			
	PH	Volume cc	Total Volume	Voidage	PH	Volume cc	Total Volume	Voidage
1	6	3	3	0.09	8	3	3	0.09
2	6	3	6	0.18	8	3	6	0.18
3	6	3	9	0.28	8	3	9	0.28
4	6	3	12	0.37	8	3	12	0.37
5	6	3	15	0.47	8	3	15	0.47
6	6	3	18	0.56	8	3	18	0.56
7	6	3	21	0.65	8	3	21	0.65
8	6	3	24	0.75	8	3	24	0.75
9	7	3	27	0.84	7.4	3	27	0.84
10	7.9	3	30	0.94	6.5	3	30	0.94
11	8	3	33	1.03	6	3	33	1.03
12	8	3	36	1.12	6	3	36	1.12
13	8	3	39	1.22	6	3	39	1.22

TABLE 6

Voidage of Different Packing Volume Test

Buffer concentration : 0.15 M

Weight of dry SP Sephadex (C-50) : 0.8 g

Packing column volume : 16 cc

	T6-A PH6---> PH8				T6-B PH8---> PH6			
No. of Test	PH	Volume cc	Total Volume	Voidage	PH	Volume cc	Total Volume	Voidage
1	6	3	3	0.19	8	3	3	0.19
2	6	3	6	0.38	8	3	6	0.38
3	6	3	9	0.56	8	3	9	0.56
4	6	3	12	0.75	8	3	12	0.75
5	7.3	3	15	0.94	7	3	15	0.94
6	8	3	18	1.13	6	3	18	1.13
7	8	3	21	1.31	6	3	21	1.31
8	8	3	24	1.50	6	3	24	1.50

TABLE 7

Voidage of Different Buffer Mixture Test

Buffer concentration : 0.15 M with 0.01% weight haemoglobin.

Weight of dry SP Sephadex (C-50) : 1.6 g

Packing column Volume : 28 cc

	T7-A PH6-->PH8				T7-B PH8-->PH6			
No. of Test	PH	Volume cc	Total Volume	Voidage	PH	Volume cc	Total Volume	Voidage
1	6	3	3	0.11	8	3	3	0.11
2	6	3	6	0.21	8	3	6	0.21
3	6	3	9	0.32	8	3	9	0.32
4	6	3	12	0.43	8	3	12	0.43
5	6	3	15	0.54	8	3	15	0.54
6	6	3	18	0.64	8	3	18	0.64
7	6	3	21	0.75	8	3	21	0.75
8	6.7	3	24	0.86	7.2	3	24	0.86
9	7.5	3.2	27.2	0.96	6.1	3	27	0.95
10	8	2.8	30	1.07	6	3	30	1.07
11	8	3	33	1.18	6	3	33	1.18
12	8	3	36	1.28	6	3	36	1.28

2. Experiment of pH Cycling Zone

A. Apparatus

The basic apparatus for pH traveling wave cycling zone consists of a Pharmacia chromatographic column (1.6 x 40 cm) with a feed system. Cyclic changes in pH are produced in a fluid which flow through a fix bed of solid adsorbant owing to periodically very pH feed. The feed system is arranged so that the pH of entering feed can be varied periodically in some fashion. The feed system consists of one four-way-valve and two 400 cc Pharamacia (R15/16) cyclinder reservoir, one for low pH buffer and the other for high pH buffer. The entering feed is adjusted to high or low pH buffer reservoirs. A periodical change of entering pH can be achieved by periodically switching the four-way-valve. The colomn system is shown schematically in Figure 4. A Pharmcia chromatographic column was packed with Sephadex gel. Preparation of the packing was the same as those in the previous voidage test. The experiment liquid is pumped from top through the solid adsorbant bed to bottom by a constant rate Pharmacia P-3 peristaltic pump. The fluid is pumped through the system using capillary tubing (0.1 cm i.d., 0.18 cm o.d.). The product stream is collected during the periods of high and low pH buffer derivation from the feed reservoir. Product is collected in graduated cylinder and measured pH value as previous voidage test method.

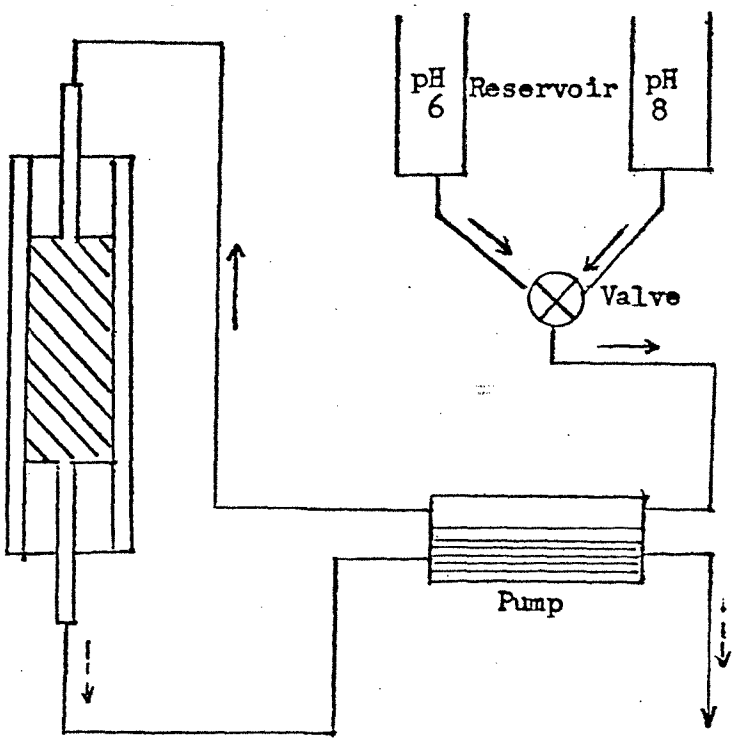


Fig. 4 PH cycling zone apparatus

B. Procedure of pH Cycling Zone

The buffer is fed at all time but with varying pH. The pH of entering feed scheme is shown in Figure 5. In this figure, the pH of feed is shown varying as a series of square wave which are represented by periodic function with period 9cc, 13cc, 30cc. This was doing for three different period to show each cycle was repeat same delay respect which represents voidage property of solid adsorbant.

The product from the cycling-zone system is time dependent on the entering pH vary continually. Thus a time dependent outlet pH is obtained with low pH in the product when feed is low, and high pH product when the feed is high. The periodic alternation of feed pH will cause a pH wave in the system. The result is continuous product of two stream of different pH buffer.

C. Experimental Process

The cycling-zone method used here essentially investigate the effect on Sephadex (C-50) ion exchanger voidage which process periodically changed pH value. Four runs were carried out on this system. Table 8 lists the experimental condition for all runs. All the periodic alteration of entering pH is same as scheme in Figure 5. The results for the cycling-zone experiments are shown in Figure 6, 7, 8 and 9. In these figure, the abscisssa represents

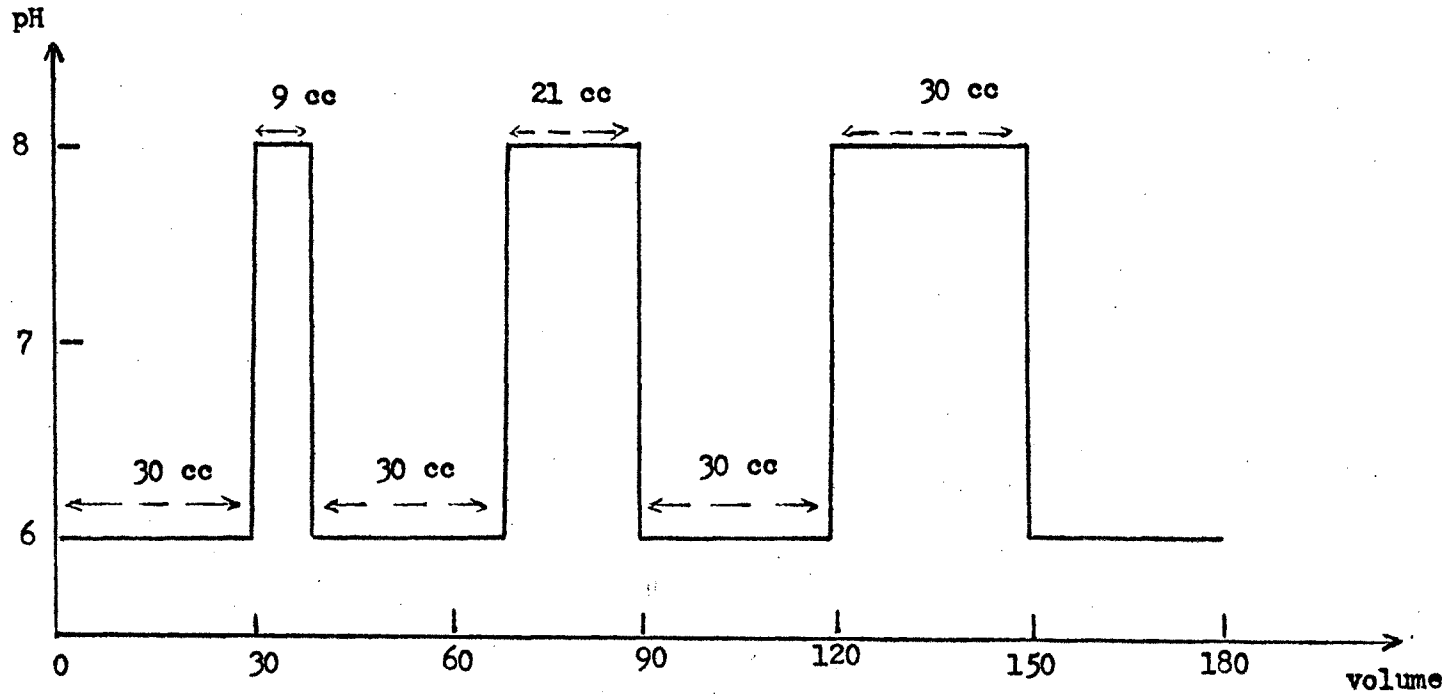


Fig. 5. pH feed diagram in cycling zone experiment

TABLE 8

A List of pH Cycling Zone Parameters for All Run

Run	C-1	C-2	C-3	C-4
Buffer Conc, M:	0.2	0.1	0.1	0.1
Packing ion exchanger:				
Height, cm:	8	8	8	8
Volume, cc:	16	16	16	16
Flow rate, cc/m:	0.5	0.5	0.5	1.0
Delay respect of pH:				
Volume No., cc:	15	15	15	12
Time No., m:	30	30	30	12
Initial voidage:				
$\varepsilon_I = \frac{v - 3}{hA}$	.75	.75	.75	---
Estimated from voidage test	.75	---	---	---
Haemoglobin and albumin:	0	0	H:0.01% A:0.05%	0

product volume that have been eluted, the ordinate represents pH value that product is colledted for late analysis.

Comparision of these respect of outlet pH curve(Fig. 6, 7) with feed pH curve(Fig. 5) indicate that respect was moved slowlier than feed. The respect was moved slowly because of stagnant liquid in the solid adsorbant and the tube. Consider the fixed-bed illustiated in Fig. 4 in which a fluid having a constant pH, pH 6 is passed into a bed of solid particles. After a long time, the effluent solute pH must eventually become pH 6. If at this time, the feed pH is changed to pH 8, solute which was previously stored on the solid is now expelled into the fluid; the effluent has a solute larger pH after some time, depending on the void volume of solid. Finally, the pH returns to pH 8. Now if the feed pH is decreased to pH 6, the solid take up solute from the fluid, and the depleted effluent solute pH becomes pH 8. After a certain period, the pH again return to pH 6 and the cycle be repeated.

It is observed that delay volume of respect (compared with feed) minus connective tube volume leaves void volume of gel. If we devide this net volume by packing volume, the answer is initial voidage. This can be governed by the following equation:

$$\varepsilon_I = \frac{V - V'}{hA}$$

ε_I = Initial voidage of gel.

V_r = Delay volume of respect as compared with entering feed pH.

V' = Volume of connective tube. It is 3cc, in this experiment.

h = Height of packing gel, cm

A = Inside area of column, cm^2

From cycling-zone outlet pH curve and previous equation, we can find initial voidage in cyclic process which is analogous to voidage relation of parametric pumping.

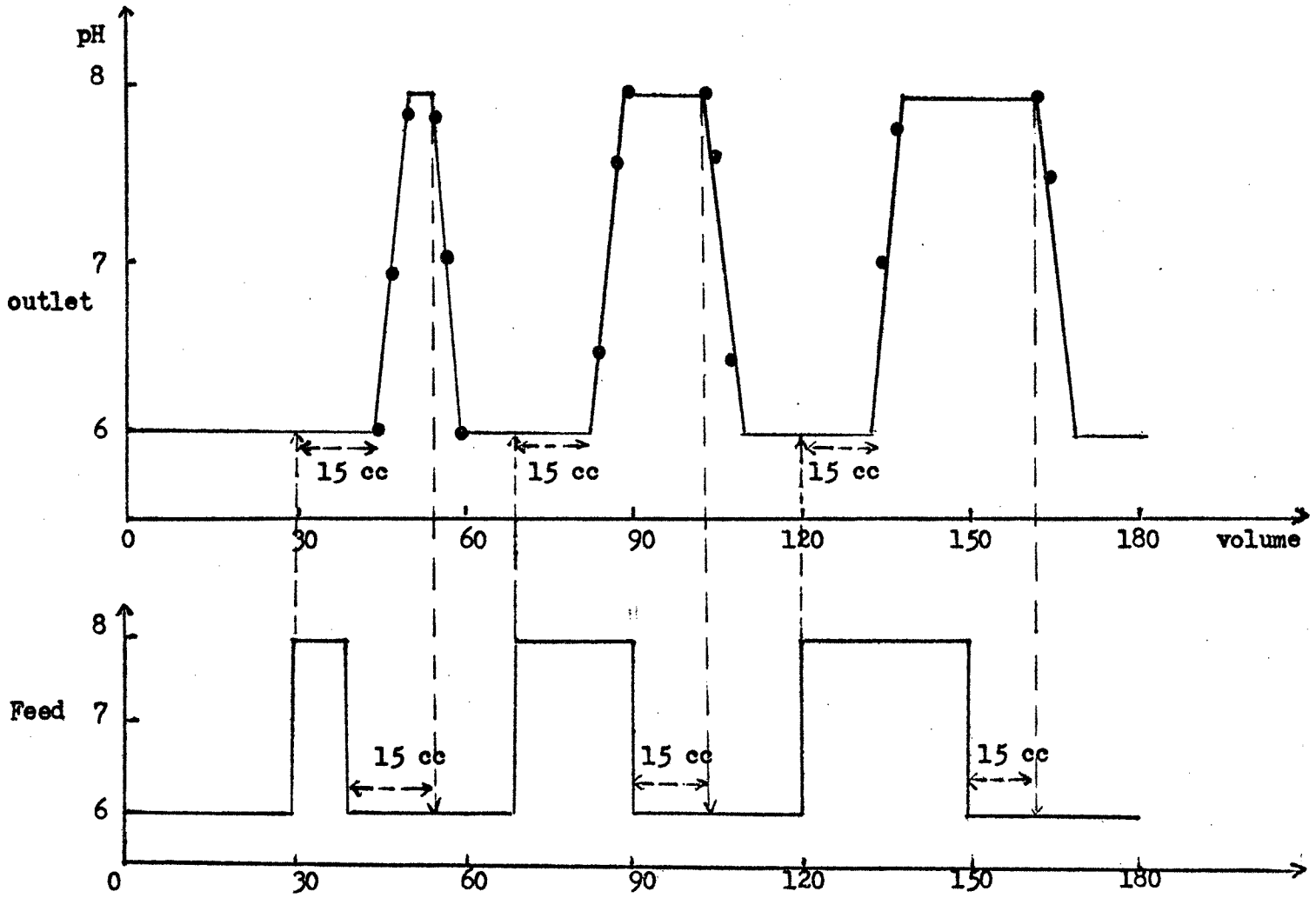


Fig. 6. Cycling zone pH outlet diagram of 0.2 M conc., packing gel 16cc and flow rate 0.5 cc/m

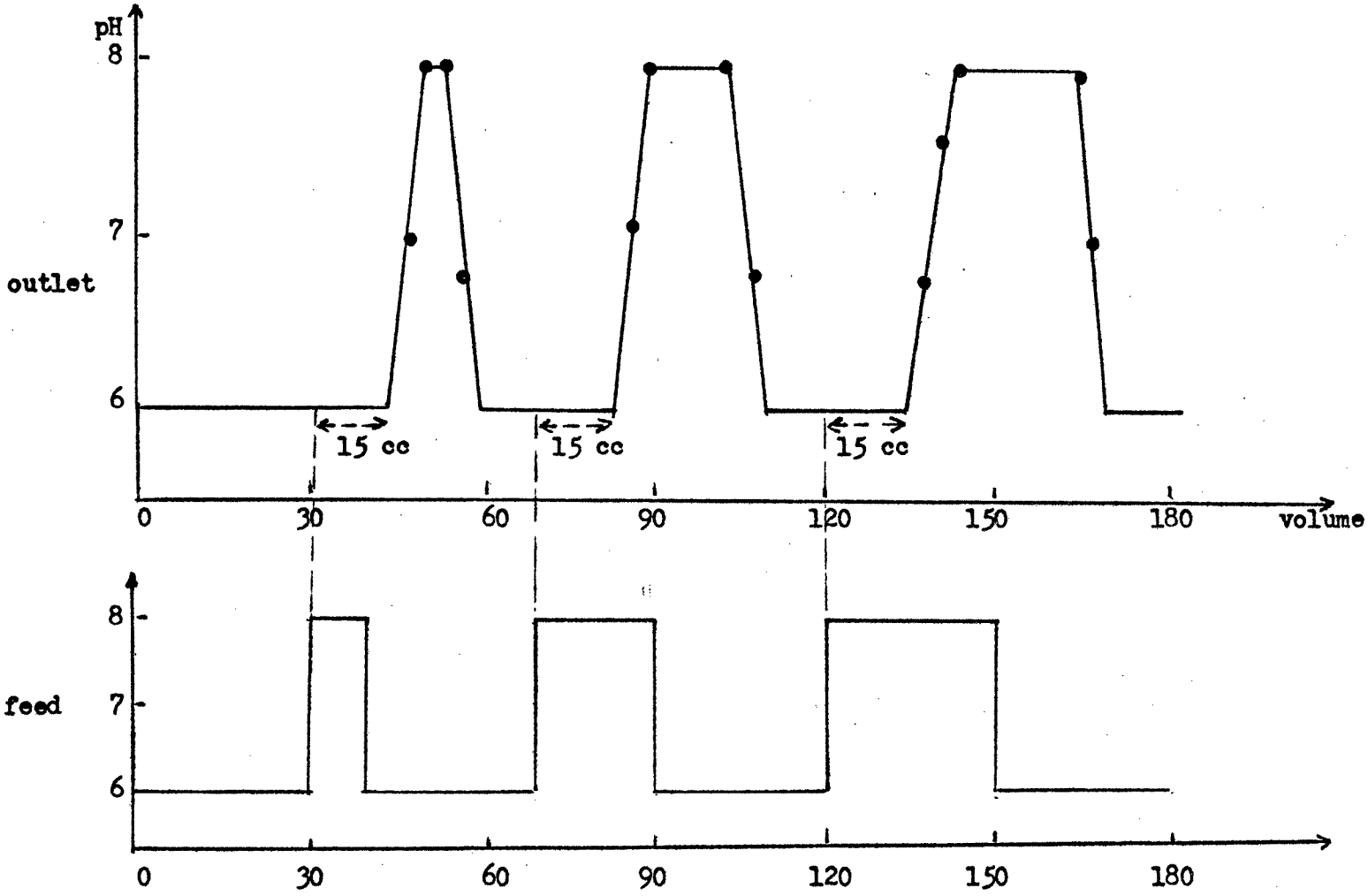


Fig. 7. Cycling zone pH diagram of 0.1 M conc., packing gel 16 cc and flow rate 0.5 cc/m

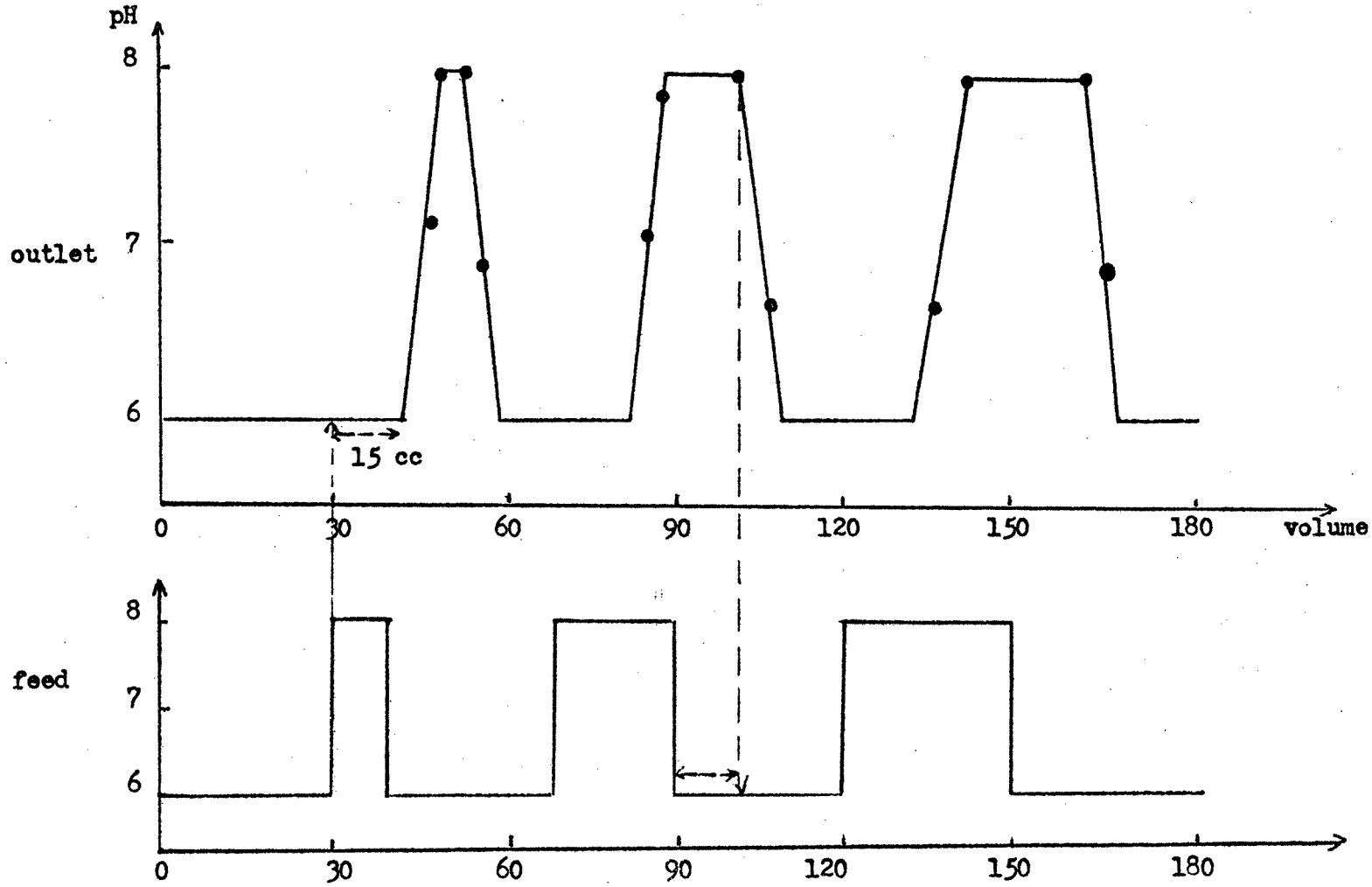


Fig. 8. Cycling zone outlet diagram of 0.1 M conc. with 0.01% haemoglobin and 0.05% albumin, packing gel 16 cc, flow rate 0.5 cc/m

