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PARAMETRIC PUMPING WITH PH AND IONIC STRENGTH: ENZYME PURIFICATION

New Jersey Institute of Technology D.ENG.SC. 1981

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PARAMETRIC PUMPING WITH pH AND IONIC STRENGTH: ENZYME PURIFICATION

by

ZIKRI M. AHMED

A Thesis
Presented in Partial Fulfillment of
the Requirements for the Degree
of
Doctor of Engineering Science

Newark, New Jersey

May, 1981

APPROVAL OF DISSERTATION

PARAMETRIC PUMPING WITH pH AND
IONIC STRENGTH: ENZYME PURIFICATION

BY

ZIKRI M. AHMED

FOR

DEPARTMENT OF CHEMICAL ENGINEERING

NEW JERSEY INSTITUTE OF TECHNOLOGY

BY

FACULTY COMMITTEE

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NEWARK, NEW JERSEY

MAY, 1981

TO DR. MED. SAFWAT AHMED

When words are hard to find,

then I can only say "Thank You."

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Newark, New Jersey
May, 1981

Zikri M. Ahmed

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ABSTRACT

The work on separation and purification of proteins by parametric pumping has been restricted so far to pH parametric pumping where the difference between the iso-electric points of the components to be separated is apparent. The present work establishes the foundations for the continuous separation and purification of proteins (including enzymes) that have similar or very close iso-electric points by using the ionic strength as a driving force for the separation process. This technique has commercial advantages over batch processes.

It was found that the most promising technique for separating alkaline phosphatase consists of the following three major steps: (I) addition and binding of all proteins in the mixture at pH 7.4 and ionic strength 0.1, (II) selective desorption of the desired enzyme at pH 7.4 and ionic strength 0.6 and (III) regeneration of the ion exchanger at pH 4.0 and ionic strength 0.1. Several factors were found to affect the purification process, including buffer pH and ionic strength, flow rate, reservoir displacement, feed concentration, product flow rate, type of adsorbent and circulation rate. It is also shown that the technique presented here can yield a larger enzyme activity recovered with high purification factor compared with the conventional adsorption process.

INTRODUCTION

The separation and purification of enzymes and proteins in general is a very complicated procedure due to several factors that affect the separation process. An example is the instability of the protein molecule which often imposes special conditions of operation that might require certain pH and ionic strength. It might also limit the choice of eluants as well. Denaturation may take place or a too strong adsorption may lead to a desorption which can cause destruction to the structure of the molecule. Another problem is the large size of the protein molecule that requires enormous adsorbent surface because of the short penetration of the molecule into the adsorbent particles; hence, the adsorption takes place on the surface.

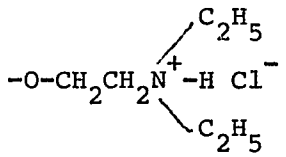
Adsorption techniques based on ion-exchange chromatography have been dominating protein separation processes. However, most of these processes are batchwise and are chemically complicated. For this reason the supply of highly purified enzymes has been quite limited. From this, the need to develop a continuous process that could be commercially applicable was obvious.

Ion Exchangers

The separation of protein components depends heavily on the selectivity of the ion exchanger. An ion exchanger consists of a crystal

lattice carrying surplus positive or negative electric charges that could be balanced by an equivalent amount of mobile ions of opposite sign, (the counter ion). The selectivity of the ion exchanger depends on the pH and the concentration of the buffer solution.

Most of the reported methods for chromatographic separation of proteins on ion exchangers used cation exchangers that contain carboxylic acid groups. Boman (1955) was the first to report the use of an anion exchanger (Dowex 2) for the chromatography of prostatic phosphatase. Since then new adsorbents derived from cellulose by the incorporation of acidic or basic groups have been developed and found to be promising in enzyme purification (Sober, 1954). Recently, DEAE-Sepharose,



is playing a major role in the protein purification processes because of its strong stability in wide pH range (2 to 10), its excellent capacity for protein adsorption (PK = 9.5) over large molecular weight range, and very good flow properties.

Parametric Pumping

A new technique in the area of separation of solute mixtures has been recently developed. This technique although still under investigation, promises a successful and economical separation technique. This is the parametric pumping technique. It has attracted considerable attention, both because it is novel and because it permits continuous operation in small equipment with very high separation factors.

The basic principle of parametric pumping is to utilize the coupling of periodic changes in some intensive variable (such as, temperature, pressure, pH, ionic strength, or electric field) and periodic changes in flow direction to separate the components of a fluid which flow past a solid adsorbent. Techniques commonly used in the separation of fluid mixtures, including adsorption, extraction, affinity chromatography and ion-exchange chromatography, might be adapted to parametric pumping. The adaptation 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.

The idea of parametric pumping (Figure 1) was first introduced by Wilhelm and his coworkers (1966).[48] In 1968 Wilhelm and Sweed separated toluene from n-heptane using silica gel as adsorbent and temperature as intensive variable in a direct mode system. High separation factors were achieved and the cyclic process proved to be valuable in separation techniques. The experiment started with the fluid in the column in equilibrium with the adsorbent at hot temperature. The same fluid that was in the bottom reservoir was then pumped through the column to the top reservoir in the first hot half cycle. In the second cold half cycle, the fluid was pumped from the top reservoir through the column and back to the bottom reservoir. The adsorbent adsorbed the solute during the cold temperature and released it during the hot temperature. Thus, during the hot half cycle, a concentrated solute was pumped into the top reservoir and this depleted the concentration of the solute in the bottom reservoir; hence, separation was obtained.