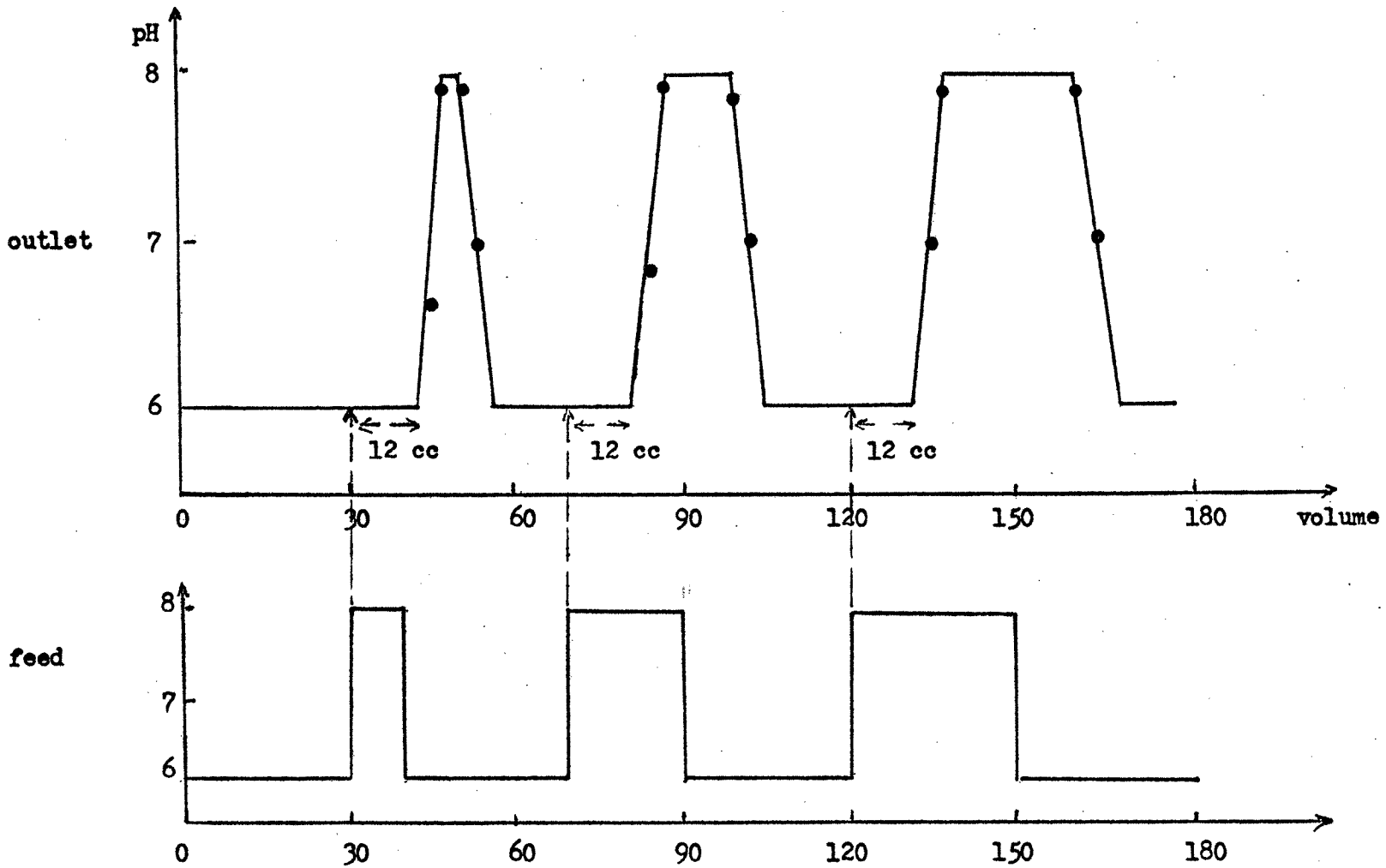


Fig. 9. Cycling zone outlet diagram of 0.1 M conc., packing gel 16 cc and flow rate 1.0cc/m

3. Experiment of Semicontinuous pH-Parametric Pump

A. Apparatus

The experimental apparatus used in this semicontinuous pH-parametric pumping is schematically in Figure 10. The equipment consists of two jacketed Pharmacia chromatographic columns (1.6 X 40 cm), one for stripping and the other for enriching. The columns were packed with SP Sephadex (C-50). Constant temperature of the column is maintained by the use of a refrigeration unit. It circilates cooling water in the jacket at 288° K, which prevents the protein from denaturing. The reservoirs with a dead volume of 6 cc at the two opposite ends of the columns were two 50 cc glass syringe operated by a dual infusion-withdrawal pump manufactured by Harvard Apparatus Co. The feed was introduced between the stripping and enriching columns by a second pump with a 50 cc syringe. To insure perfect mixing in the reservoirs, small magnetic stirrers were placed in the reservoir syringes. The fluid is pumped through the system using capillary tubing (0.1 cm i.d., 0.18 cm o.d.). The product take-off value were micrometer capillary values used both to regulate flow and impose a small back pressure on the system. The samples are measured on a Beckmann DU spectrophotometer.

The change of pH was accomplished by two Bio-Fiber Beaker(manufactured by Bio-Red Laboratory). One was for high pH and the other for low pH. To eliminate stagnation of the buffer, magnetic stirrers are

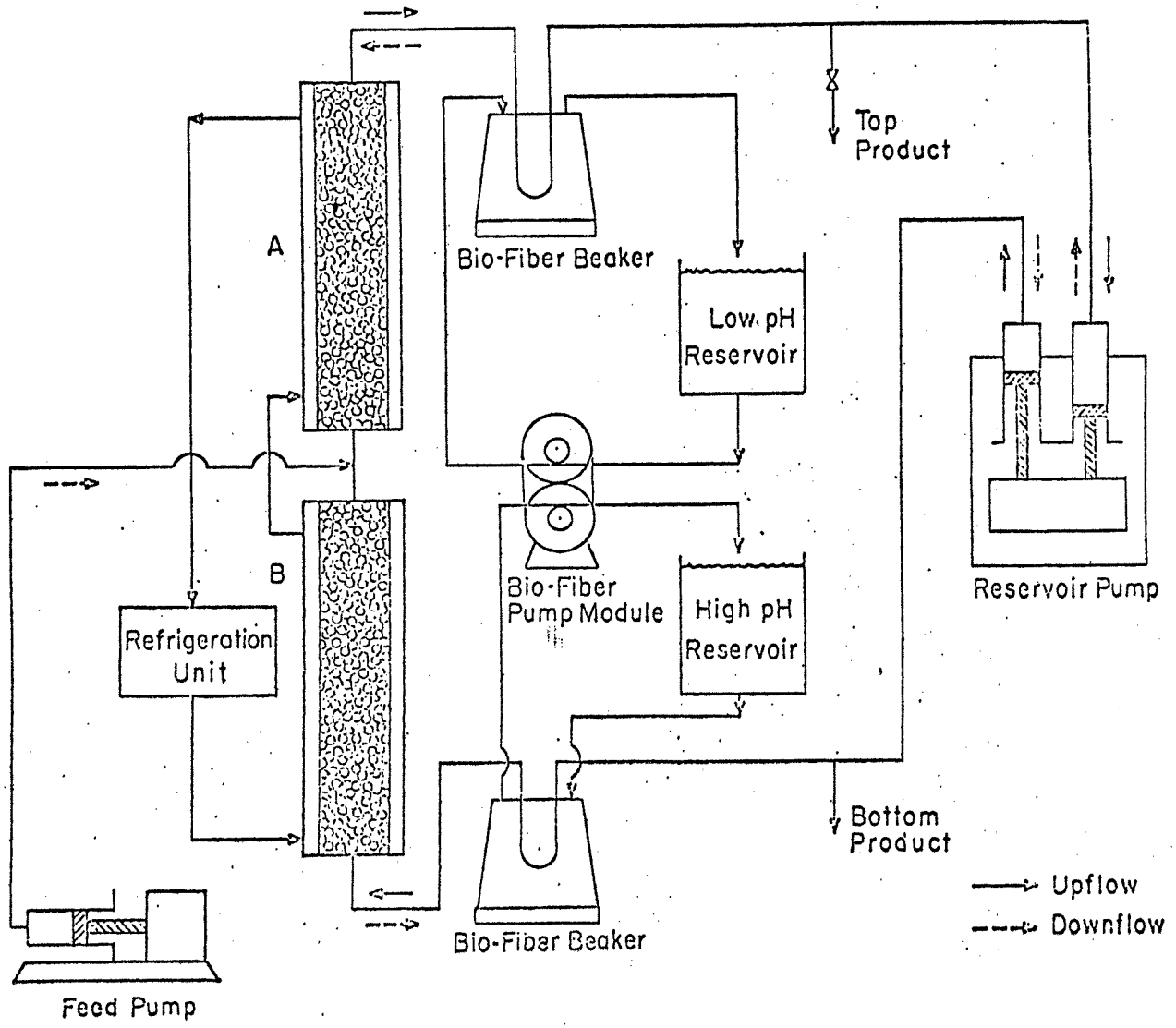


Fig. 10: Experimental Apparatus for semicontinuous pH-parametric pumping

placed in the bottom of both dialysis cells. The buffers were mixtures of monobasic and dibasic sodium phosphate (Colowick and Kaplan, 1955). Buffer is circulated in the jaeket past of the dialysis cell, while the protein solution which wishes to changes its pH is passed through the tube bundles of this beaker. A 2000 cm^3 reservoir is used for the circulation of fresh buffer through each dialysis cell. A Bio-Red peristaltic pump is used to circulate the buffer at 0.33 cm^3 per second.

B. Experimental Procedure

Preparatory method is same as previous voidage test experiment. Prior to each run, all the air is removed from the connecting lines. This is done by filling all the lines with feed mixture. Low 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 entire system, including the interstitial column volumne, the bottom reservoir, the top dead volume and feed syringe were filled with feed mixture. Except the bottom reservoir filled with high pH feed mixture, the others filled with low pH feed mixture.

During the first half-cycle the fluid in bottom reservoir was pumped through the high pH beaker and into the bottom of column B. At the same time, solution that emerged from column A flowed through

the low pH beaker and filled the top reservoir. On the next half-cycle, the solution in the top reservoir flowed back through the low pH beaker, and passed through columns A and B, and the high pH beaker to the bottom reservoir. Simultaneously, the feed pump was activated and the product take-off valves were opened and adjusted for the desired product flow rates. This procedure was repeated for each cycle.

C. Method of Product Concentration Measurment and Calibration

Samples for analysis were taken from the product streams at the end of each cycle and analyzed spectrophotometrically. Haemoglobin will absorb light at (wavelength) of 403 mμ and 280 mμ, while albumin will absorb light only at 280 mμ. From the following calibration curves, we know that the concentration of the single protien system (haemoglobin-water and albumin-water) was linearly proportional to the absorbance ℓ at 280 mμ and 403 mμ. That is

$$\ell A(280) = \beta A(280) \times \eta A \quad \text{----------(1)}$$

$$\text{and } \ell H(280) = \beta H(280) \times \eta H \quad \text{----------(2)}$$

$$\ell H(403) = \beta H(403) \times \eta H \quad \text{----------(3)}$$

where A and H indicate albumin and haemoglobin, respectively.

ℓ = absorbance

β = slope

η = concentration (weight percent)

Initially, the four cells that are to be used for the measurements are filled with buffer 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. The absorbance is found by the simple relationship that it is equal to the common Log of one hundred divided by the transmission Log(100/T). That is:

$$T/100 = R/C \qquad (4)$$

$$\lambda = \text{Log}(100/T) = \beta \cdot \eta \qquad (5)$$

where

T = Transmission of correction

R = Transmission reading of spectrophotometer

C = Correctional factor of cell

100 = Set number of reference cell

Equation (4) put into equation (5), we can get

$$\lambda = \text{Log}(C/R) = \beta \cdot \eta \qquad (6)$$

In order to determine the concentration of the samples, the calibration curves must be prepared. A calibration tables of absorbance λ readings corresponding to haemoglobin and albumin at pH 6 and pH 8 were made by measuring carefully prepared solution of know concentration (Table 9, 10 and 11). If the absorbance λ is plotted against haemoglobin

TABLE 9

Caliberation Curve Experiment-Haemoglobin at PH 6 Buffer

1 : Correction Factor of Cell:

Buffer	λ	Cell 1st	Cell 2nd	Cell 3rd	Cell 4th	T
PH 6 (0.035 M)	403 μ	100	98	97.3	100.2	24°C
PH 6 (0.035 M)	280 μ	100	100.1	98.1	102.9	24°C

2 : Test Result:

No. of Test	Haemoglobin at PH 6 Buffer Wavelength (λ) = 403 μ η	R	ℓ	β	Haemoglobin at PH 6 Buffer Wavelength (λ) = 280 μ η	R	ℓ	β
1	0.020	5.2	1.275	63.75	0.020	38.9	0.410	20.5
2	0.016	10.2	0.979	61.19	0.016	49.1	0.300	18.75
3	0.012	18.3	0.738	61.5	0.012	59.1	0.241	20.08
4	0.010	23.1	0.627	62.7	0.010	61.2	0.213	21.3
5	0.008	31.0	0.497	62.1	0.008	66.2	0.171	21.37
6	0.004	55.5	0.256	64.0	0.004	79.5	0.112	28.0

* $\ell = \text{Log}(C/R)$, where: ℓ = absorbance
R = transmission
C = correctional factor of cell
η = concentration(weight percent)
β = slop(ℓ/η)

TABLE 10

Caliberation Curve Experiment-Haemoglobin at PH 8 Buffer

1 ; Correction Factor of Cell:

Buffer	λ	Cell 1st	2nd	3rd	4th	T
PH 8 (0.035 M)	403μ	100	98.2	97.9	99.3	24°C
PH 8 (0.035 M)	280μ	100	98.8	97.1	99.2	24°C

2 ; Test Result:

No. of Test	Haemoglobin at PH 8 Buffer Wavelength (λ) = 403μ				Haemoglobin at PH 8 Buffer Wavelength (λ) = 280μ			
	η	R	ℓ	β	η	R	ℓ	β
1	0.020	11.5	0.931	46.55	0.020	45.2	0.339	16.95
2	0.016	17.8	0.740	46.25	0.016	53.3	0.260	16.25
3	0.012	28.1	0.548	45.66	0.012	61.9	0.205	17.08
4	0.010	35.1	0.447	44.70	0.010	68.5	0.159	15.90
5	0.008	44.5	0.342	42.75	0.008	74.8	0.113	14.12
6	0.004	65.5	0.181	45.25	0.004	85.5	0.064	16.00

* $\ell = \text{Log}(C/R)$,where: ℓ = absorbance
R = transmission
C = correctional factor of cell
η = concentration(weight percent)
β = slop(ℓ/η)

TABLE 11

Caliberation Curve Experiment-Albumin at PH 6 & PH 8 Buffer

1 ; Correction Factor of Cell:

Buffer	λ	Cell 1st	2nd	3rd	4th	T
PH 6 (0.035 M)	280 μ	100	100.1	98.1	102.9	24°C
PH 8 (0.035 M)	280 μ	100	98.8	97.1	99.2	24°C

2 : Test Result:

	Albumin at PH 6 Buffer Wavelength (λ) =280 μ				Albumin at PH 8 Buffer Wavelength (λ) =280 μ			
No. of Test	η	R	ℓ	β	η	R	ℓ	β
1	0.050	51.5	0.288	5.76	0.050	53.0	0.270	5.40
2	0.045	60.3	0.211	4.69	0.045	55.8	0.240	5.33
3	0.035	65.5	0.196	5.60	0.035	65.7	0.179	5.11
4	0.025	74.0	0.131	5.24	0.025	69.9	0.150	6.00
5	0.015	86.9	0.052	3.47	0.015	80.6	0.080	5.33
6	0.010	92.2	0.047	4.70	0.010	90.8	0.038	3.80

* ℓ = Log (C/R) , where: ℓ = absorbance
R = transmission
C = correctional factor of cell
η = concentration(weight percent)
β = slop(ℓ/η)

and albumin on normal cartesian coordinates (Figure 11 and 12), the absorbance was found to be a linear function of concentration. It Have the slope

for pH 6	$\beta A(280) = 5.29$
	$\beta H(280) = 21.69$
	$\beta H(403) = 60$
for pH 8	$\beta A(280) = 6.06$
	$\beta H(280) = 16.05$
	$\beta H(403) = 45.19$

In the case of binary protein system (haemoglobin-albumin-water), the analysis was somewhat complicated. The ℓ at 280 mμ was the sum of that in haemoglobin-water and in albumin-water. Therefore, for an unknown binary protein mixture the haemoglobin concentration was determined directly from ℓ at 403 mμ (Eq. 3). Knowing the haemoglobin concentration, for haemoglobin at λ = 280 mμ was determined by Eq. 2. Subtraction of this ℓ from the total ℓ obtained at 280 mμ in haemoglobin -albumin-water gave the contribution of ℓ for albumin alone hence its concentration by Eq. 1. A workable expression for the haemoglobin and albumin concentration as a function of reading results could be calculated from previous principle, as following:

Fig. 11. Caliberation curve of haemoglobin at pH 6 and pH 8 buffer

	pH	μ.	Exp.	α
(1)	6	403	○	60
(2)	8	403	—	45.19
(3)	6	280	⬡	21.668
(4)	8	280	—	16.05

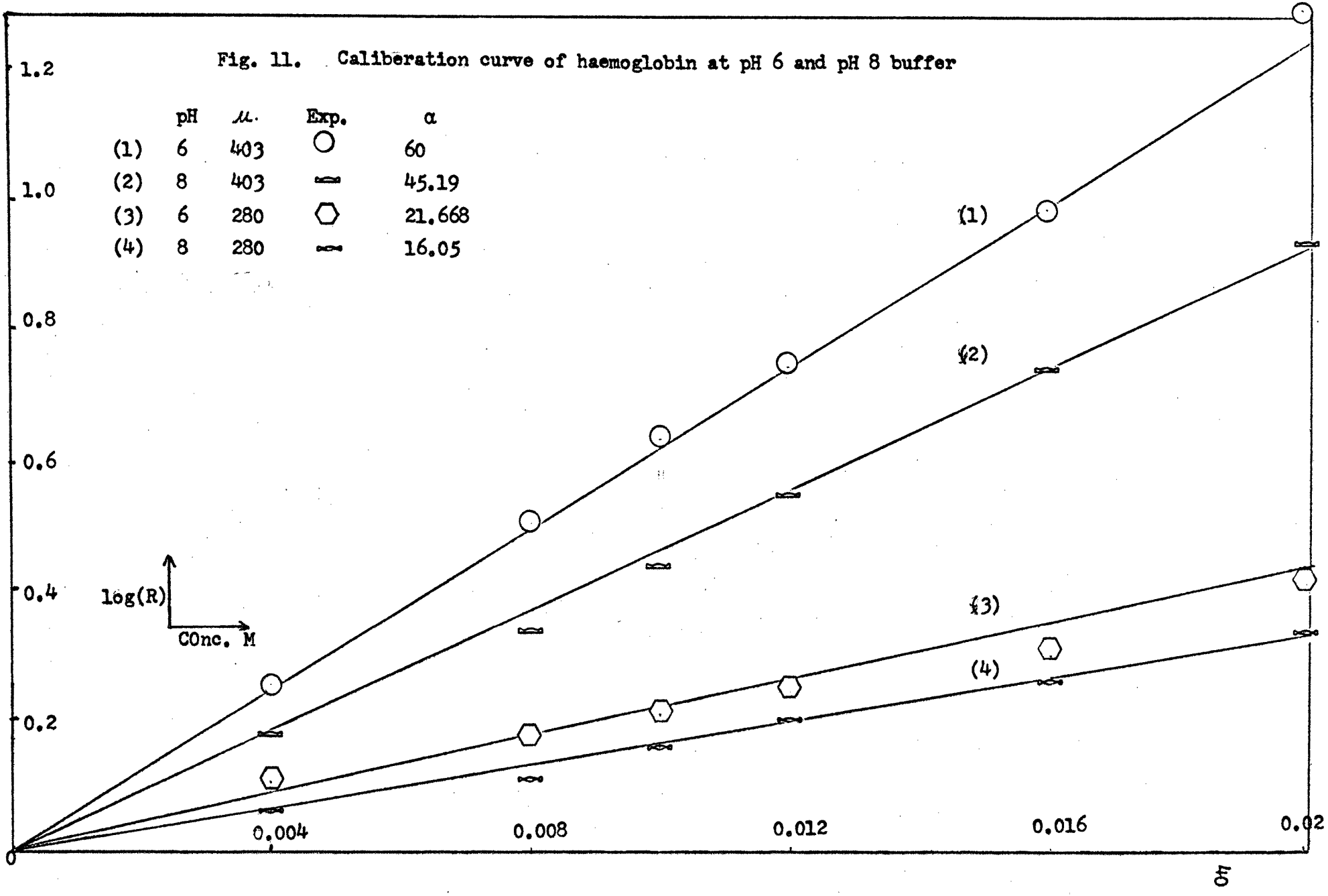
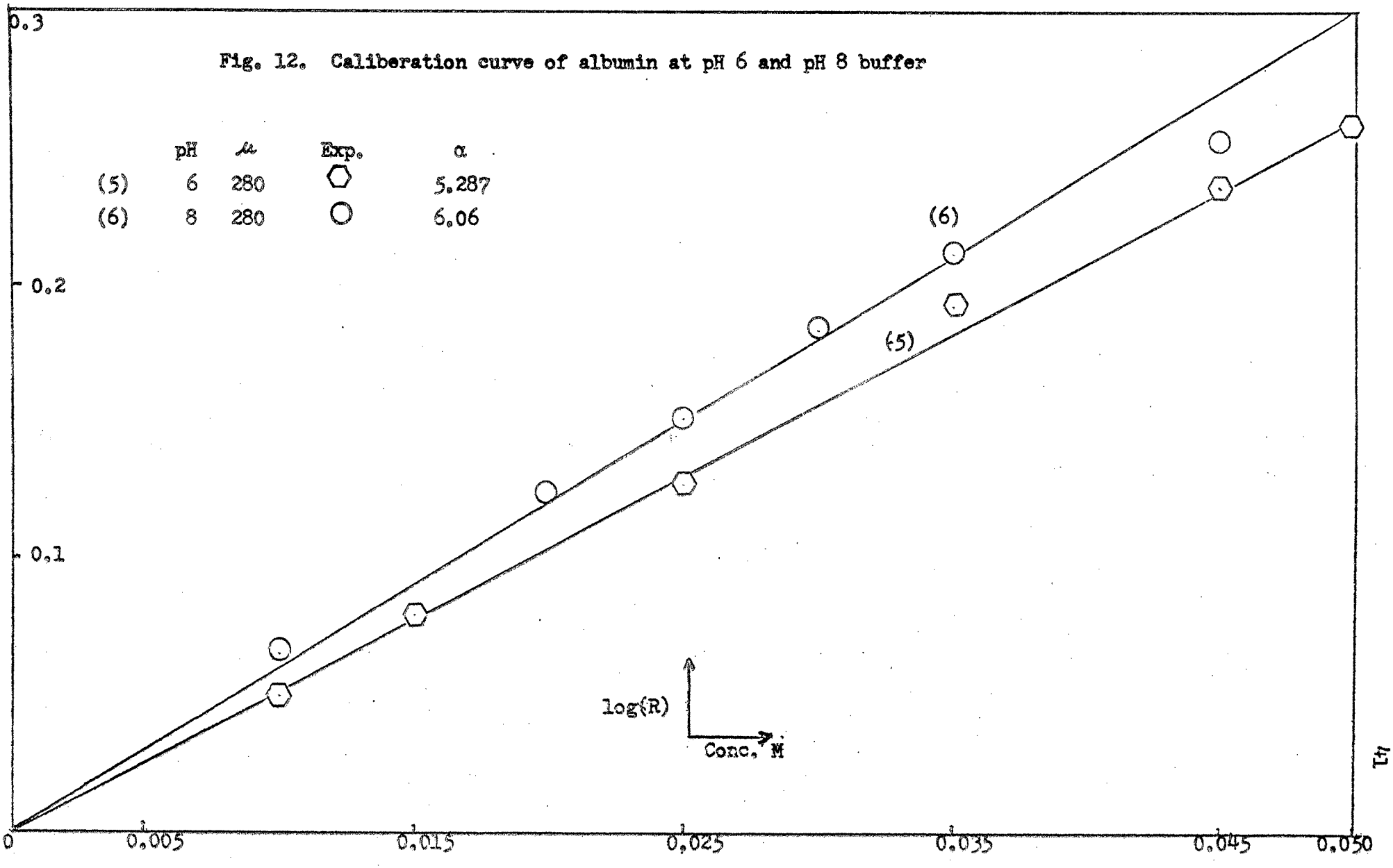


Fig. 12. Caliberation curve of albumin at pH 6 and pH 8 buffer



$$\eta H = \frac{\text{Log } (C(403)/ R(403))}{\beta A(403)} \qquad (7)$$

$$\eta A = \frac{\text{Log } (C(280)/ R(280)) - (\beta H(280))(\eta H)}{\beta A(280)} \qquad (8)$$

If equation 7 and 8 concentration divided by the average feed concentration $\langle \eta H \rangle F$ and $\langle \eta A \rangle F$ which result are $y_{(nH)}$ and $y_{(nA)}$ where n is the cycle number.

C. Experimental Process

All semi-continuous pH-parametric pumping experiments were carried out in the apparatus depicted in Figure 10. Table 12 summarizes the parameters for selected experiments. In order to determine the effect of displacement volume on separation, series of experiments were carried out with different displacement volume of 18, 20 and 24 cc at 0.035 M buffer and 32 cc at 1.5 M buffer. In the next series of experiments, the purpose was to study the effect of product flow rate. In order to research the cycle time, the location of feed introduced and albumin effect on semi-continuous pH-parametric pumping separation, series of experiments were carried out.

TABLE 12

A List of Semicontinuous pH-Parametric Pumping Parameters for All Run

Run	$Q(\frac{\pi}{w})$ cc	Q cc/m	Column H_B	Column H_T	Buffer pH_h	Buffer pH_l	Buffer $Conc_M$	Product (cc/m) ϕ_B	Product (cc/m) ϕ_T	Product (cc/m) pH_B	Product (cc/m) pH_T	Feed(weight%) pH	Feed(weight%) A.	Feed(weight%) H.	Start	$\frac{\pi}{w}$ m.
															*	
L 2	20	0.5	9	9	8.3	6	0.035	4	4	7.9	6.1	6	0.05	0.01	U	40
L 3	24	0.5	8	8	8.6	6.2	0.035	4	4	7.8	6.2	6	0.05	0.01	U	48
L 6	21	3.0	8	8	8.5	6	0.035	4	4	7.3	6.1	6	0.05	0.01	U	7
L 7	20	0.5	8	8	8.9	6	0.035	4	4	8.2	6.2	6	0.05	0.01	U	40
L 8	24	0.5	8	8	8.9	6	0.035	4	4	8.1	6.1	6	0.05	0.01	U	48
L 9	18	o.5	8	8	8.9	6	0.035	4	4	7.9	6.2	6	0.05	0.01	U	36
L10	20	0.5	8	8	8.9	6	0.200	1.5	6.5	8	6	6	0.05	0.01	U	40
L11	20	0.5	8	8	8.9	6	0.200	6.5	1.5	7.4	6.2	6	0.05	0.01	U	40
L12	32	0.5	8	8	8.9	4.9	0.150	4	4	7.9	5.2	6	0.05	0.01	U	64
L13	20	0.5	14	9	8.9	4.9	0.200	4	4	8.6	4.9	4.9	0.05	0.01	U	40
L14	20	0.5	8	8	8.9	6	0.200	4	4	7.4	6.2	6	0.05	0.01	U	40
L15	15	0.5	8	8	8.9	6	0.200	4	4	8	6	6	0.05	0	U	30

* Upflow first.

CHAPTER III

RESULTS AND DISCUSSION

1. Voidage Value of Ion Exchanger at Different Buffer Concentration

A. The Effect of Aqueous Buffer Concentration on Voidage at Different pH Value

As far as the amount of the aqueous buffer concentration is concerned, we can draw pH verses voidage on normal cartesian coordinates (Fig. 3). Comparing with the low buffer concentration that

(1). The initial voidage at high buffer concentration is larger.

(2). The pH varied traces from pH 6 to pH 8 and from pH 8 to pH 6 are very close. Neglecting their small difference, we assume their pH varied traces to be equal.

(3). pH change rate at high buffer concentration is faster.

(4). The initial voidage change rate at high buffer concentration range is smaller.

The ion exchanger swells in aqueous solvent. Its voidage depends on the swelling properties of SP Sephadex (C-50). The degree of swelling varies with the ionic composition of the swelling medium. At low buffer concentration, repulsion between the ions of the fixed charged group is maximum and the ion exchanger swells greatly. The degree of swelling decreases with increasing buffer concentration, because of diminishing repulsion of ions.

Consequently, the initial voidage is larger at high buffer concentration.

The extent to which an ion exchanger is charged depens on pH. Repulsion between charged group is maximum at pH values, when the ion exchanger is fully charged and decreases as the charged groups become neutalized. This variation in repulsion results in a pH dependent swelling. From literature[1], we know that at constant ionic strength, SP Sephadex (C-50) ion exchangers are fully charged over a very wide pH range and have swelling properties indepent of pH. In the present experiments pH 8 and pH 6 aqueous buffer mix from different fractions of aqueous monobasic sodium phosphate and dibasic sodium phosphate at a given concentration. As a result, pH 6 and pH 8 aqueous buffer ionic strengths are different at the given concentration. Hence, there is a slight variation of voidage from pH 6 to pH 8 at the given buffer concentration.

From previous discussion, we know that the ion exchanger swelling of high concentration is larger than that of low concentration. The flow becomes closer to the plug flow as the size of the particles through which the flow passes becomes smaller. That is, the range of pH change between the low and high pH is smaller.

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1. Pharmacia Fine Chemicals, "Sephadex Ion Exchangers — A Guide to Ion Exchange Chromatography" (1975).

Therefore, pH varied traces at high buffer concentration are faster than those at low buffer concentration.

Fig. 13 indicates that voidage curves associated with the 16 cc, 28 cc packing volumes and the aqueous buffer with 0.01% haemoglobin are identical at buffer concentration of 0.15 M. From those experiments it is shown that the volume of packed ion exchanger and whether haemoglobin exists or not have no effect on the value of the ion exchanger voidage.

B. Initial Voidage and Swelling Factor of Ion Exchanger

Initial voidage from buffer concentration of 0.035 M to 0.20 M could be determined from the predicted curve of initial voidage verses buffer concentration on normal cartesian cordinate as shown in Fig. 14. The other initial voidage could be obtained extrapolation.

Swelling factor(S) is defined as the volume obtained from one gram dry ion exchanger. It can be expressed by the following equation:

$$S = \frac{(1 - @_I)\, hA}{g_1} \qquad (9)$$

S = swelling factor, cc/g.

$@_I$ = initial voidage, dimensionless.

h = height of packing ion exchanger, cm.

A = inside area of column, cm^2.

g_1 = dry weight of packing ion exchanger, g.

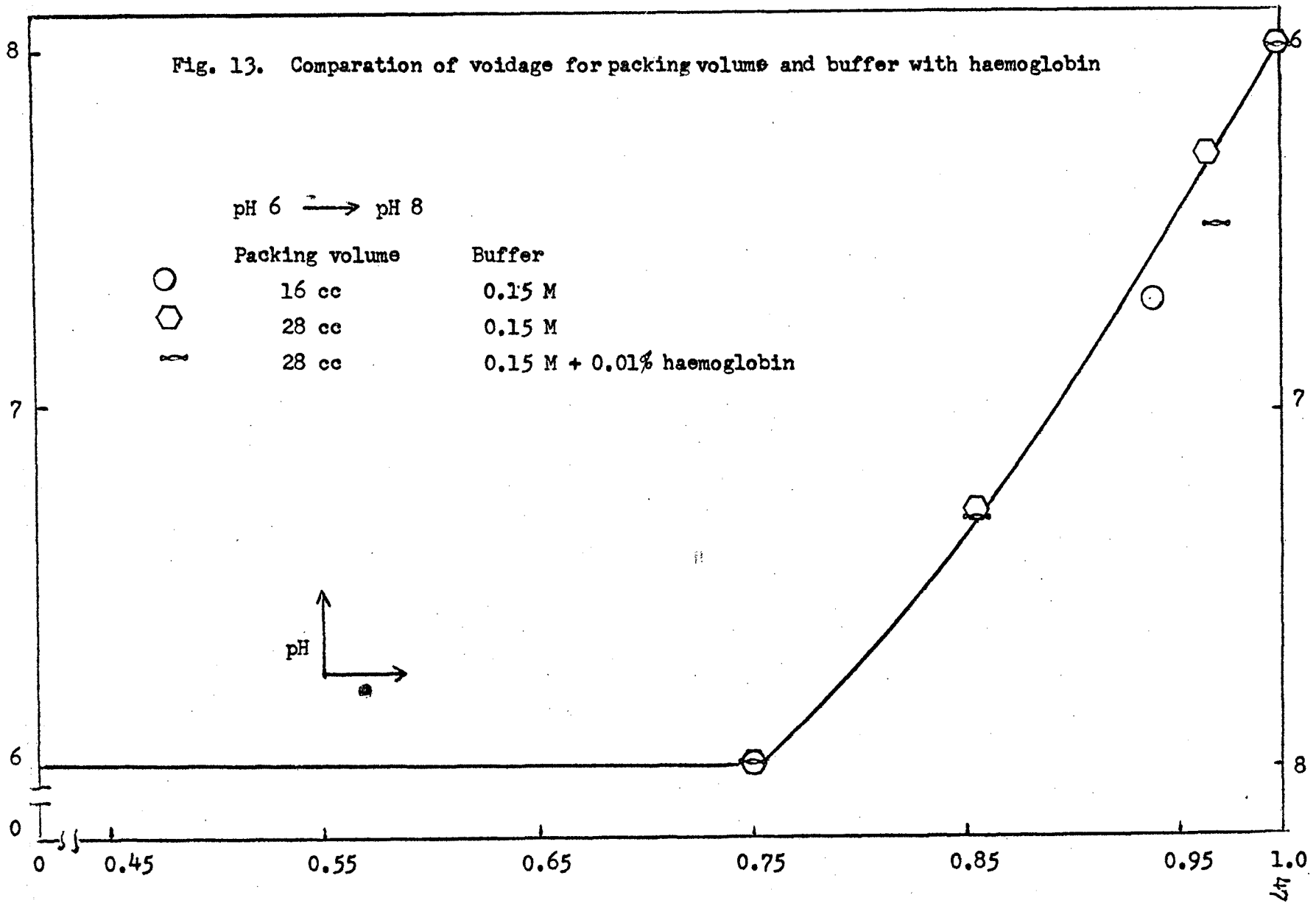

Fig. 13. Comparation of voidage for packing volume and buffer with haemoglobin

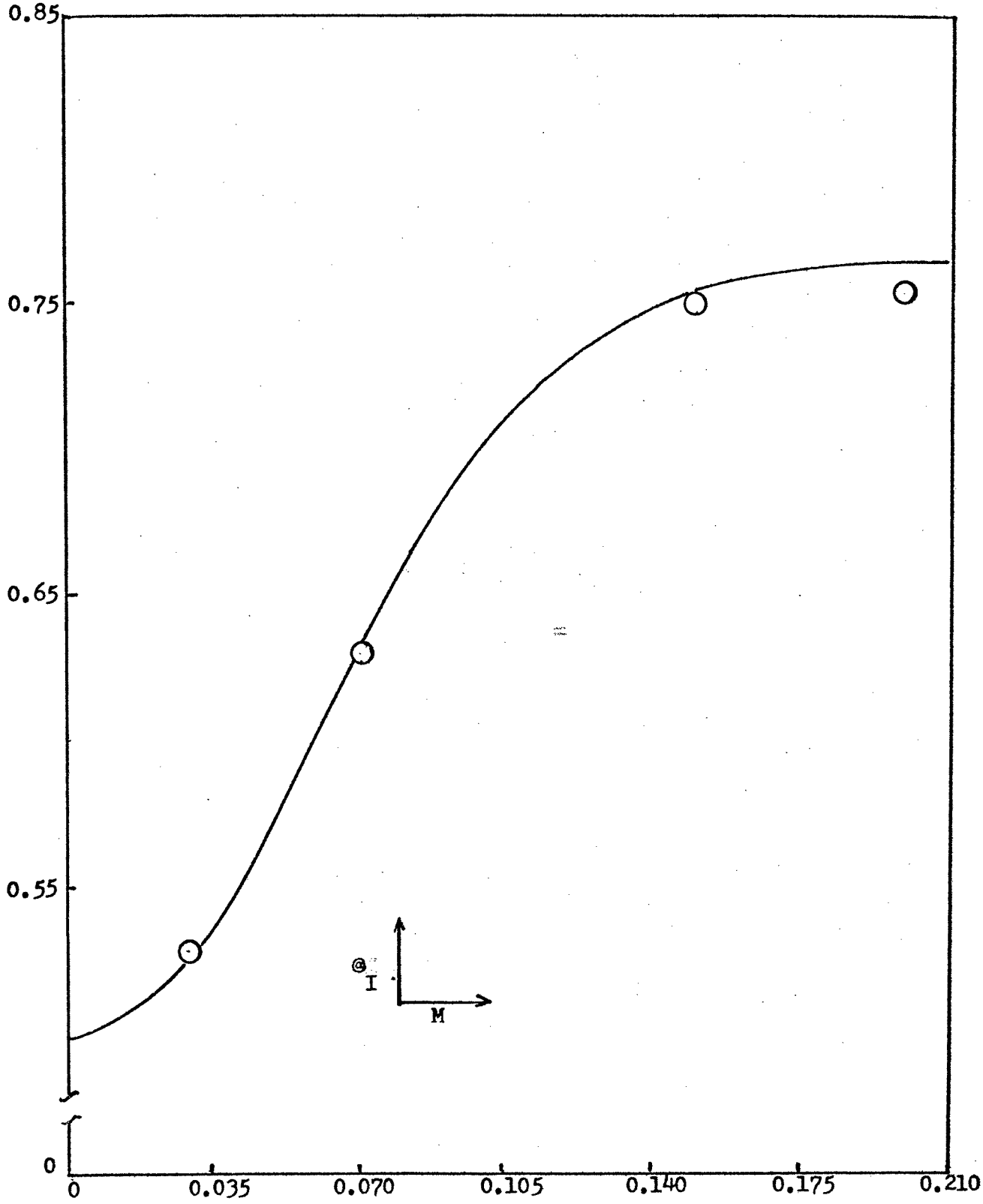

Fig. 14. Initial voidage vs. buffer concentration

h, g_1, $\varnothing_I$ and A values at different concentration are listed in Table 13, S is calcuated from equation 9. Figure 15 shows swelling factor verses buffer concentration on normal corrdinate. From this curve, we know that ion exchanger swelling decreases from low to high buffer concentration and varies slowly at high concentration range. Therefore, the initial voidage change varies slowly at high concentration range.

C. Average voidage of Ion Exchanger at Different Buffer Concentration

During the first upflow half-cycle at semicontinuous pH-parametric pumping, pH of ion exchanger which initially mixs with low aqueous pH buffer varies as shown in Figure 3 from low to high pH. These curves are quite linear. To faciliate the computation of voidage, average value is used to represent the voidage distribution in a small range. Average voidage is the sum of the initial and final voidage divided by two. Since the initial and final voidage differ by an insignificant amount, the average voidage should be close to the real voidage distribution in the column. Table 14 lists the average voidage of different buffer concentration. Fig. 16 is the average voidage against the buffer concentration graph on normal cartesian coordiate. We can determine the average voidage from this curve.

TABLE 13

Swelling Factor of Ion Exchanger at Constant Buffer Concentration

Buffer Concentratoon M	$@_I$	h cm	A cm^2	g_1 g.	S cc/g.
0.035	0.54	14	2	1.12	11.5
0.070	0.63	12	2	1.20	7.4
0.150	0.75	14	2	1.60	4.4
0.200	0.75	16	2	2.00	4.0

* $$S = \frac{(1-@_I)hA}{g_1}$$

S = swelling factor

$@_I$ = initial voidage

h = height of packing column

A = inside area of column

g_1 = weight of dry SP Sephadex (C-50)

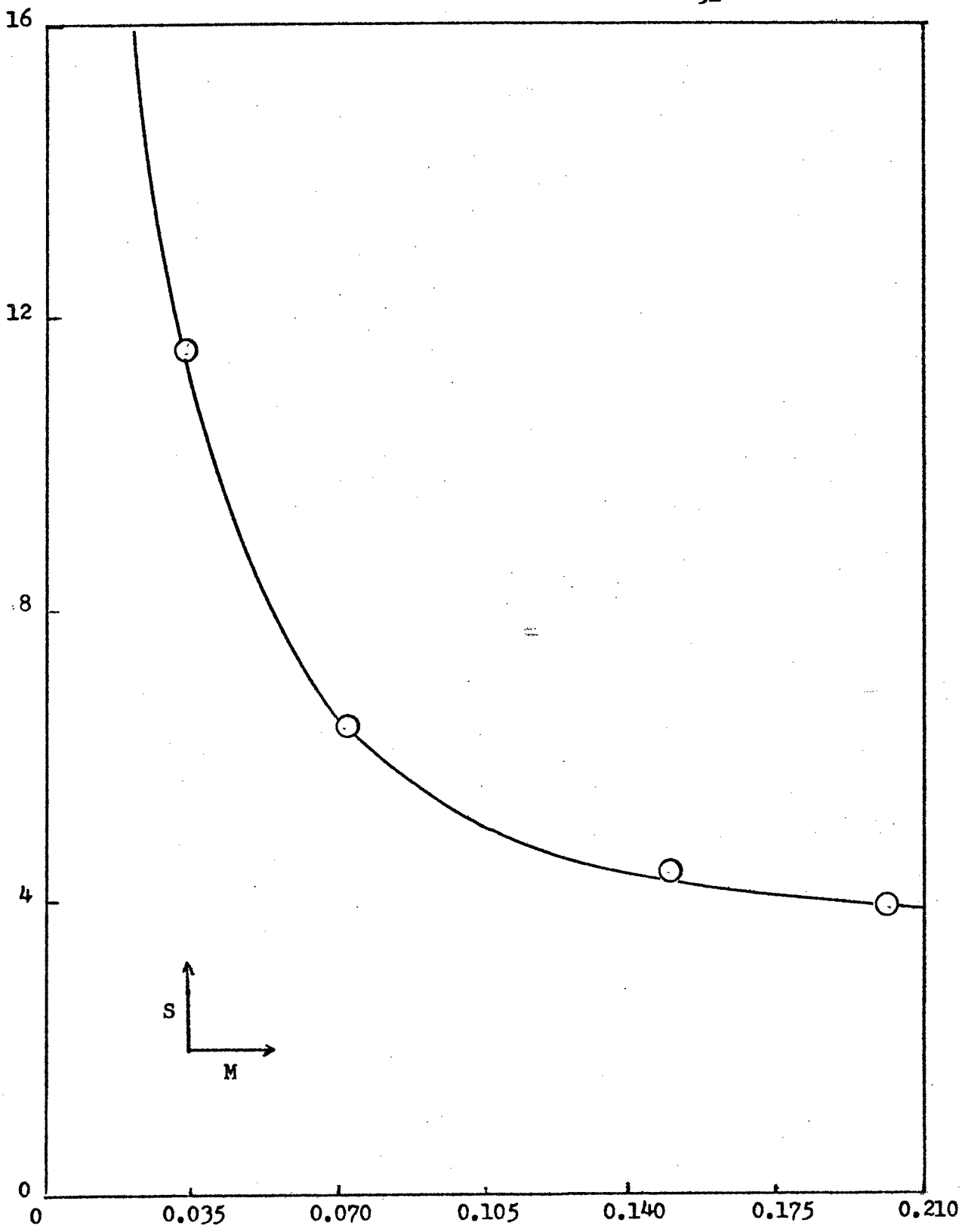

Fig. 15. Swelling factor curve at different buffer concentration

TABLE 14

Average Voidage of Ion Exchanger at Constant Buffer Concentration

Buffer Concentration M	$@_I$	$@_F$	$@_A$
0.035	0.54	0.96	0.750
0.070	0.63	1.00	0.875
0.150	0.75	1.00	0.875
0.200	0.75	1.00	0.875

$$* \quad @_A = \frac{@_I + @_F}{2}$$

$@_A$ = average voidage

$@_I$ = initial voidage

$@_F$ = final voidage

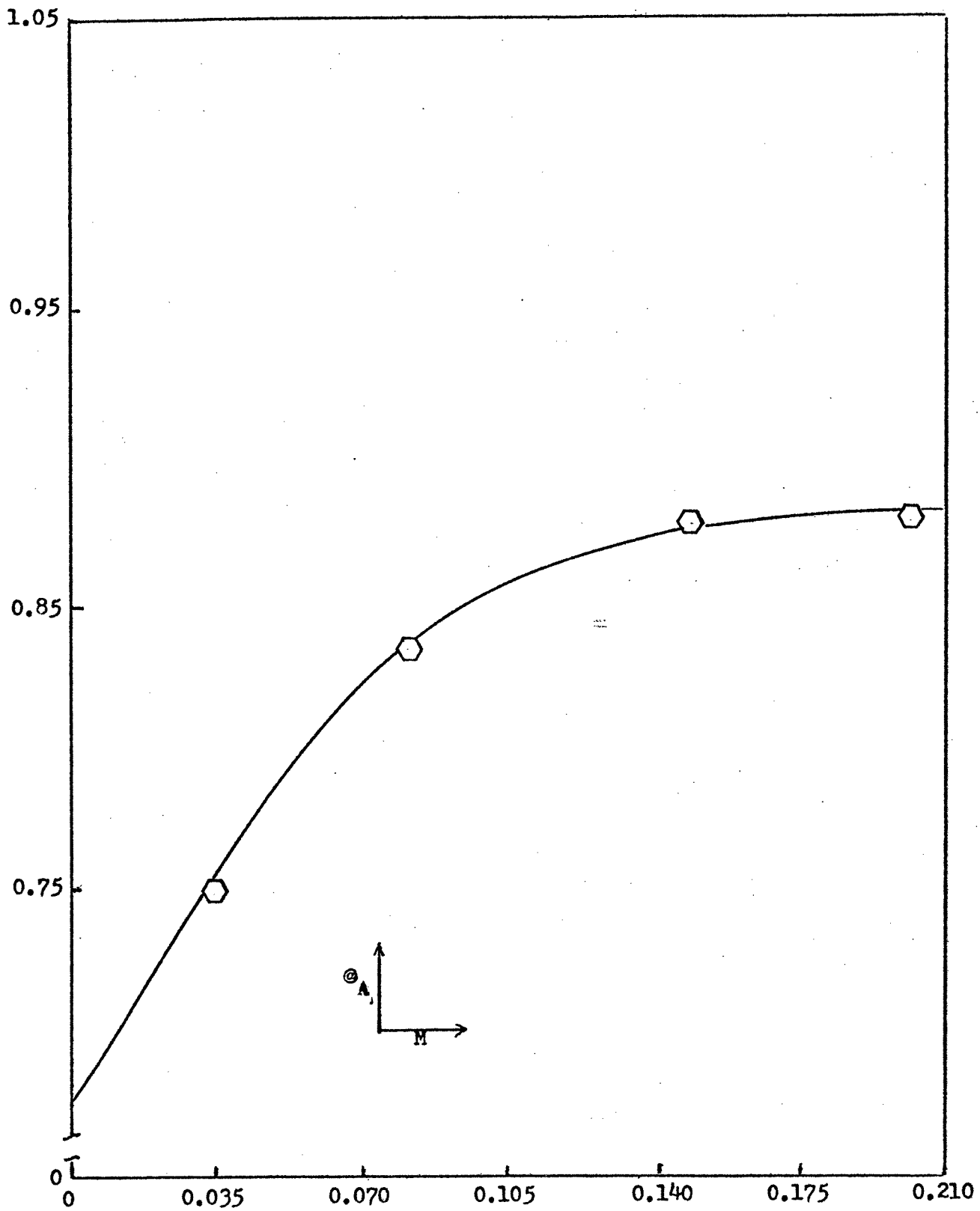

Fig. 16. Average voidage curve at different buffer concentration

Assume that there are no others effects, it is observed that the maximum displacement volume in pH-parametric pumping will be the fluid does not move from one reservoir through the column into the other reservoir with mixing. It can be expressed by the following equation:

$$\text{Maximum (or Optional) displacement volume} = Q\left(\frac{\tau}{w}\right)(@_A) \quad (10)$$

where: Q = reservoir displacement rate, cc/m.

$\frac{\tau}{w}$ = duration of half cycle, m.

$@_A$ = average voidage, dimensionless.

Hence, the optional displacement volume of different buffer concentration in semicontinuous pH-parametric pump could be predicted from previous equation (10) and average curve Figure 16.

2. Voidage Properties in pH Cycling Zone Process

We are investigating cycling zone pH outlet curve in Fig. 6 and Fig. 7, to see that every delay volume of respect is equal in each experiment. Neglecting their small difference, the pH varied traces from pH 6 to pH 8 or reverse and different period cycle are same. This illustrates the voidage can be constant consideredly.

Table 8 shows the calculated initial voidage from this experiment. Comparing of these results with that of previous test method, it shows that the initial voidage for n zones agree with that obtain from draining the interstitial aqeous. The type of operation illustrated in voidage test method will be refered to as a single zone process. The pH cycling zone could be concidered by operation two or more single zone in series. The adventage of the cycling zone process is that chang pH continuously which similar to parametric pumping process.

The same experiment was repeated with both hamoglobin (0.01%) and albumin (0.05%). By comparing Fig. 7 and Fig. 8, we see that pH outlet curve with and without hamoglobin, albumin is same. As previous test method, the hamoglonbin and albumin have no effect on voidage in cycling zone process.

The effect of flow rate on the outlet pH distribution was

also studied for flow rate 0.5 cc/m and 1.0 cc/m. Fig. 7 and Fig. 9 show that the lower flow rate gives slightly lenger period to respect pH change.

There occurs different respect time, owing to the fluid velocity that move through the fixed-bed. This result indicate the flow rate is effect on properities of voidage and performance of pH-parametric pumping.

3. Semicontinuous pH-Parametric Pump

A. The Effect of Displacement Volume

Figure 17 shows the effect of displacement volume ($Q(\frac{\pi}{w})$) on separation. Separation factor (S.F.) is defined as the quotient of the bottom and top concentration. $\langle Y_B \rangle n / \langle Y_T \rangle n$ (S.F.) for haemoglobin increases as displacement volume increases, while albumin remains approximately unity. Thus, separation could be improved with large displacement volume. However, there are optional displacement volumes. If the displacement becomes excessive, fluid moves from one reservoir through the column, and into the other reservoir with mixing. Consequantly, the S.F. will decline, or seperation may not be found at all.

From the voidage test, the optional displacement volume of buffer concentration with 0.035 M is 0.75 times the volume of packing ion exchanger. Hence, the optional displacement volume of 32 cc gel at 0.035 M is 24 cc. The capacity of ion exchanger combines with haemoglobin at buffer concentration of 0.035 M is very large. During the upflow half-cycle, the larger the displacement volume is, the more the haemoglobin are bound to ion exchanger. During the downflow half-cycle, there are more haemoglobins turned back to liquid phase. From the experimental result, we see that the

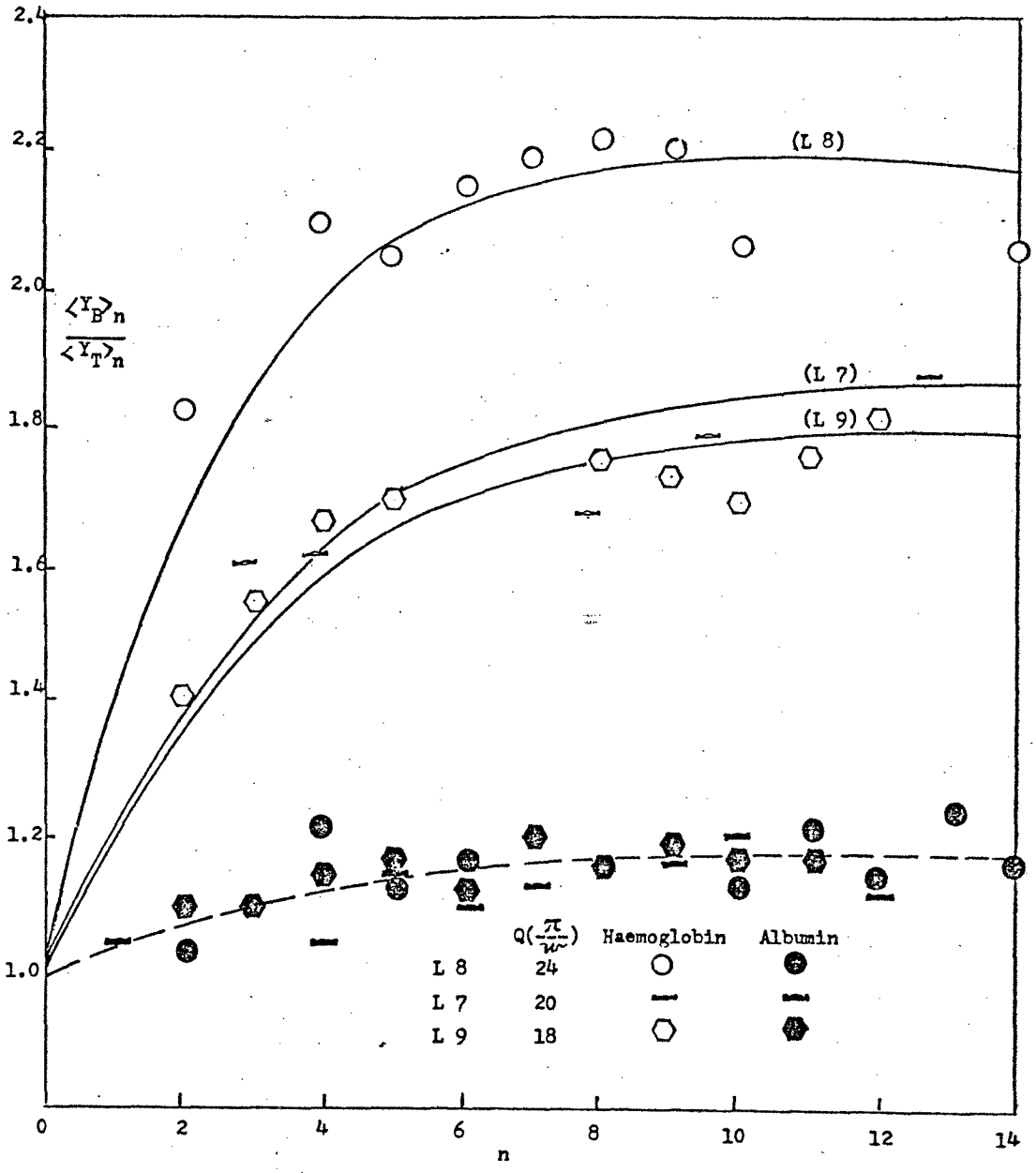

Fig. 17. Effect of displacement volume on separation

y(nHB)(i.e. bottom haemoglobin concentration divides by the average feed concentration,where n is the cycle number) of run L 8 (displacement volume = 24 cc) is larger than that of run L 7 (displacement volume = 20 cc). The y(nHB) of run L 7 (displacement volume = 20 cc) is larger than that of run L 9 (displacement volume = 18 cc). Consequently, under the optional displacement volume, S.F. for haemoglobin increases as displacement volume increases.

The optional displacement volume of 16 cm height gel at 0.15 M concentration is 28 cc. Figure 18 showes the experimental result associated with a volume at run L 12 (displacement volume = 32 cc). For the displacement volume beyond the optional value, fluid moves from one reservoir through the column, and into the other reservoir with mixing. For the steady state ($n \rightarrow \infty$), the y(nHT)(i.e.top haemoglobin concentration divides by the average feed concentration, where n is the cycle number) and y(nHB) will meet and become equal, while the albumin remains approximately unity.

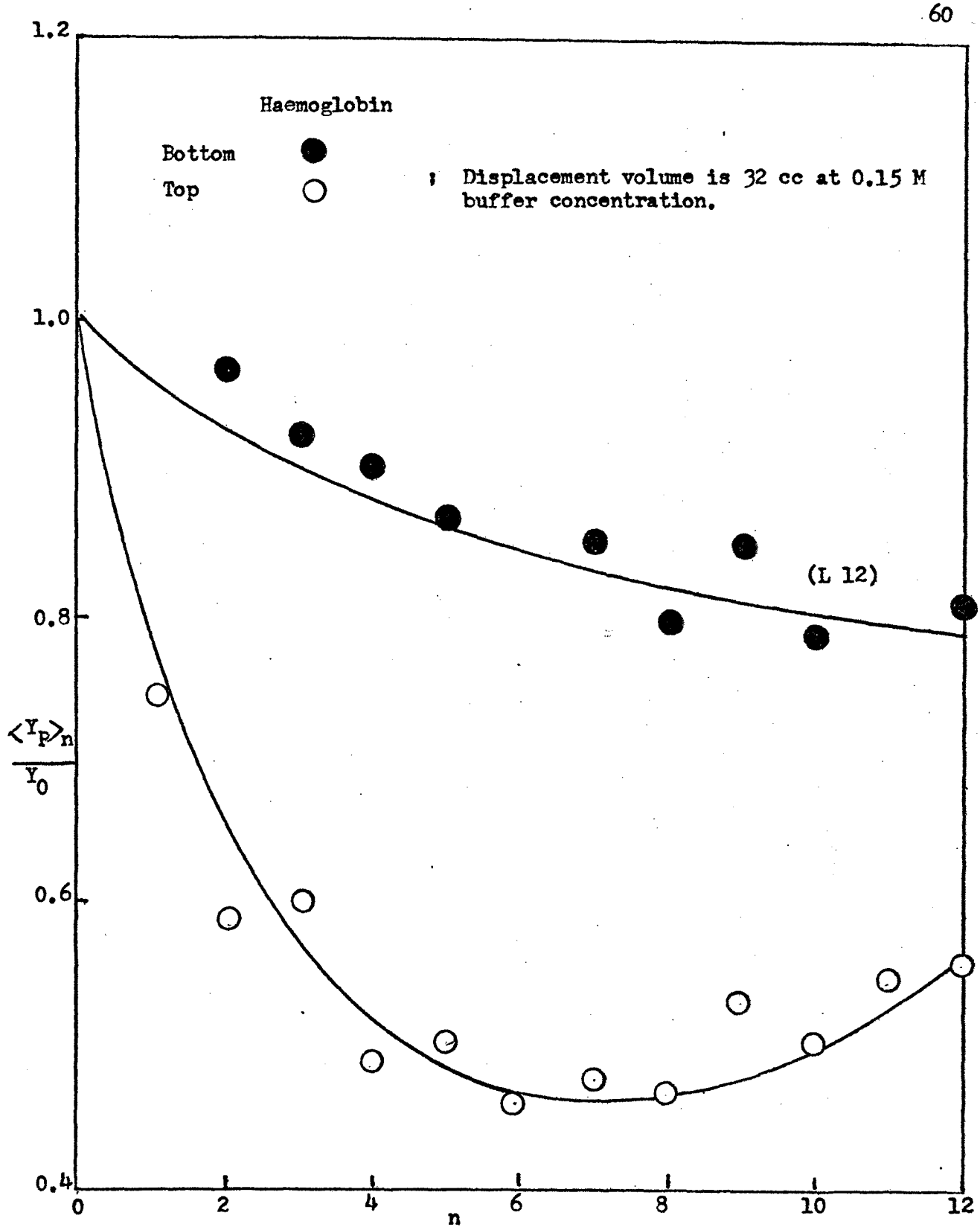

Fig. 18. Effect of over maximum displacement volume on separation

B. The Effect of Product Flow Rate

It should be pointed out that at the steady state the mass balance formula is

(Aqueous feed in) = (Top product out) + (Bottom product out)

that is

$$(\phi_T + \phi_B)\ Q(\frac{\pi}{w})Y_0 = \phi_T Q(\frac{\pi}{w})Y_T + \phi_B Q(\frac{\pi}{w})Y_B \qquad (11)$$

so the bottom product concentration is

$$\frac{\langle Y_B \rangle_\infty}{Y_0} = 1 + \frac{\phi_T}{\phi_B}\left(1 - \frac{\langle Y_T \rangle_\infty}{Y_0}\right) \qquad (12)$$

Where

ϕ_T = top product volume flow rate /reservoir displacement rate, dimensionless.

ϕ_B = bottom product volume flow rate/reservoir displacement rate, dimensionless.

Y_0 = concentration of solute in the feed, Kg moles/cu. cm.

Y_B = concentration of solute in the bottom product during downflow, Kg moles/cu. cm.

Y_T = concentration of solute in the top product during downflow, Kg moles/cu. cm.

$\langle\ \rangle_n$ = average concentration at n^{th} cycle.

From theory and experiment, we know that $\frac{\langle Y_T \rangle_\infty}{Y_0}$ is always less than one and larger than zero. So, there are three cases between ϕ_T and ϕ_B.

Case 1. $\phi_B \rangle \phi_T$:

Figure 19 shows the effect of ϕ_T on the concentration transients. The decrease of ϕ_T produced a decrease in steady-state top product concentration, and at the same time the transient time for depletion of the solute (haemoglobin) from the top reservoir or the top product stream became longer.

When ratio of ϕ_T and ϕ_B close to zero (i.e. $\phi_B \rangle\rangle \phi_T$), the equation 12 become $\langle Y_B \rangle \alpha / Y_0$ equal to one. That is to say that at steady state ($n \longrightarrow \infty$) haemoglobin predominates in the bottom product while the haemoglobin supplied by the feed stream.

So: $$\langle Y_B \rangle \alpha / Y_0 = 1 \qquad (\text{If } \phi_B \rangle\rangle \phi_T)$$

Case 2. $\phi_T \rangle \phi_B$:

For a given value of ϕ_T by adjustment of ϕ_B to an arbitrarily low value, we may obtain an arbitrarily high degree of enrichment in the bottom product stream.

Since $\langle Y_T \rangle \alpha / Y_0$ is always less one, from equation 12 we know $\langle Y_B \rangle \alpha / Y_0 \rangle \langle Y_T \rangle \alpha / Y_0$. Fig. 20 showes that bottom concentration is always larger than top concentration. For this case of haemoglobin $\langle Y_T \rangle n / Y_0$ falls monotonically and levels off to a certant value, while $\langle Y_B \rangle n / Y_0$ first increases and then decreases for a certain number of cycle and levels out to a steady value.

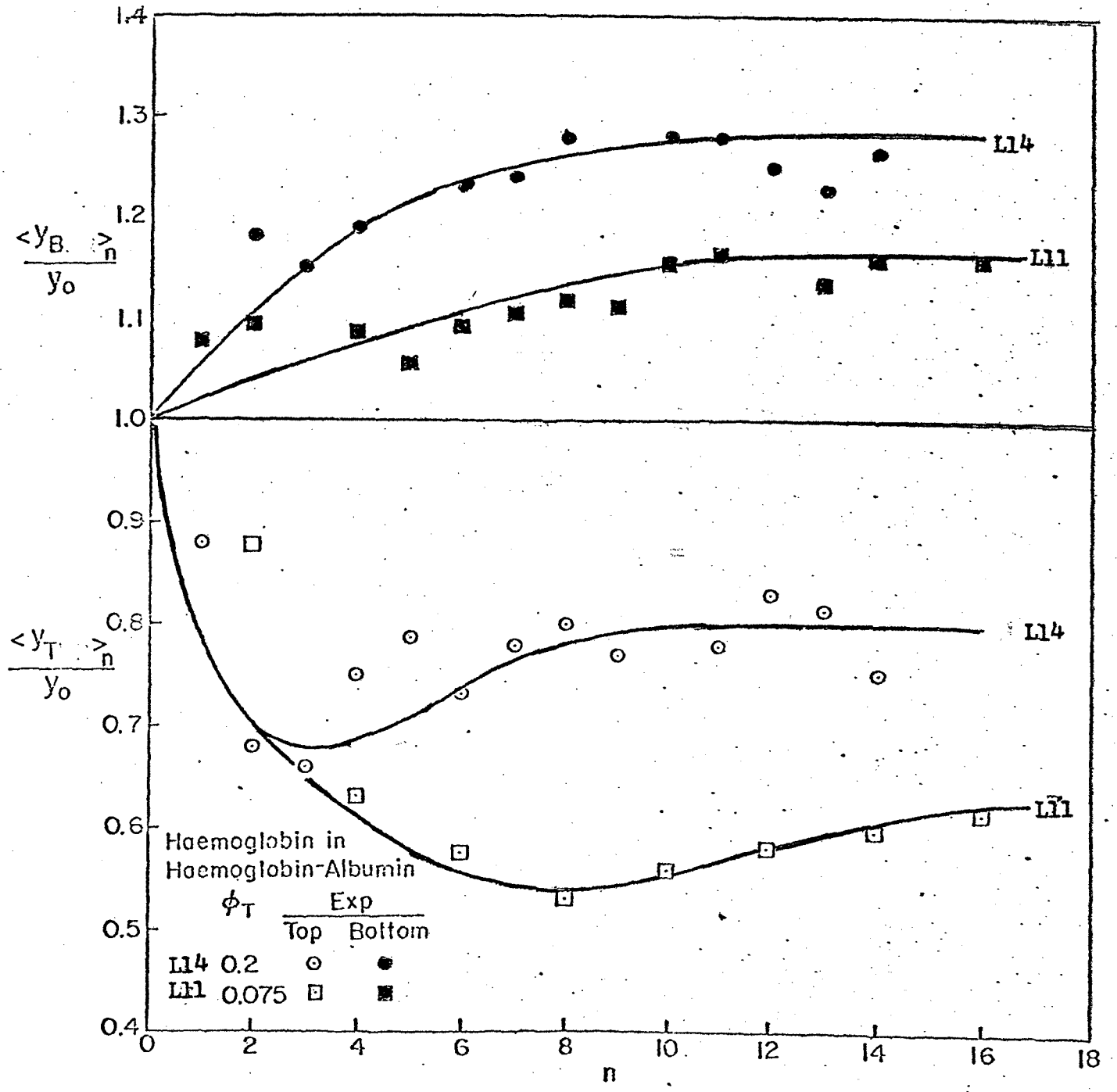

Fig. 19. Effect of top product flow rate on separation

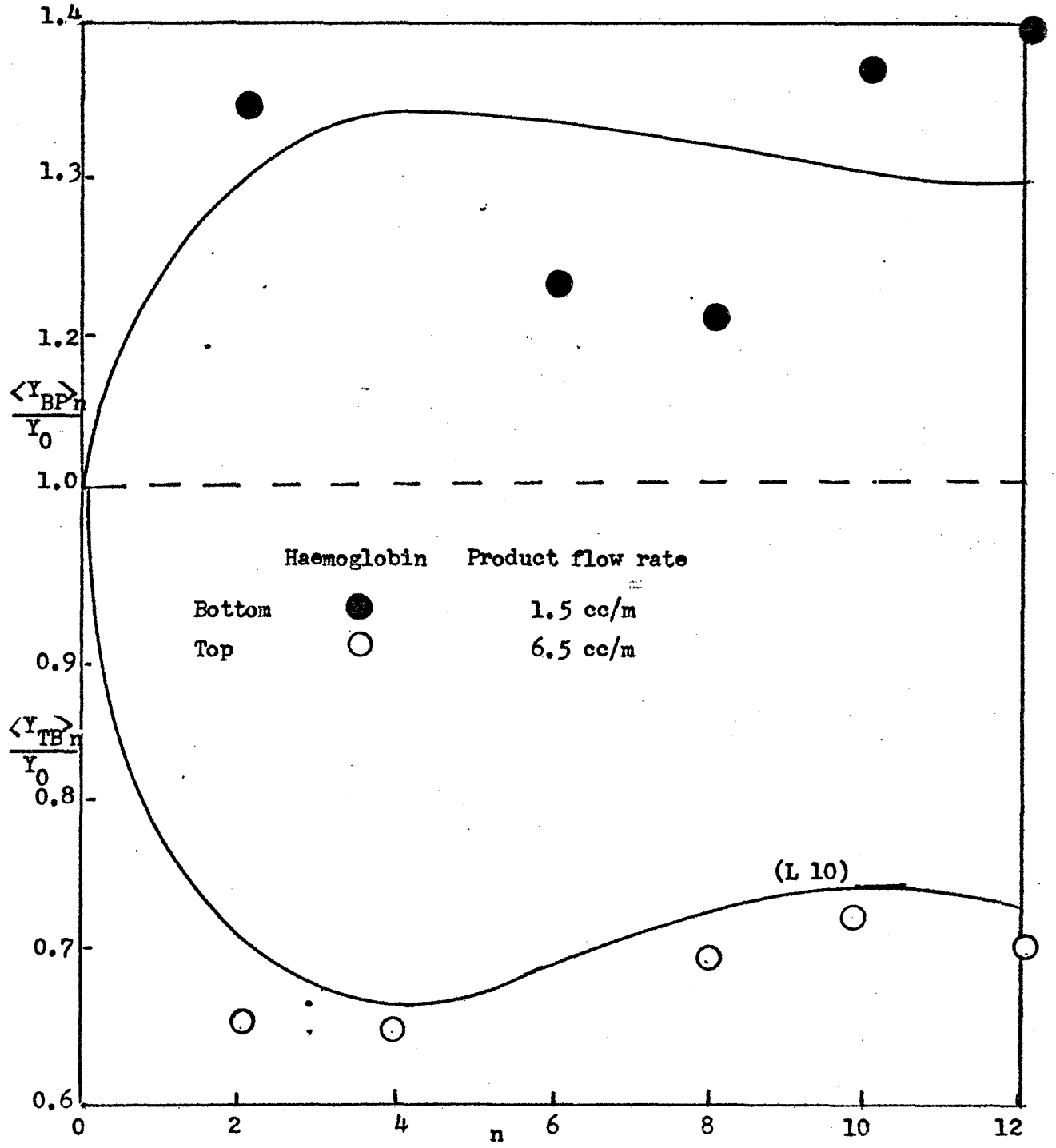

Fig. 20. Result of top product flow rate is greater than bottom flow rate.

Case 3. $\phi_T = \phi_B$:

At this case, we can get $\langle Y_B \rangle \infty/Y_0 = \langle Y_T \rangle \infty/Y_0$ from equation 12. This is to say that at the steady state ($n \longrightarrow \infty$) haemoglobin in top and bottom will aqual concentration. Fig. 19 showes that there are nearly same concentration of top and bottom at $\phi_T = \phi_B$.

So the components removed can be concentration to any desired practical level by setting the flow rate of the product stream containing these components at the required value.

C. The Effect of The Other Factor

(a). Location of Feed Introduced

Location of feed introduced is also effect on separation. Fig. 21 showes the bottom column higher than the top column at buffre concentration 0.2 M, we could get very good separtion. At this experiment run L 13, 0.2 M low pH feed was introduced between bottom column (enriching section) of 14 cm height and top column (stripping section) of 9 cm height. During a high pH half-cycle, there are more haemoglobin bound to ion exchanger at higher length enriching section. During a low pH half-cycle, the difference of pH between downflow and ion exchanger solution will increase after feed mixed with downflow. Since enriching section of longer length had more haemoglobib bound and past through more low pH flow, there are more amount of haemoglobin could be turned back to liquid phase. So the separation is very good at this feed type.

Prelimnary experiments had been conduted with feed introduction at the top of the column. With a buffer concentration of 0.035 M, the capacity of the column for haemoglobin was very large. Intrduction of this feed stream into the top of the column led, during the early cycles of operation, to the accumulation in the uppermost layers ofpacking of substantially all of the

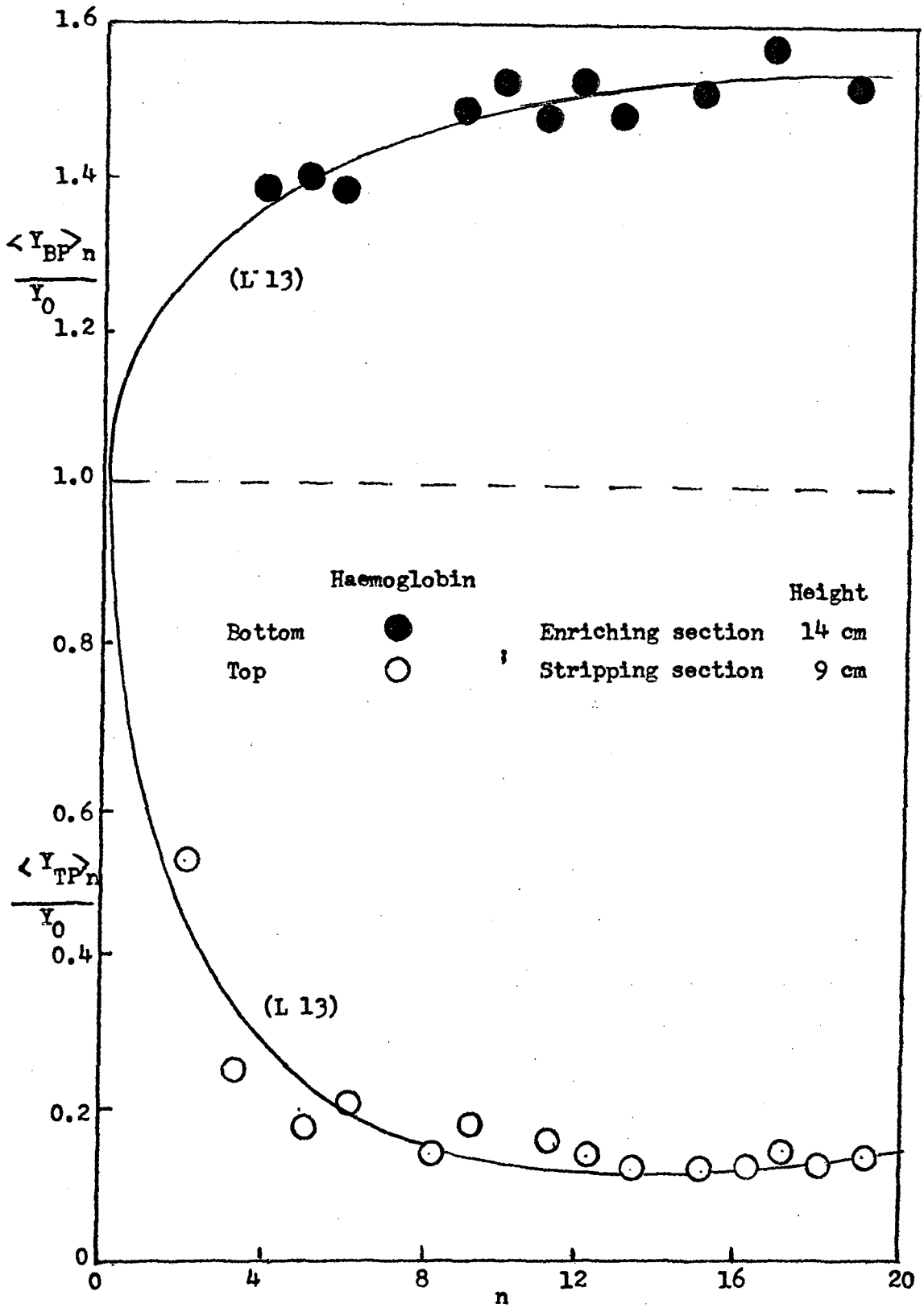

Fig. 21. Concentration transients for haemoglobin. The enriching section is longer than stripping section.

haemoglobin. The resulting layer persisted in later cycles, as determined visually, and led eventually to excessive pressure drop and inability to pump bottom reservoir liquid into the column. In this arrangement of parametric pumping, the linear velocity in downflow was greater than that in upflow. Hence, the liquid from the bottom reservoir, for which the pH was high, never reached the top most layers of the column, and therefore, the haemoglobin was never caused to transfer back into the liquid phase when it would be accessible for further downward movement.

So, the optimized location of feed stream introduced is between $1 > HT/HB > 0$ (HT=height of bottom column, HB=height of top column).

(b). The Effect of Flow Rate at Low Buffer Concentration of 0.035 M

Fig. 22 showes that the separation factor (S.F.) of flow rate (Q) 3.0 cc/m is larger than that of flow rate 0.5 cc/m at buffer concentration of 0.035 M.

From pH-parametric pumping principle, we know that the separated component between a mobile and an unmobile phase should conveniently and practically be brought about by variaation of pH variable. That is separated component must be easy to bound to ion exchanger and turn back to liquid phase.

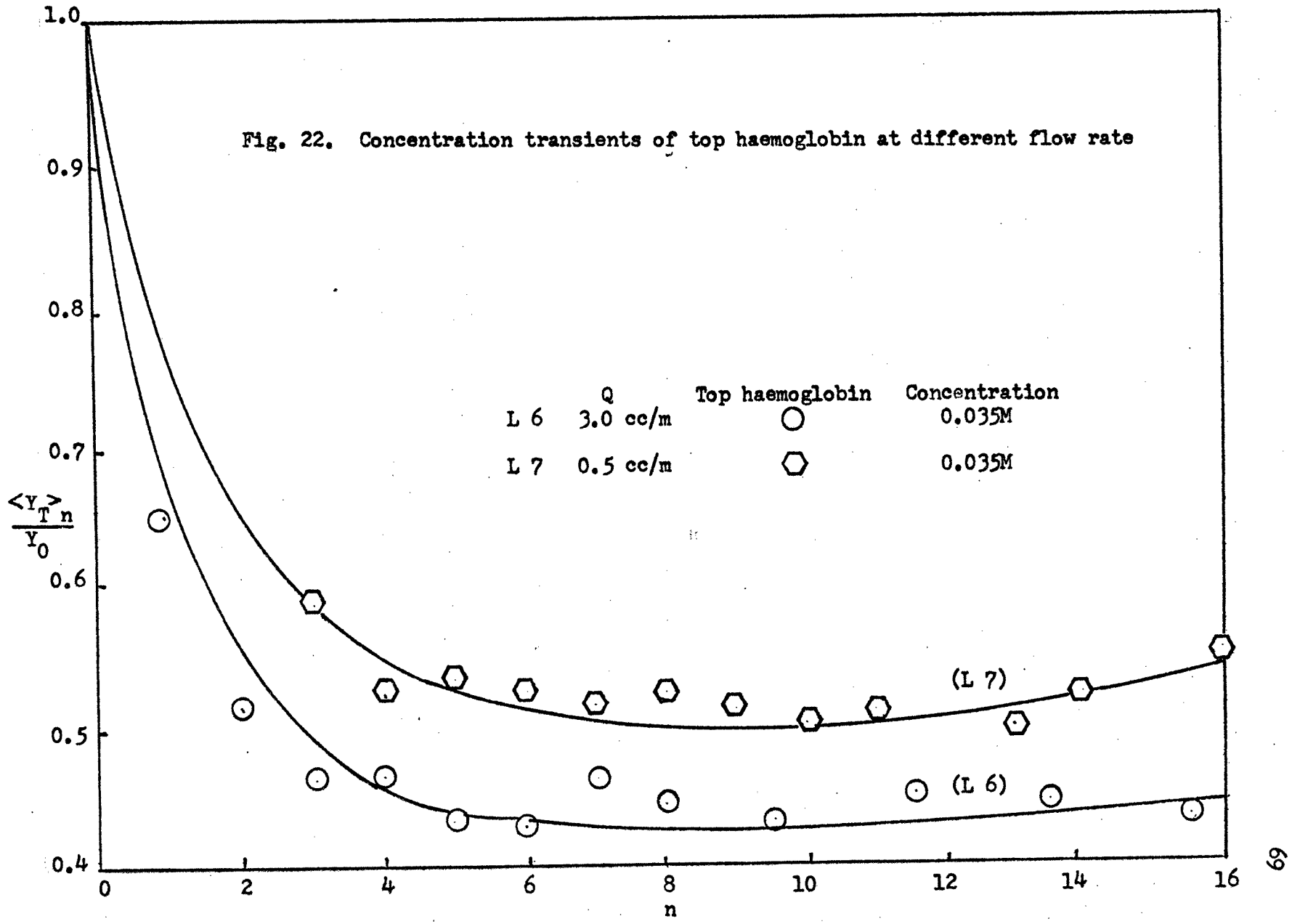

Fig. 22. Concentration transients of top haemoglobin at different flow rate

During upflow half-cycle, flow rate is slow (i.e. 0.5 cc/m) at buffer concentration 0.035 M, there are many haemoglobin bound around same ion exchanger. Since repulsion of position charge haemoglobin ion (S^+) each other which cause less uptake of haemoglobin, the efficiency of bound to ion exchanger decreases resulting high concentration of the haemoglobin in the top product stream. When flow rate is fast, haemoglobin will be more chance to bound to every ion exchanger. Fig. 23 showes that y(nHT) of run L 6 (flow rate 3.0 cc/m) is larger than y(nHT) of run L 7 (flow rate 0.5 cc/m). The better separation is, the faster flow rate is at low buffer concentretation 0.035 M.

(c). Property of Albumin in Semicontinuous pH-Parametric Pump

The albumin net charge is always negative during both up and down flow, ion exchanger and movement of solute between phase do not occure to any appreciable extent. This is clearly showen in Figure 24. For albumin, $\langle Y_T \rangle n / Y_0$ and $\langle Y_B \rangle n / Y_0$ are essentially independent of n, and the concentration of the top and bottom product stream may be considered to be con tant.

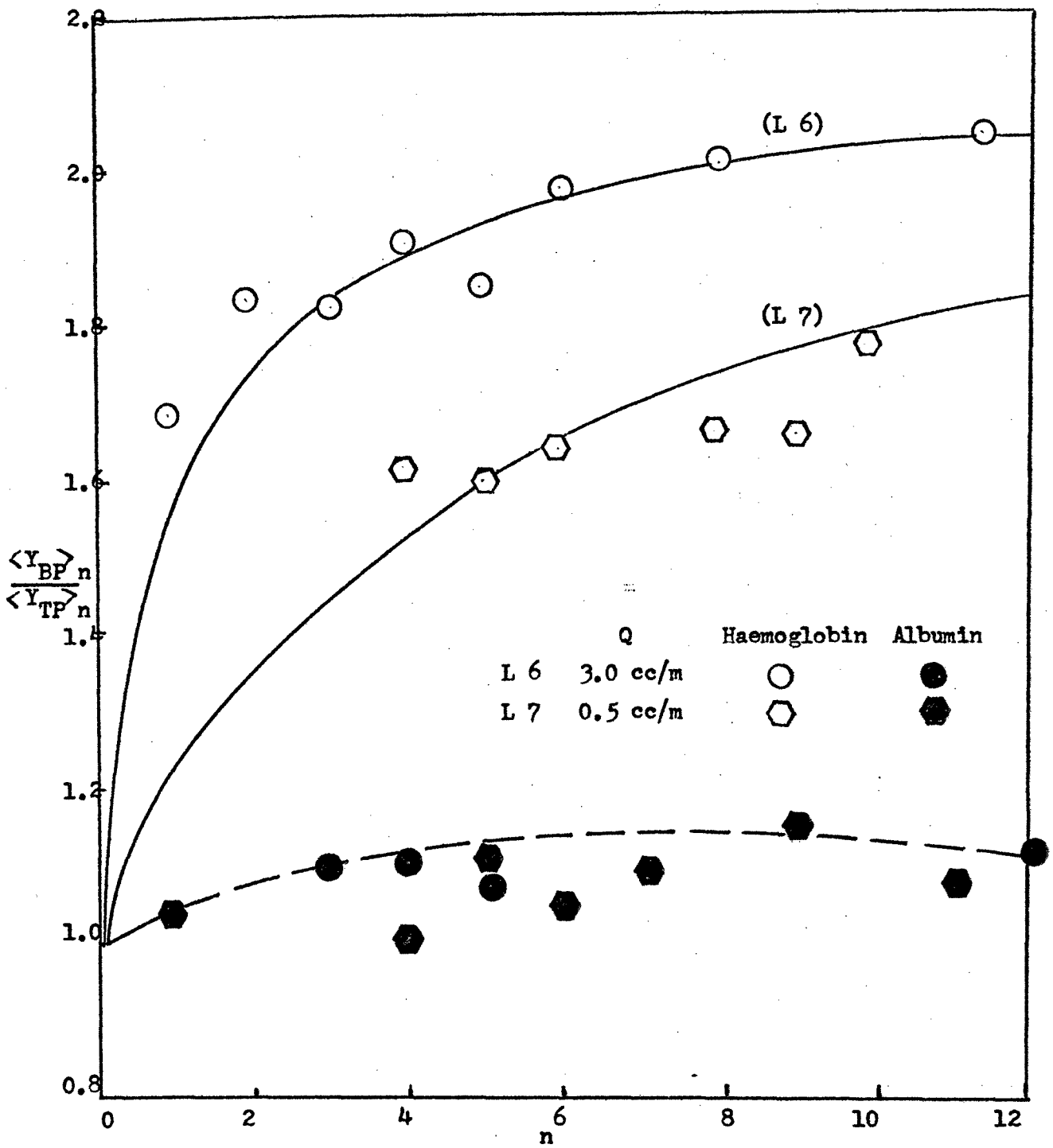

Fig. 23 Effect of flow rate on separation at low buffer concentration (0.035 M)

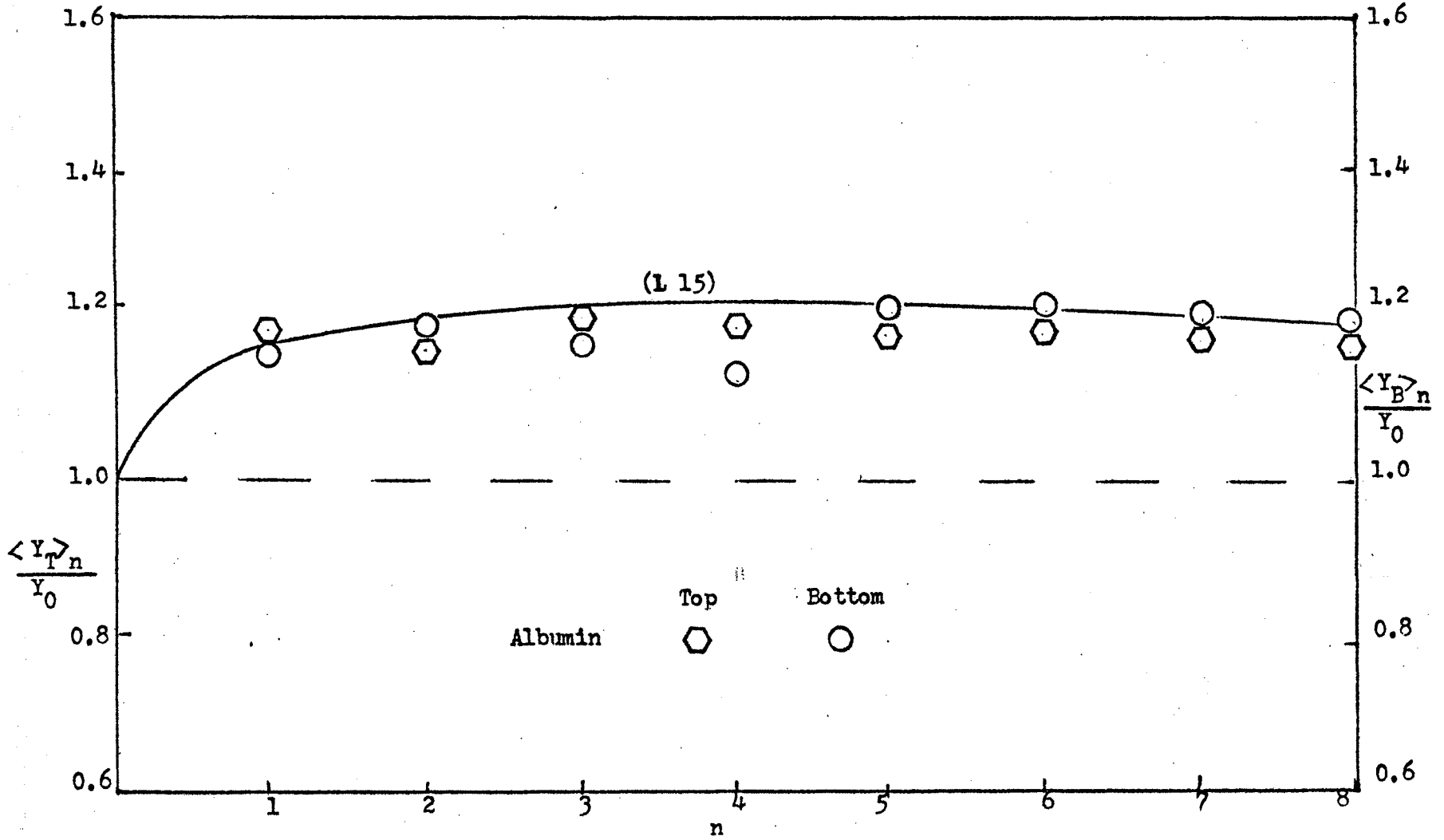

Fig. 24. Concentration transients for albumin

CHAPTER IV

CONCLUSION

It has been experimentally shown that

(1). The voidage in draining the interstitial aqueous is almost the same as pH cycling zone which is as pH process as pH-parametric pumping.

(2). The optional displacement volume in semicontinuous pH-parametric pump is $Q(\frac{\pi}{w})(\ \varepsilon_A\ )$. Where

Q = reservoir displacement rate, cu. cm/sec.

$\frac{\pi}{w}$ = duration of half cycle, sec.

ε_A = average voidage, dimensionless. It can be picked from voidage test result Fig. 16.

(3). The separation factor for haemoglobin is higher at higher displacement volumes, while that for albumin is unaffected. The separation could thus be improved by increasing the displacement volume, although at the point at which breakthrough from one reservoir to the other occurs the separation would decline or become nonexistent.

(4). The components removed can be concentrated to any desired level by setting the flow rate of the product stream containing these components at the require value.

TABLE 15

Semi-Continuous PH-Parametric Pumping Run L 2

1 : Parameters:

$Q(\frac{\pi}{w})$ = 20 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 40 m

ϕ_T = 4 cc/m

ϕ_B = 4 cc/m

H_T = 9 cm

H_B = 9 cm

Start = Upflow first

Buffer:

PH_L = 6

PH_H = 8.3

Conc. = 0.035 M

Feed:

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.3	100	98.2	99.4	96.1	---	45.19	28 C
PH 6	100	97.2	98.8	92.2	0.0096	60	28 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.3	100	101.8	106.1	99	---	16.05	6.06
PH 6	100	98.8	102.5	100.8	0.0591	21.67	5.29

3 : Sample Analysis at $\lambda = 4034\,A$ (Run L 2)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Transmission Cell	Haemoglobin conc. y_H	$\langle y_H \rangle_B / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4	8	35.1	33.1	3	0.0105	1.100	1.83
1 T	4	6.1	27	44.5	3	0.0057	0.601	
2 B	4	8.05	35.1	34.9	4	0.0097	1.014	1.99
2 T	4	6.1	27	47	4	0.0048	0.508	
3 B	4	8.25	35.1	36	3	0.0097	1.017	1.90
3 T	4.1	6.4	27	48.6	3	0.0051	0.534	
4 B	4	7.9	35.1	37.5	4	0.0090	0.942	1.72
4 T	3.9	6.1	27	44.6	4	0.0052	0.547	
5 B	4	7.9	37.5	37.9	3	0.0092	0.965	1.58
5 T	4.2	6.1	25.9	43.9	3	0.0058	0.612	
6 B	4	7.95	37	37.5	3	0.0090	0.942	1.71
6 T	4	6.1	25.9	44.5	4	0.0053	0.549	
7 B	4	7.95	37	39.2	4	0.0089	0.931	1.59
7 T	4	6.1	25.9	45.5	3	0.0056	0.584	
8 B	4	7.9	36.5	34.9	3	0.0097	1.014	1.93
8 T	4	6.1	25.9	45.9	4	0.0050	0.526	
9 B	4	7.9	36.5	38.2	3	0.0092	0.957	1.65
9 T	4	6	25.2	45.8	3	0.0055	0.579	

Continued, 403 μ (Run L 2)

10 B	4	7.8	36.5	39	4	0.0086	0.903	1.73
10 T	4	6	25.2	46.2	4	0.0050	0.521	
11 B	4	7.8	37	35.2	3	0.0099	1.039	1.92
11 T	4.1	6	25.5	48.2	3	0.0052	0.541	
12 B	4.1	7.85	37	34.4	4	0.0098	1.028	2.08
12 T	4	6	25.5	47.9	4	0.047	0.493	
R B			36.5	33.6	3	0.0104	1.083	1.96
R T			23.5	46.9	3	0.0053	0.552	

4 : Sample Analysis at $\lambda = 280\mu$ (Run L2)

Sample	y_H	Transmission Feed	Transmission Sample	Cell	Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0105	35.1	25.5	3	0.0743	1.257	1.19
1 T	0.0057	30.5	36.2	3	0.0621	1.050	
2 B	0.0097	35.1	27.8	4	0.0653	1.105	1.16
2 T	0.0048	30.5	40.0	4	0.0562	0.952	
3 B	0.0097	34.3	28.9	3	0.0675	1.142	1.25
3 T	0.0051	31.1	41.2	3	0.0539	0.913	
4 B	0.0090	34.3	30.8	4	0.0598	1.012	0.95
4 T	0.0052	31.1	36.0	4	0.0632	1.070	
5 B	0.0092	35.1	28.2	3	0.0706	1.194	1.14
5 T	0.0058	28.2	36.2	3	0.0617	1.044	
6 B	0.0090	33.1	22.9	3	0.0810	1.372	1.39
6 T	0.0053	28.2	38.2	4	0.0579	0.981	
7 B	0.0089	33.1	28.1	3	0.0716	1.212	1.21
7 T	0.0056	30.5	39.5	3	0.0554	0.937	
8 B	0.0097	35.1	25.3	3	0.0720	1.219	1.15
8 T	0.0050	30.5	36.8	4	0.0622	1.054	
9 B	0.0090	36.2	29.2	3	0.0681	1.152	1.27
9 T	0.0055	30.2	40.6	3	0.0535	0.905	

Continued. 280 μ (Run L 2)

10 B	0.0086	36.2	29.4	4	0.0642	1.086	1.21
10 T	0.0050	30.2	41.2	4	0.0530	0.896	
11 B	0.0099	33.2	23.1	3	0.0830	1.405	1.38
11 T	0.0052	28.5	38.2	3	0.0597	1.011	
12 B	0.0098	33.2	24.9	4	0.0729	1.234	1.21
12 T	0.0047	28.5	38.2	4	0.0604	1.022	
R B	0.0104	34.2	22	3	0.0852	1.442	1.52
R T	0.0053	29.1	39.9	3	0.0558	0.944	

TABLE 16

Semi-Continuous PH-Parametric Pumping Run L 3

1 : Parameters:

$Q(\frac{\pi}{w})$ = 24 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 48 m

ϕ_T = 4 cc/m

ϕ_B = 4cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer:

PH_L = 6.2

PH_H = 8.6

Conc.= 0.035 M

Feed:

PH = 6.2

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.6	100	95.2	98.2	96.7	---	45.19	28 C
PH 6	100	97.1	99.5	96.9	0.0097	60	28 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.6	100	92	97.1	95.5	---	16.05	6.06
PH 6	100	96	102.2	106	0.0641	21.67	5.29

3 ; Sample Analysis at $\lambda = 403\ \mu$ (Run L 3)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Transmission Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4	8.4	38.1	34	3	0.0099	1.020	1.59
1 T	4	6.15	24.8	42.1	3	0.0062	0.642	
2 B	4	8.0	38.1	39	4	0.0087	0.899	1.70
2 T	4.2	6.2	24.8	47.8	4	0.0051	0.527	
3 B	4	7.8	37.2	42.2	3	0.0081	0.837	1.51
3 T	3.9	6.2	25.8	47.5	3	0.0053	0.552	
4 B	3.9	7.8	37.2	43.2	4	0.0077	0.798	1.72
4 T	4.1	6.2	25.8	52.1	4	0.0045	0.463	
5 B	3.9	7.8	36	39.2	3	0.0088	0.907	1.88
5 T	4	6.2	24.1	52.2	3	0.0046	0.481	
6 B	4	7.8	36	40.2	4	0.0084	0.869	1.89
6 T	4.1	6.2	24.1	52.3	4	0.0044	0.460	
7 B	4	7.7	36.8	43.4	3	0.0078	0.809	1.63
7 T	4	6.2	24.8	51.2	3	0.0048	0.495	
8 B	3.4	7.8	36.8	43.2	4	0.0077	0.798	1.65
8 T	2.5	6.2	24.8	50.8	4	0.0046	0.482	
9 B	3.8	7.7	37.4	43.9	3	0.0077	0.798	1.61
9 T	4	6.1	24.1	51.2	3	0.0048	0.495	

Continued. 403 μ (Run L 3)

10 B	2.6	7.9	37.4	44.8	4	0.0074	0.763	1.60
10 T	4.1	6.4	24.1	51.1	4	0.0046	0.477	
11 B	3.2	7.7	37.9	45.5	3	0.0074	0.762	1.25
11 T	4	6.2	24.2	44.1	3	0.0058	0.607	
12 B	4	7.8	37.9	44.8	4	0.0074	0.762	1.49
12 T	4	6.2	24.2	48.9	4	0.0049	0.510	
13 B	4	7.75	37.4	44.1	3	0.0077	0.793	1.66
13 T	4.1	6.25	24.1	52.5	3	0.0046	0.477	
14 B	4	7.85	37.4	42.3	4	0.0079	0.819	1.76
14 T	4	6.25	24.1	51.9	4	0.0045	0.466	
15 B	3.6	7.85	37.4	41	3	0.0084	0.865	1.90
15 T	4.1	6.2	25.1	54.1	3	0.0044	0.454	
16 B	4	7.75	37.4	40.5	4	0.0083	0.862	1.87
16 T	4	6.1	25.1	52.2	4	0.0045	0.461	
17 B	4.1	7.75	37.4	40.5	3	0.0085	0.877	1.49
17 T	4	6.1	23.9	45.3	3	0.0057	0.587	
18 B	4	7.75	37.4	40.1	4	0.0084	0.872	1.59
18 T	4	6.1	23.9	46.5	4	0.0053	0.548	

Continued. 4034 (Run L 3)

19 B	3.6	7.75	37.4	40.1	3	0.0086	0.887	1.72
19 T	3.8	6.05	25.1	49.8	3	0.0050	0.516	
20 B	3.4	7.7	37.4	39.1	4	0.0087	0.897	1.81
20 T	3.9	6.05	25.1	49.9	4	0.0048	0.495	
21 B	3.5	7.75	37.8	40.3	3	0.0085	0.882	1.77
21 T	3.8	6.05	24.1	51	3	0.0048	0.498	
22 B	3.2	7.7	37.8	43.1	4	0.0077	0.800	1.56
22 T	4.1	6.0	24.1	48.8	4	0.0049	0.512	
23 B	3.3	7.75	37.8	42	3	0.0081	0.835	1.73
23 T	4.2	6.0	26.2	52.1	3	0.0047	0.483	
24 B	3.5	7.7	37.8	40.9	4	0.0082	0.852	1.96
24 T	4.0	6.05	26.2	54.1	4	0.0042	0.435	
25 B	1.6	7.65	39.9	40	3	0.0086	0.889	1.45
25 T	4.0	6.1	25.4	43.8	3	0.0059	0.612	
26 B	1.4	7.85	39.9	40	4	0.0085	0.874	1.33
26 T	3.9	6.4	25.4	40.2	4	0.0063	0.656	
27 B	2.4	7.8	39.2	40.8	3	0.0084	0.870	1.43
27 T	3	6.15	27.6	44.1	3	0.0059	0.607	

Continued. 4034 (Run L 3)

28 B	2.5	7.85	39.2	40.2	4	0.0084	0.869	1.54
28 T	4	6.2	27.6	45.4	4	0.0055	0.565	
29 B	1.6	7.85	--	--	-	--	--	--
29 T	4	6.15	27.1	49.1	3	0.0055	0.565	
30 B	4	7.8	37.8	43.1	4	0.0077	0.800	1.64
30 T	3.9	6.25	27.1	50.5	4	0.0047	0.486	
R B		7.8	37.8	47.1	3	0.0069	0.712	1.37
R T		6.25	26.1	49.5	3	0.0050	0.521	

4 ; Sample Analysis at λ = 280 μ (Run L 3)

Sample	y_H	Transmission Feed	Transmission Sample	Cell	Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0099	34.1	21.9	3	0.0805	1.255	1.20
1 T	0.0062	25.2	33.2	3	0.0669	1.043	
2 B	0.0087	34.1	29.1	4	0.0621	0.968	0.94
2 T	0.0051	25.2	36.8	4	0.0660	1.029	
3 B	0.0081	37.8	33.1	3	0.0556	0.867	0.87
3 T	0.0053	28.2	36.1	3	0.0637	0.994	
4 B	0.0077	37.8	30.7	4	0.0609	0.950	1.04
4 T	0.0045	28.2	41.6	4	0.0584	0.911	
5 B	0.0088	35.3	26.9	3	0.0687	1.072	1.16
5 T	0.0046	25.1	39.5	3	0.0592	0.923	
6 B	0.0084	35.3	26.9	4	0.0685	1.068	1.15
6 T	0.0044	25.1	41.2	4	0.0596	0.929	
7 B	0.0078	38.1	31.8	3	0.0593	0.925	1.11
7 T	0.0048	26.2	42.1	3	0.0532	0.830	
8 B	0.0077	38.1	31.5	4	0.0591	0.922	0.99
8 T	0.0046	26.2	40.8	4	0.0595	0.928	
9 B	0.0077	35.8	32.1	3	0.0589	0.919	1.01
9 T	0.0048	25.1	39.5	3	0.0584	0.911	

Continued. 280 μ (Run L 3)

10 B	0.0074	35.8	33.5	4	0.0555	0.866	0.88
10 T	0.0046	25.1	39.2	4	0.0628	0.979	
11 B	0.0074	35.1	33.8	3	0.0548	0.855	0.77
11 T	0.0058	24.5	32.1	3	0.0713	1.112	
12 B	0.0074	35.1	34.2	4	0.0539	0.841	0.89
12 T	0.0049	24.5	39.8	4	0.0604	0.942	
13 B	0.0077	35.6	36.1	3	0.0525	0.819	0.94
13 T	0.0046	26.1	41.2	3	0.0557	0.869	
14 B	0.0079	35.6	33.8	4	0.0535	0.834	0.87
14 T	0.0045	26.1	40.1	4	0.0614	0.957	
15 B	0.0084	35.1	30.2	3	0.0614	0.957	1.13
15 T	0.0044	26.9	42.5	3	0.0540	0.843	
16 B	0.0083	35.1	31.2	4	0.0582	0.908	0.99
16 T	0.0045	26.9	41.5	4	0.0585	0.914	
17 B	0.0085	35.1	31.3	3	0.0586	0.914	0.91
17 T	0.0057	24.8	35.2	3	0.0642	1.001	
18 B	0.0084	35.1	32.8	4	0.0543	0.847	0.86
18 T	0.0053	24.8	37.9	4	0.0627	0.979	

Continued. 280 μ (Run L 3)

19 B	0.0086	35.1	31.6	3	0.0576	0.899	1.05
19 T	0.0050	25.4	40.9	3	0.0547	0.854	
20 B	0.0087	35.1	31.6	4	0.0562	0.877	0.97
20 T	0.0048	25.4	41.3	4	0.0577	0.901	
21 B	0.0085	36.1	32.8	3	0.0552	0.862	0.92
21 T	0.0048	25.1	42	3	0.0533	0.932	
22 B	0.0077	36.1	33.1	4	0.0555	0.866	0.93
22 T	0.0049	25.1	40.1	4	0.0597	0.932	
23 B	0.0081	35.8	32.2	3	0.0576	0.899	1.07
23 T	0.0047	28.8	42	3	0.0538	0.839	
24 B	0.0082	35.8	30.9	4	0.0591	0.922	1.12
24 T	0.0042	28.8	45.3	4	0.0526	0.821	
25 B	0.0086	37.1	22.9	3	0.0807	1.259	1.10
25 T	0.0059	31.2	34	3	0.0662	1.033	
26 B	0.0085	37.1	22.9	4	0.0798	1.245	0.96
26 T	0.0063	29.1	28.2	4	0.0829	1.294	
27 B	0.0084	37.8	28.3	3	0.0661	1.031	0.99
27 T	0.0059	31.2	34	3	0.0662	1.033	

Continued. 280 μ (Run L 3)

28 B	0.0084	37.8	27.8	4	0.0662	1.032	0.97
28 T	0.0055	31.2	35.2	4	0.0680	1.061	
R B	0.0069	36.2	31.5	3	0.0499	0.778	0.79
R T	0.0050	28.8	36.8	3	0.0634	0.983	

TABLE 17

Semi-Continuous PH-Parametric Pumping Run L 6

1 : Parameters:

$Q(-\frac{\pi}{w})$ = 21 cc

Q = 3.0 cc/m

$-\frac{\pi}{w}$ = 7 m

ϕ_T = 4 cc/m

ϕ_B = 4 cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer:

PH_L = 6

PH_H = 8.5

Conc. = 0.035 M

Feed:

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.5	100	99.5	98.5	102	---	45.19	26 C
PH 6	100	96.1	99.8	100.1	0.0095	60	26 C

At wavelength = 280

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.5	100	103.1	101.5	105.5	---	16.05	6.06
PH 6	100	95.5	101.5	101.9	0.0623	21.67	5.29

3 : Sample Analysis at $\lambda = 403\,\mu$ (Run L 6)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Transmission Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4.5	7.3	32.8	33.1	3	0.0104	1.103	1.69
1 T	4.9	6.15	25.8	42.5	3	0.0062	0.650	
2 B	3	7.2	32.8	40.2	4	0.0089	0.942	1.84
2 T	4.6	6.15	25.8	51.2	4	0.0048	0.511	
3 B	4.6	7.2	33.2	43.9	3	0.0081	0.853	1.83
3 T	3.2	6.2	25.8	54.2	3	0.0044	0.465	
4 B	1.9	7.3	33.2	42.1	4	0.0085	0.895	1.91
4 T	2.8	6.2	25.8	54.2	4	0.0044	0.468	
5 B	2.4	7.2	32.8	44.8	3	0.0075	0.797	1.85
5 T	3.3	6.2	25.8	56.1	3	0.0041	0.431	
6 B	2.8	7.3	32.8	43.9	4	0.0081	0.853	1.99
6 T	3.4	6.3	25.8	57.1	4	0.0040	0.427	
7 B	2.5	7.4	32.1	43.8	3	0.0078	0.820	1.76
7 T	3.7	6.3	26.1	54.2	3	0.0044	0.465	
8 B	2.5	7.3	32.1	42.2	4	0.0085	0.893	2.02
8 T	3.5	6.15	26.1	56.1	4	0.0042	0.441	
9-10 B	2.4	7.4	32.1	43.5	3	0.0078	0.826	1.89
9-10 T	6.1	6.15	26.1	56.3	c*	0.0041	0.437	

Continued. 4034c (Run L 6)

B	4.5	7.2	32.1	40.1	4	0.0089	0.944	2.07
11-12								
T	6.2	6.15	26.1	55.05	c	0.0043	0.455	
B	4.2	7.35	33.1	43	3	0.0076	0.838	1.89
13-14								
T	6	6.2	25.8	55.8	c	0.0042	0.444	
B	5.1	7.3	33.1	46	4	0.0076	0.805	1.88
15-16								
T	6.3	6.1	25.8	58	c	0.0048	0.428	
B	2.4	7.2	31.2	40.1	3	0.0086	0.909	1.98
17-18								
T	6.4	6.1	25.2	54.7	c	0.0043	0.459	
B	3.2	7	31.2	47.1	4	0.0074	0.781	1.63
19-20								
T	6.5	6.1	25.9	53.4	c	0.0045	0.478	
R B		7.2	33.8	40.8	3	0.0084	0.884	2.01
R T		6.7	25.8	56	3	0.0042	0.440	

* c = correctional transmission of sample

4 ; Sample Analysis at $\lambda = 280 A$ (Run L 6)

Sample	y_H	Transmission Feed	Transmission Sample Cell		Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0104	35.5	25.1	3	0.0726	1.165	1.21
1 T	0.0062	27.8	36	3	0.0597	0.959	
2 B	0.0089	35.5	29.5	4	0.0677	1.087	1.23
2 T	0.0048	27.8	41.1	4	0.0549	0.881	
3 B	0.0081	34.1	33.2	3	0.0586	0.941	1.11
3 T	0.0044	27.8	43	3	0.0525	0.843	
4 B	0.0085	34.1	33.2	4	0.0603	0.968	1.11
4 T	0.0044	27.8	42.2	4	0.0544	0.873	
5 B	0.0075	35.5	35.9	3	0.0546	0.876	1.09
5 T	0.0041	28.1	45.1	3	0.0498	0.800	
6 B	0.0081	35.5	35.2	4	0.0572	0.918	1.19
6 T	0.0040	28.1	46.5	4	0.0480	0.771	
7 B	0.0078	36.1	34.2	3	0.0573	0.919	1.22
7 T	0.0044	27.9	46.2	3	0.0466	0.748	
8 B	0.0085	36.1	34.2	4	0.0582	0.934	1.21
8 T	0.0042	27.9	46.2	4	0.0477	0.766	
9-10 B	0.0078	35.8	35.1	3	0.0554	0.889	1.03
T	0.0041	27.9	42.5	c*	0.0534	0.858	

Continued, 280μ (Run L 6)

11-12	B	0.0089	35.8	32.1	4	0.0617	0.990	1.10
	T	0.0043	27.2	40.8	c	0.0560	0.899	
13-14	B	0.0076	35.2	35	3	0.0562	0.901	1.11
	T	0.0042	29	44	c	0.0502	0.806	
15-16	B	0.0076	35.2	35	4	0.0589	0.946	1.06
	T	0.0048	27.8	41.8	c	0.0552	0.887	
17-18	B	0.0086	32.1	31	3	0.0622	0.998	1.10
	T	0.0043	27.8	40.7	c	0.0562	0.902	
19-20	B	0.0074	32.1	35.2	4	0.0590	0.948	0.97
	T	0.0045	27.8	38.2	c	0.0606	0.973	
R	B	0.0084	35.8	33.2	3	0.0578	0.928	1.09
R	T	0.0042	27.1	43.2	3	0.0529	0.850	

c* = correctional transmission of sample

TABLE 18

Semi-Continuous PH-Parametric Pumping Run L 7

1 : Parameters:

$Q(\frac{\pi}{w})$ = 40 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 40 m

ϕ_T = 4 cc/m

ϕ_B = 4 cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer:

PH_L = 6

PH_H = 8.9

Conc. = 0.035 M

Feed:

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.9	100	97.1	100.1	99	0.0104	45.19	22 C
PH 6	100	101.9	103.5	103.8	0.0107	60	22 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.9	100	95.8	99.8	97.1	0.0485	16.05	6.06
PH 6	100	103	105.2	106.2	---	21.67	5.29

3 : Sample Analysis at $\lambda = 4034$ (Run L 7)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Transmission Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4	8.3	32.8	35.2	3	0.0101	0.943	1.71
1 T	4	6.1	23.2	45.5	3	0.0059	0.551	
2 B	4	8.3	32.2	33.1	3	0.0107	1.002	2.04
2 T	3.9	6.05	23.1	50.2	3	0.0052	0.489	
3 B	3.9	8.3	32.8	34.2	4	0.0102	0.954	1.60
3 T	4	6.1	23.2	43.2	4	0.0063	0.593	
4 B	4	8.15	34.1	39.5	3	0.0090	0.841	1.62
4 T	4	6.05	22.8	48	3	0.0055	0.519	
5 B	4	8.1	32.5	38.9	3	0.0091	0.855	1.60
5 T	3.9	6.1	23.5	47.1	3	0.0056	0.532	
6 B	4	8.2	32.2	38.1	4	0.0092	0.860	1.63
6 T	3.8	6.2	23.1	48	4	0.0056	0.523	
7 B	4	8.15	32.5	40	4	0.0087	0.814	1.58
7 T	4	6.25	23.5	48.2	4	0.0055	0.514	
8 B	4	8.1	34.1	38	3	0.0093	0.869	1.65
8 T	4	6.25	22.8	47.5	4	0.0056	0.523	
9 B	4	8.2	32	38.9	3	0.0091	0.855	1.65
9 T	3.9	6.25	22.8	47.8	3	0.0055	0.514	

Continued. 4034 (Run L 7)

10 B	4	8.1	32.2	38.2	3	0.0093	0.871	1.77
10 T	4.1	6.2	23.1	50	3	0.0052	0.492	
11 B	3.9	8.1	32	39.2	4	0.0089	0.832	1.65
11 T	4	6.2	22.8	49.2	4	0.0054	0.505	
12 B	4	8.1	34.1	38.8	4	0.0090	0.841	1.77
12 T	3.9	6.2	22.4	51.2	3	0.0051	0.476	
13 B	4	8.1	32.1	36.1	3	0.0098	0.922	1.86
13 T	3.9	6.2	23.2	49.8	3	0.0053	0.494	
14 B	3.8	8.1	32.2	34.8	4	0.0100	0.939	1.72
14 T	4	6.2	23.1	46.2	4	0.0058	0.547	
15 B	3.7	8.15	32.1	35	4	0.0099	0.933	1.57
15 T	4	6.25	23.2	43.2	4	0.0063	0.593	
16 B	4	8.15	34.1	37.5	3	0.0095	0.888	1.61
16 T	3.9	6.3	22.4	45.9	4	0.0059	0.552	
17 B	3.6	8.3	32.1	38.1	3	0.0093	0.874	1.62
17 T	4.8	6.35	22.8	46.8	3	0.0057	0.537	
18 B	3.8	8.25	32.1	36.5	3	0.0097	0.912	1.67
18 T	3.4	6.4	23.1	46.2	3	0.0058	0.545	

Continued. 403 A (Run L 7)

19 B	3.7	8	32.1	36.2	4	0.0096	0.903	1.82
19 T	3.3	6.2	22.8	49.8	4	0.0053	0.497	
20 B	3.7	8.3	34.1	33.1	4	0.0105	0.984	2.05
20 T	3.7	6.2	21.9	51.2	3	0.0051	0.476	
21 B	4	8.4	32.1	36.1	3	0.0098	0.922	2.11
21 T	4	6.2	23.1	54.2	3	0.0046	0.437	
22 B	4.2	8.4	34.1	40.1	4	0.0087	0.813	1.90
22 T	3.2	6.2	21.9	55.2	4	0.0046	0.427	
23 B	3.6	8.3	32.1	38.9	4	0.0089	0.839	2.02
23 T	3.1	6.2	23.1	56.2	4	0.0044	0.415	
R B			32.1	40.5	4	0.0085	0.802	1.77
R T			23.1	53.2	4	0.0048	0.452	

4 : Sample Analysis at $\lambda = 280\mu$ (Run L 7)

Sample	y_H	Transmission Feed	Transmission Sample	Cell	Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0101	32.8	28.9	3	0.0620	1.278	1.04
1 T	0.0059	26.8	38	3	0.0594	1.226	
2 B	0.0107	31.2	28.2	3	0.0622	1.282	0.97
2 T	0.0052	25.5	37.2	3	0.0640	1.319	
3 B	0.0102	32.8	27.1	4	0.0644	1.327	0.80
3 T	0.0063	26.8	29.2	4	0.0802	1.654	
4 B	0.0090	36.1	33	3	0.0554	1.143	1.00
4 T	0.0055	26.8	40.8	3	0.0552	1.139	
5 B	0.0091	32.8	31.8	3	0.0578	1.193	1.12
5 T	0.0056	26.8	42.5	3	0.0515	1.062	
6 B	0.0092	31.2	30.8	4	0.0579	1.194	1.06
6 T	0.0056	25.5	41.5	4	0.0542	1.118	
7 B	0.0087	32.8	33.8	4	0.0525	1.084	1.08
7 T	0.0055	26.8	44.8	4	0.0483	0.997	
8 B	0.0093	37	35.2	3	0.0500	1.032	0.92
8 T	0.0056	26.8	41.5	4	0.0542	1.118	
9 B	0.0091	32.8	31.2	3	0.0592	1.221	1.15
9 T	0.0055	25.2	42.8	3	0.0513	1.058	

Continued. 2804 (Run L 7)

10 B	0.0093	31.5	31	3	0.0591	1.213	1.22
10 T	0.0052	26.2	45.1	3	0.0482	0.993	
11 B	0.0089	32.8	32.1	4	0.0557	1.149	1.06
11 T	0.0054	25.2	43	4	0.0521	1.075	
12 B	0.0090	36.1	34.1	4	0.0511	1.053	1.09
12 T	0.0051	25.8	46.1	3	0.0468	0.966	
13 B	0.0098	32.1	29.8	3	0.0606	1.250	1.26
13 T	0.0053	25.8	45	3	0.0480	0.990	
14 B	0.0100	31.5	28.2	4	0.0621	1.281	1.08
14 T	0.0058	26.2	39.4	4	0.0576	1.189	
15 B	0.0099	32.1	30	4	0.0579	1.194	0.94
15 T	0.0063	25.8	36.8	4	0.0612	1.262	
16 B	0.0095	35.8	30	3	0.0609	1.257	1.06
16 T	0.0059	25.8	39.5	4	0.0570	1.176	
17 B	0.0093	32.3	31.1	3	0.0589	1.215	1.07
17 T	0.0057	24.2	40.5	3	0.0550	1.135	
18 B	0.0097	31	27.5	3	0.666	1.375	1.22
18 T	0.0058	25.8	40.5	3	0.546	1.126	

Continued. 280 μ (run L 7)

19 B	0.0096	32.3	32.8	4	0.0523	1.079	0.96
19 T	0.0053	24.2	42	4	0.0544	1.123	
20 B	0.0105	37	32	4	0.0517	1.067	1.02
20 T	0.0051	24.2	43.9	3	0.0508	1.049	
21 B	0.0098	32.1	29.5	3	0.0594	1.224	1.46
21 T	0.0046	25.2	51	3	0.0406	0.837	
22 B	0.0087	35.8	33.9	4	0.0523	1.079	1.08
22 T	0.0046	24.2	47.1	4	0.0479	0.998	
23 B	0.0089	32.1	31.2	4	0.0577	1.191	1.19
23 T	0.0044	25.2	47.2	4	0.0485	1.001	
R B	0.0085	31	30.8	4	0.0597	1.232	1.02
R T	0.0048	25.8	41.2	4	0.0581	1.198	

TABLE 19

Semi-Continuous PH-Parametric Pumping Run L 8

1 : Parameters:

$Q(\frac{\pi}{w})$ = 24cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 48 m

ϕ_T = 4 cc/m

ϕ_B = 4 cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer:

PH_L = 6

PH_H = 8.9

Conc. = 0.035 M

Feed:

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.9	100	101.9	101.2	103.8	---	45.19	22 C
PH 6	100	95.5	97.1	97.9	0.0097	60	22 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.9	100	100	99.5	100.9	---	16.05	6.06
PH 6	100	92.1	97.1	98.8	0.0554	21.67	5.29

3 : Sample Analysis at $\lambda = 403\mu$ (Run L 8)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Transmission Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4	8	37.1	31.1	3	0.0113	1.165	1.81
1 T	4.2	6	24.9	41.1	3	0.0062	0.641	
2 B	4	8.2	37.1	33.8	4	0.0106	1.093	1.82
2 T	3.8	6	24.9	43.9	4	0.0058	0.598	
3 B	4	8.05	36.1	35.8	3	0.0099	1.021	1.73
3 T	4	6	24.8	44	3	0.0057	0.599	
4 B	4	8.1	36.1	36.5	4	0.0100	1.035	2.09
4 T	4	6	24.8	50.5	4	0.0048	0.493	
5 B	4	8	36.2	37.8	3	0.0094	0.969	2.04
5 T	4	6.1	24.2	51	3	0.0046	0.474	
6 B	4	8	36.2	37.2	4	0.0098	1.016	2.15
6 T	4	6.1	24.2	52	4	0.0045	0.472	
7 B	3.8	8.05	36.2	36	3	0.0099	1.021	2.19
7 T	4	6.1	24.5	52.1	3	0.0045	0.464	
8 B	4	8	36.2	37	4	0.0099	1.022	2.22
8 T	4	6.1	24.5	52.8	4	0.0044	0.460	
9 B	4	8	37.2	35.5	3	0.0100	1.031	2.20
9 T	4	6	24.8	51.8	3	0.0045	0.468	

Continued. 403 A (Run L 8)

10 B	4	7.95	37.2	34.8	4	0.0105	1.082	2.06
10 T	4	6	24.8	48.5	4	0.0051	0.524	
11 B	4	8	37.2	35.2	3	0.0101	1.041	1.89
11 T	4	6	25	46.5	3	0.0053	0.549	
12 B	4	8	37.2	35.5	4	0.0103	1.063	1.94
12 T	4.1	6	25	47	4	0.0053	0.547	
13 B	4	8.1	37.2	36.2	3	0.0099	1.020	1.88
13 T	3.9	6.1	24.8	47	3	0.0052	0.541	
14 B	4	8.1	37.2	35.2	4	0.0104	1.071	2.04
14 T	4	6.05	24.8	48.5	4	0.0050	0.524	
15 B	3.9	8.05	37.2	33.8	3	0.0105	1.082	2.11
15 T	4	6.05	24.8	48.8	3	0.0049	0.513	
R B			37.2	36.2	4	0.0101	1.043	1.96
R T			24.8	48	4	0.0051	0.531	

4 : Sample Analysis at $\lambda = 280\,\mu$ (Run L 8)

Sample	y_H	Transmission Feed	Transmission Sample	Cell	Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0113	33.5	21.1	3	0.0809	1.460	1.14
1 T	0.0062	28.5	30.2	3	0.0705	1.273	
2 B	0.0106	33.5	27	4	0.0654	1.180	1.03
2 T	0.0058	28.5	34.1	4	0.0696	1.257	
3 B	0.0099	33.2	27.9	3	0.0659	1.189	1.23
3 T	0.0057	28.5	38.1	3	0.0534	0.965	
4 B	0.0100	33.2	28	4	0.0641	1.157	1.22
4 T	0.0048	28.5	41	4	0.0525	0.947	
5 B	0.0094	32.5	33	3	0.0542	0.978	1.13
5 T	0.0046	28.2	43.2	3	0.0476	0.860	
6 B	0.0098	32.5	33.2	4	0.0537	0.969	1.16
6 T	0.0045	28.2	45	4	0.0690	1.247	
7 B	0.0099	32	28.8	3	0.0626	1.129	1.30
7 T	0.0045	28.8	45.8	3	0.0474	0.856	
8 B	0.0099	32	27.2	4	0.0667	1.204	1.52
8 T	0.0044	28.8	46.5	4	0.0438	0.790	
9 B	0.0100	34.8	30.2	3	0.0599	1.081	1.29
9 T	0.0045	28.1	44.2	3	0.0462	0.834	

Continued. 280 μ (Run L 8)

10 B	0.0105	34.8	29.1	4	0.0600	1.083	1.13
10 T	0.0051	28.1	40.2	4	0.0529	0.956	
11 B	0.0101	34.2	29.1	3	0.0613	1.106	1.22
11 T	0.0053	28.5	40.5	3	0.0501	0.904	
12 B	0.0103	34.2	30.5	4	0.0584	1.054	1.14
12 T	0.0053	28.5	40.8	4	0.0699	1.262	
13 B	0.0099	34.2	28	3	0,0646	1.166	1.25
13 T	0.0052	27.8	40	3	0.0515	0.930	
14 B	0.0104	34.2	30.1	4	0.0594	1.072	1.15
14 T	0.0050	27.8	41.1	4	0.0704	1.271	
15 B	0.0105	33.2	24.2	3	0.734	1.326	1.43
15 T	0.0049	27.2	40.8	3	0.0604	0.923	
R B	0.0101	33.2	26.2	4	0.0688	1.243	1.26
R T	0.0051	27.2	39.5	4	0.0604	0.982	

TABLE 20

Semi-Continuous PH-Parametric Pumping Run L 9

1 : Parameters:

$Q(\frac{\pi}{w})$ = 18 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 36 m

ϕ_T = 4 cc/m

ϕ_B = 4 cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer:

PH_L = 6

PH_H = 8.9

Conc. = 0.035 M

Feed:

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.9	100	96.1	99.8	99.9	---	45.19	21 C
PH 6	100	94.9	96	96.3	0.0099	60	21 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.9	100	92.5	100.8	98.7	---	16.05	6.06
PH 6	100	92.4	95.8	94.2	0.0652	21.67	5.29

3 : Sample Analysis at $\lambda = 4034$ (Run L 9)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Transmission Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4	8.1	34	31.1	3	0.0112	1.132	1.49
1 T	4	6.15	24.1	34.2	3	0.0074	0.754	
2 B	4	8	34	36.2	4	0.0097	0.985	1.40
2 T	4	6.15	24.1	39.8	4	0.0069	0.703	
3 B	4	7.9	34.1	37.1	3	0.0095	0.960	1.54
3 T	3.8	6.15	24	40.9	3	0.0062	0.624	
4 B	4	7.85	34.1	39.7	4	0.0088	0.896	1.66
4 T	4	6.15	24	46.2	4	0.0053	0.537	
5 B	4	7.9	34.6	38.8	3	0.0091	0.917	1.69
5 T	3.7	6.15	24.4	45.8	3	0.0053	0.541	
6 B	4	8	34.6	38.5	4	0.0091	0.925	1.81
6 T	3.9	6.15	24.4	47.9	4	0.0050	0.510	
7 B	4	8	34.6	39.8	3	0.0088	0.892	1.60
7 T	4.5	6.15	23.7	44.8	3	0.0055	0.557	
8 B	4	7.7	34.6	40.1	4	0.0087	0.886	1.74
8 T	3.7	6	23.7	48.1	4	0.0050	0.507	
9 B	4	7.7	34.8	40.8	3	0.0086	0.868	1.72
9 T	4	6.15	24.4	48.2	3	0.0049	0.503	

Continued. 403A (Run L 9)

10 B	4	7.8	34.8	41.8	4	0.0084	0.846	1.69
10 T	4	6.15	24.4	48.5	4	0.0049	0.501	
11 B	4	7.8	34.8	42	3	0.0083	0.840	1.75
11 T	4	6	23.8	49.8	3	0.0047	0.479	
12 B	4	7.8	34.8	40.8	4	0.0086	0.869	1.81
12 T	4	6.15	23.8	50	4	0.0047	0.479	

4 : Sample Analysis at λ = 280 μ(Run L 9)

Sample	y_H	Transmission Feed	Transmission Sample Cell		Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0112	32	20.5	3	0.0844	1.295	0.964
1 T	0.0074	25.8	22.8	3	0.0876	1.343	
2 B	0.0097	32	25.3	4	0.0718	1.102	1.094
2 T	0.0069	25.8	30	4	0.0657	1.007	
3 B	0.0095	32.5	26.5	3	0.0705	1.082	1.091
3 T	0.0062	25.8	32	3	0.0646	0.992	
4 B	0.0088	32.5	29.5	4	0.0632	0.969	1.145
4 T	0.0053	25.8	36.9	4	0.0552	0.847	
5 B	0.0091	34.1	28.9	3	0.0654	1.003	1.156
5 T	0.0053	25.3	36.9	3	0.0566	0.868	
6 B	0.0091	34.1	29.3	4	0.0629	0.965	1.123
6 T	0.0050	25.3	37.1	4	0.0560	0.859	
7 B	0.0088	34.1	30	3	0.0635	0.974	1.200
7 T	0.0055	25.8	38.2	3	0.0529	0.812	
8 B	0.0087	34.1	31.2	4	0.0594	0.912	1.15
8 T	0.0050	25.8	39.1	4	0.0517	0.793	
9 B	0.0086	34.1	29.2	3	0.0660	1.012	1.177
9 T	0.0049	25.2	37.9	3	0.0561	0.860	

Continued. 280 μ (Run L 9)

10 B	0.0084	34.1	30.2	4	0.0626	0.960	1.154
10 T	0.0049	25.2	38.1	4	0.0543	0.832	
11 B	0.0083	33.5	31.5	3	0.0614	0.941	1.160
11 T	0.0047	25.3	39.8	3	0.0529	0.811	
12 B	0.0086	33.5	30.2	4	0.0621	0.952	1.20
12 T	0.0047	25.3	39.8	4	0.0515	0.790	

TABLE 21

Semi-Continuous PH-Parametric Pumping Run L 10

1 : Parameters:

$Q(\frac{\pi}{w})$ = 20 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 40 m

ϕ_T = 6.5 cc/m

ϕ_B = 1.5 cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer:

PH_L = 6

PH_H = 8.9

Conc. = 0.2 M

Feed:

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 ; Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.9	100	97.2	101.1	99.2	---	45.19	21 C
PH 6	100	98.2	99.8	93.2	0.0091	60	21 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.9	100	97.1	102	100.8	---	16.05	6.06
PH 6	100	99.8	102.2	96.6	0.0525	21.67	5.29

3: Sample Analysis at $\lambda = 403\mu$ (Run L 10)

Sample		Volume cc	PH	Transmission Feed	Transmission Sample	Transmission Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1-2	B	3.2	8.05	30.5	30.7	3	0.0114	1.258	1.94
	T	13	5.9	27.8	44.2	3	0.0059	0.648	
3-4	B	3.1	8.05	30.5	31.1	4	0.0111	1.225	1.87
	T	13	6.0	27.8	41.0	4	0.0059	0.653	
5-6	B	3	7.0	30.5	33.5	3	0.0106	1.166	1.48
	T	13	6.0	27.6	37	3	0.0072	0.789	
7-8	B	3	8.0	30.5	34.8	4	0.0100	1.106	1.59
	T	13	6.0	27.6	38.8	4	0.0063	0.697	
9-10	B	3	8.0	30.5	35.1	3	0.0101	1.117	1.54
	T	12.9	6.0	27.5	40	3	0.0066	0.727	
11-12	B	3	8.05	30.5	34.1	4	0.0102	1.128	1.63
	T	13	6.0	27.5	39	4	0.0063	0.693	
13-14	B	3	8.0	30.2	34.4	3	0.0104	1.138	1.52
	T	13	6.0	27.8	39	3	0.0068	0.747	
15-16	B	3	8.0	30.2	34.2	4	0.0102	1.124	1.58
	T	13	6.0	27.8	38	4	0.0065	0.713	
R	B		8.0	30.2	33.2	3	0.0105	1.156	1.38
R	T		6.0	27.8	34.8	3	0.0076	0.838	

4 : Sample Analysis at $\lambda = 280\,\mu$ (Run L 10)

Sample	V_1 cc	V_2 cc	Transmission Feed	Transmission Sample Cell		Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
B	1.8	1.4	32.1	25	3	0.0706	1.344	1.08
1-2 T	6.4	6.6	33.8	34.5	3	0.0650	1.238	
B	1.5	1.6	32.1	24.7	4	0.0714	1.359	1.23
3-4 T	6.5	6.5	33.8	35.5	4	0.0580	1.108	
B	1.5	1.5	30.3	27.8	3	0.0650	1.239	1.10
5-6 T	6.5	6.5	34.2	34.8	3	0.0590	1.123	
B	1.5	1.5	30.3	29	4	0.0628	1.196	1.10
7-8 T	6.5	6.5	34.2	35.2	4	0.0571	1.087	
B	1.5	1.5	33.5	30.7	3	0.0592	1.129	0.88
9-10 T	6.4	6.5	32	32.5	3	0.0670	1.277	
B	1.5	1.5	33.5	25	4	0.0729	1.388	1.06
11-12 T	6.5	6.5	32	32	4	0.0684	1.303	
B	1.5	1.5	31	28.5	3	0.0638	1.215	1.02
13-14 T	6.5	6.5	34.1	35.7	3	0.0623	1.186	
B	1.5	1.5	31	28	4	0.0648	1.234	1.08
15-16 T	6.5	6.5	34.1	33.7	4	0.0598	1.140	
R B			30.8	27.8	3	0.0653	1.244	0.94
R T			32.8	30	3	0.0695	1.324	

TABLE 22

Semi=Continuous PH-Parametric Pumping Run L 11

1 ; Parameters;

$Q(\frac{\pi}{w})$ = 20 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 40 m

ϕ_T = 1.5 cc/m

ϕ_B = 6.5 cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer;

PH_L = 6

PH_H = 8.9

Conc. = 0.2 M

Feed;

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 ; Correction Factor of Cell and Average Concentration of Feed;

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.9	100	111.5	107.8	109.5	---	45.19	24 C
PH 6	100	97.8	98.9	94.8	0.009	60	24 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.9	100	111	106.8	109	---	16.05	6.06
PH 6	100	96	97.8	93.7	0.0488	21.67	5.29

3 : Sample Analysis at λ = 4034 (Run L 11)

Sample		Volume cc	PH	Transmission Feed	Transmission Sample	Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1	B	6.5	8.15	35.8	35.5	3	0.0097	1.078	
2	B	6.5	7.6	35.8	35.9	4	0.0098	1.096	
1-2	B							1.087	1.239
	T	4.5	5.9	28	33.2	3	0.0079	0.877	
3	B	6.5	7.5	36	39.2	3	0.0087	0.973	
4	B	6.5	7.4	36	36.2	4	0.0097	1.087	
3-4	B							1.030	1.625
	T	3	6.0	28	43.1	4	0.0057	0.634	
5	B	5.9	7.4	40.2	40.2	3	0.0094	1.053	
6	B	6	7.4	40.2	39.2	4	0.0098	1.097	
5-6	B							1.075	1.859
	T	3	6.0	28	48.2	3	0.0052	0.578	
7	B	5.9	7.4	41.2	38.1	3	0.0099	1.110	
8	B	5.6	7.3	41.2	38.2	4	0.0101	1.124	
7-8	B							1.117	2.119
	T	3	6.0	28	49.2	4	0.0047	0.527	
9	B	5	7.6	40.8	38.1	3	0.0099	1.111	
10	B	5.6	7.6	40.8	37.1	4	0.0104	1.156	

Continued. 403μ (Run L11)

9-10	B							1.133	2.010
	T	3	6.2	28	49	3	0.0051	0.564	
11	B	5.4	7.4	35.2	36.2	3	0.0105	1.165	
12	B	5.2	7.4	35.2	32.8	4	0.0116	1.287	
11-12	B							1.226	2.125
	T	3	6.1	28	45.8	4	0.0052	0.577	
13	B	5	7.5	36	33.7	3	0.0102	1.134	
14	B	4.6	7.3	36	33.8	4	0.0104	1.160	
13-14	B							1.147	1.912
	T	3	6.2	27.6	46.9	3	0.0054	0.600	
15	B	5	7.3	36	28.2	3	0.0119	1.324	
16	B	5	7.3	36	33.8	4	0.0104	1.160	
15-16	B							1.242	2.019
	T	3	6.2	27.6	44.1	4	0.0055	0.615	
R	B			35.8	33.2	3	0.0103	1.150	1.767
R	T			27.5	44	3	0.0058	0.651	

4 : Sample Analysis at $\lambda = 280\mu$ (Run L 11)

Sample		V_1 cc	V_2 cc	Transimission Feed	Transimission Sample Cell		Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1	B			32.5	29.1	3	0.0622	1.274	
2	B			32.5	29.7	4	0.0678	1.390	
1-2	B							1.332	1.059
	T	1.5	3	33.8	31.2	3	0.0614	1.258	
3	B			33.5	33.9	3	0.0539	1.104	
4	B			33.5	34.8	4	0.0568	1.162	
3-4	B							1.133	0.916
	T	1.4	1.6	33.8	33.8	4	0.0604	1.237	
5	B			39.9	41	3	0.437	0.896	
6	B			39.9	38.8	4	0.481	0.985	
5-6	B							0.940	0.761
	T	1.4	1.6	33.8	36.2	3	0.0603	1.236	
7	B			38.5	37.8	3	0.0606	1.243	
8	B			38.5	37.1	4	0.0504	1.033	
7-8	B							1.169	0.973
	T	1.5	1.5	38.5	37	3	0.0507	1.039	
9	B			39	36.5	3	0.0507	1.039	
10	B			39	35.8	4	0.0522	1.070	

Continued. 280M (Run L 11)

9-10	B							1.055	0.697
	T	1.5	1.5	33.8	31.2	3	0.0738	1.513	
11	B			34.2	35.2	3	0.0517	1.060	
12	B			33.8	32.2	4	0.0566	1.161	
11-12	B							1.110	0.714
	T	1.7	1.3	33.8	28.7	4	0.0759	1.555	
13	B			32.8	30.8	3	0.0568	1.164	
14	B			32.8	31.8	4	0.0614	1.258	
13-14	B							1.211	0.782
	T	1.5	1.5	33.8	29.8	3	0.0754	1.546	
15	B			34	29.2	3	0.0561	1.150	
16	B			34	33	4	0.0587	1.203	
15-16	B							1.176	0.784
	T	1.5	1.5	33.8	29.2	4	0.0732	1.500	
R	B			32.5	31.5	3	0.0549	1.125	0.878
R	T			33	34.2	3	0.625	1.281	

TABLE 23

Semi-Continuous PH-Parametric Pumping Run L 12

1 : Parameters:

$Q(\frac{\pi}{w})$ = 32 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 64 m

ϕ_T = 4 cc/m

ϕ_B = 4 cc/m

H_T = 8 cm

H_B = 8 cm

Start = Upflow first

Buffer:

PH_L = 4.9

PH_H = 8.9

Conc. = 0.15 M

Feed:

PH = 6

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.9	100	99.8	98.2	90	---	45.19	24 C
PH 6	100	102.2	98.8	94.5	0.01	60	24 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.9	100	99	97.2	94.2	---	16.05	6.06
PH 6	100	99.8	99	96.2	0.0546	21.67	5.29

3 : Sample Analysis at $\lambda = 403\mu$ (Run L 12)

Sample	Volume cc	PH	Tranamission Feed	Sample	Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4	8.2	34.2	34.8	3	0.0101	1.012	1.364
1 T	4	6	25.8	35.4	3	0.0074	0.742	
2 B	4	8.1	34.2	32.8	4	0.0097	0.970	1.655
2 T	4	5.6	25.8	42	4	0.0058	0.586	
3 B	4	8	34.2	37.2	3	0.0093	0.932	1.541
3 T	4	5.3	25.2	42.8	3	0.0060	0.605	
4 B	4	7.9	34.2	35.2	4	0.0090	0.902	1.841
4 T	4	5.2	25.2	48	4	0.0049	0.490	
5 B	4	7.9	33.8	39.4	3	0.0087	0.877	1.681
5 T	4	5.1	25.8	48	3	0.0052	0.522	
6 B	4	8	33.8	40.2	4	0.0077	0.774	1.683
6 T	4	5.05	25.8	50	4	0.0046	0.460	
7 B	4	7.95	33.8	39.8	3	0.0086	0.867	1.653
7 T	4	5.1	25.8	47.8	3	0.0052	0.525	
8 B	4	7.8	33.8	39.8	4	0.0078	0.784	1.590
8 T	4	5.1	25.8	47.9	4	0.0049	0.493	
9 B	4	7.7	33.8	39.8	3	0.0086	0.867	1.616
9 T	4	5.1	25.2	47	3	0.0053	0.537	

Continued. 403μ (Run L 12)

10 B	4	7.7	33.8	39.8	4	0.0078	0.784	1.552
10 T	4	5.05	25.2	47	4	0.0050	0.505	
11 B	4	7.7	32.2	39.8	3	0.0086	0.867	1.589
11 T	4.4	5.05	25	46.5	3	0.0054	0.545	
12 B	4	7.8	32.2	39.8	4	0.0080	0.803	1.451
12 T	4	5.2	25	44	4	0.0055	0.553	
R B		7.7	32.2	39.8	3	0.0078	0.784	1.459
R T		5.05	25	47	3	0.0053	0.537	

4 : Sample analysis at $\lambda = 280\ \mu$ (Run L 12)

Sample	y_H	Transmission Feed	Transmission Sample Cell		Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0101	34.2	28.1	3	0.0621	1.138	1.002
1 T	0.0074	31.8	32.2	3	0.0619	1.136	
2 B	0.0097	34.2	29.9	4	0.0565	1.035	0.954
2 T	0.0058	31.8	35	4	0.0592	1.085	
3 B	0.0093	34.2	31.2	3	0.0568	1.040	0.964
3 T	0.0060	30.8	35.8	3	0.0589	1.079	
4 B	0.0090	34.2	25.2	4	0.0706	1.294	1.247
4 T	0.0049	30.8	37.8	4	0.0566	1.037	
5 B	0.0087	34.2	31	3	0.0588	1.077	1.034
5 T	0.0052	30.8	38.2	3	0.0569	1.042	
6 B	0.0077	34.2	33.8	4	0.0552	1.011	1.045
6 T	0.0046	30.8	40.2	4	0.0528	0.967	
7 B	0.0086	34.8	33.2	3	0.0542	0.992	0.911
7 T	0.0052	30.8	37	3	0.0595	1.090	
8 B	0.0078	34.8	34	4	0.0523	0.959	0.935
8 T	0.0049	30.8	38.1	4	0.0560	1.025	
9 B	0.0086	34.2	34	3	0.0525	0.961	0.862
9 T	0.0053	30	36,2	3	0.0609	1.115	

Continued. 280μ (Run L 12)

10 B	0.0078	34.2	33.8	4	0.0527	0.966	0.910
10 T	0.0050	30	37	4	0.0579	1.062	
11 B	0.0086	32.2	30.8	3	0.0595	1.091	0.813
11 T	0.0054	30.2	31	3	0.0732	1.341	
12 B	0.0080	32.2	31	4	0.0584	1.070	0.794
12 T	0.0055	30.2	29.2	4	0.0736	1.347	
R B	0.0078	31	31	3	0.0612	1.121	0.855
R T	0.0053	30.2	31.2	3	0.0716	1.311	

TABLE 24

Semi-Continuous PH-Parametric Pumping Run L 13

1 : Parameters:

$Q(\frac{\pi}{w})$ =20 cc

Q = 0.5 cc/m

$\frac{\pi}{w}$ = 30 m

ϕ_T = 4 cc/m

ϕ_B = 4 cc/m

H_T = 9 cm

H_B = 14 cm

Start = Upflow first

Buffer:

PH_L = 4.9

PH_H =8.9

Conc. = 0.2 M

Feed:

PH = 4.9

Haemoglobin = 0.01 %

Albumin = 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 403 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_H \rangle_F$	β_H	Temp.
PH 8.9	100	98	96	97	0.0091	45.19	24 C
PH 6	100	96	99	98.5	---	60	24 C

At wavelength = 280 μ

Buffer	Cell 1st	2nd	3rd	4th	$\langle y_A \rangle_F$	β_H	β_A
PH 8.9	100	96	99	98.5	0.0546	16.05	6.06
PH 6	100	96	99	98.5	0.0626	21.67	5.29

3 : Sample Analysis at $\lambda = 403\mu$ (Run L 13)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Cell	Haemoglobin conc. y_H	$\langle y_H \rangle / \langle y_H \rangle_F$	$\langle y_H \rangle_B / \langle y_H \rangle_T$
1 B	4	8.9	39.5	17.5	3	0.0163	1.79	
1 T	4.1	--	--	--	-	--	--	
2 B	4	8.7	39.5	21	4	0.0147	1.61	3.117
2 T	4.1	5.0	42	51	4	0.0047	0.516	
3 B	4	8.7	39.2	25.9	3	0.0125	1.383	5.720
3 T	4	4.8	42	73.4	3	0.0022	0.242	
4 B	4	8.5	39.2	26	4	0.0126	1.389	11.49
4 T	4	4.9	42	84	4	0.0011	0.122	
5 B	4	8.3	38	25.2	3	0.0128	1.41	8.019
5 T	4	4.8	41.7	79	3	0.0016	0.176	
6 B	3.9	8.4	38	26.3	4	0.0125	1.377	6.961
6 T	4.1	4.8	41.7	75.8	4	0.0018	0.198	
7 B	4	8.4	38	18	3	0.0161	1.76	20.02
7 T	4	4.8	41.5	88	3	0.0008	0.088	
8 B	4	8.3	38	18.5	4	0.0159	1.749	12.24
8 T	4	4.8	41.5	82.2	4	0.0013	0.143	
9 B	4	8.5	38.2	23.5	3	0.0135	1.485	7.949
9 T	3.9	4.8	41	78	3	0.0017	0.187	

Continued. 403 μ (Run L 13)

10 B	4	8.9	38.2	22.9	4	0.0139	1.524	7.704
10 T	4.1	4.9	41	75.8	4	0.0018	0.198	
11 B	4	8.9	37.5	24	3	0.0133	1.463	8.875
11 T	4	4.9	41.2	80.2	3	0.0015	0.165	
12 B	4	8.8	37.5	21.5	4	0.0138	1.524	10.66
12 T	4	4.9	41.2	81.3	4	0.0013	0.143	
13 B	4	8.8	37.8	24	3	0.0133	1.460	13.28
13 T	4	4.9	42	85.5	3	0.0010	0.110	
14 B	4	8.7	37.8	25	4	0.0130	1.43	8.133
14 T	4	4.8	42	81.2	4	0.0013	0.176	
15 B	3.9	8.5	37.6	23.4	3	0.0135	1.49	13.56
15 T	4	4.9	42.1	85.7	3	0.0010	0.110	
16 B	3.9	8.5	37.6	24.8	4	0.0131	1.44	11.91
16 T	4.1	4.9	42.1	83.5	4	0.0011	0.121	
17 B	4	8.6	37	22	3	0.0141	1.55	10.85
17 T	4	4.9	42.5	82	3	0.0013	0.143	
18 B	4	8.6	37	21	4	0.0147	1.61	13.33
18 T	4	4.9	42.5	81	4	0.0011	0.121	

Continued. 403 μ (Run L 13)

19 B	4	8.5	37	23.0	3	0.0134	1.48	9.62
19 T	4	4.9	41.5	81.5	3	0.0014	0.154	
20 B	4	8.6	37	21.5	4	0.0144	1.59	7.61
20 T	4	4.9	41.5	74.8	4	0.0019	0.209	
R B			37	25	3	0.0130	1.43	7.65
R T			42	77.5	3	0.0017	0.187	

4 ; Sample Analysis at $\lambda = 280\mu$ (Run L 13)

Sample	y_H	Transmission Feed	Transmission Sample	Cell	Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	0.0163	33.4	11.3	3	0.112	2.05	--
1 T	---	32.8	--	-	--	--	
2 B	0.0147	33.4	12.2	4	0.1107	2.02	1.10
2 T	0.0047	32.8	23	4	0.0998	1.828	
3 B	0.0125	34	19.5	3	0.0831	1.52	1.68
3 T	0.0022	33	48.6	3	0.0494	0.904	
4 B	0.0126	34	19.6	4	0.0822	1.50	2.278
4 T	0.0011	33	60.2	4	0.0357	0.658	
5 B	0.0128	30.5	18.2	3	0.0873	1.60	2.517
5 T	0.0016	32.5	59,8	3	0.0347	0.635	
6 B	0.0125	30.5	18.6	4	0.0862	1.578	1.877
6 T	0.0018	32.5	51.2	4	0.0459	0.841	
7 B	0.0161	31	12.5	3	0.1056	1.930	3.776
7 T	0.0008	33	67.5	3	0.0279	0.511	
8 B	0.0159	31	14	4	0.0977	1.788	2.562
8 T	0.0013	33	58	4	0.0381	0.698	
9 B	0.0135	31.8	16	3	0.0948	1.736	1.736
9 T	0.0017	33.3	46.7	3	0.0546	0.873	

Continued, 280 μ (Run L 13)

10 B	0.0139	31.8	16.7	4	0.0903	1.654	1.716
10 T	0.0018	33.8	47.2	4	0.0526	0.963	
11 B	0.0133	31.5	16.5	3	0.0931	1.704	2.095
11 T	0.0015	33.2	53.4	3	0.0444	0.813	
12 B	0.0138	31.5	14	4	0.1030	1.886	2.445
12 T	0.0013	33.2	55	4	0.0421	0.771	
13 B	0.0133	32	15.8	3	0.0962	1.760	3.099
13 T	0.0010	33	64.4	3	0.0310	0.567	
14 B	0.0130	32	18.2	4	0.0865	1.584	2.021
14 T	0.0013	33	54.5	4	0.0428	0.783	
15 B	0.0135	31.6	15.2	3	0.0983	1.800	3.276
15 T	0.0010	33.5	65	3	0.0300	0.549	
16 B	0.0131	31.6	20	4	0.0795	1.460	3.054
16 T	0.0011	33.5	67.5	4	0.0261	0.478	
17 B	0.0141	31.6	20	3	0.0795	1.460	3.054
17 T	0.0013	33.6	60.6	3	0.0347	0.635	
18 B	0.0147	32.4	13.1	4	0.1056	1.930	2.993
18 T	0.0011	33.6	60.4	4	0.0352	0.644	

Continued. 280 μ (Run L 13)

19 B	0.0134	32	16	3	0.0948	1.737	2.748
19 T	0.0014	34.4	60.5	3	0.0345	0.631	
20 B	0.0144	32	13	4	0.1067	1.950	2.720
20 T	0.0019	34.4	55.4	4	0.0391	0.716	

TABLE 25

Semi-Continuous Ph-Parametric Pumping Run L 15

1 : Parameters:

$Q(\frac{\pi}{w})$	= 15 cc	Buffer:	
Q	= 0.5 cc/m	PH_L	= 6
$\frac{\pi}{w}$	= 30 m	PH_H	= 8.9
ϕ_T	= 4 cc/m	Comc.	= 0.2 M
ϕ_B	= 4 cc/m	Feed:	
H_T	= 8 cm	PH	= 6
H_B	= 8 cm	Haemoglobin	= 0 %
Start	= Upflow first	Albumin	= 0.05 %

2 : Correction Factor of Cell and Average Concentration of Feed:

At wavelength = 280 μ

Buffer	Cell				$\langle y_A \rangle_F$	β_H	β_A
	1st	2nd	3rd	4th			
PH 8.9	100	100.8	96.7	96.7	0.0463	16.05	6.06
PH 6	100	102.2	101.8	101.6	0.0600	21.67	5.29

3 : Sample Analysis at $\lambda = 280\,\mu$ (Run L 15)

Sample	Volume cc	PH	Transmission Feed	Transmission Sample	Cell	Albumin conc. y_A	$\langle y_A \rangle / \langle y_A \rangle_F$	$\langle y_A \rangle_B / \langle y_A \rangle_T$
1 B	4.5	8.2	53.8	45.8	3	0.0535	1.154	0.933
1 T	3.5	6	49.8	41.2	3	0.0743	1.237	
2 B	4	8.2	53.8	45	4	0.0648	1.182	1.005
2 T	4	6	49.8	43	4	0.0706	1.176	
3 B	3.8	8	53.2	44.8	3	0.551	1.189	0.988
3 T	4	6	51.5	42.2	3	0.0723	1.204	
4 B	4	8	53.2	46.8	4	0.0520	1.122	0.934
4 T	4	6	51.5	42.2	4	0.0721	1.201	
5 B	4	8	52.8	44.2	3	0.0561	1.210	1.007
5 T	4	6	49.2	42.2	3	0.0721	1.201	
6 B	4	8	52.8	45	4	0.0548	1.182	0.984
6 T	4	6	49.2	42.2	4	0.0721	1.201	
7 B	4	8	52.8	44.8	3	0.0551	1.189	1.003
7 T	4	6	49.2	42.8	3	0.0711	1.185	
8 B	4	8	52.8	44.8	4	0.0551	1.189	1.033
8 T	4	6	49.2	43.8	4	0.0691	1.151	
9 B	4	7.8	52.8	42.7	3	0.0585	1.263	1.021
9 T	4	6	49.2	41.2	3	0.0743	1.237	

Continued. 280 μ (Run L 15)

10 B	4	7.8	52.8	35.2	4	0.0724	1.564	1.267
10 T	4	6	49.2	41.2	4	0.0741	1.234	
11 B	4	7.8	52	36.2	3	0.0704	1.520	1.171
11 T	4	6	49.2	39.4	3	0.0779	1.298	
12 B	4	7.8	52	35.2	4	0.0724	1.564	1.267
12 T	4	6	49.2	41.2	4	0.0741	1.234	
13 B	4	7.8	51.2	36.2	3	0.0704	1.520	1.221
13 T	4	6	48.2	41	3	0.0747	1.244	
R B			51.2	36.2	4	0.0704	1.520	1.285
R T			48.2	42.8	4	0.0710	1.182	

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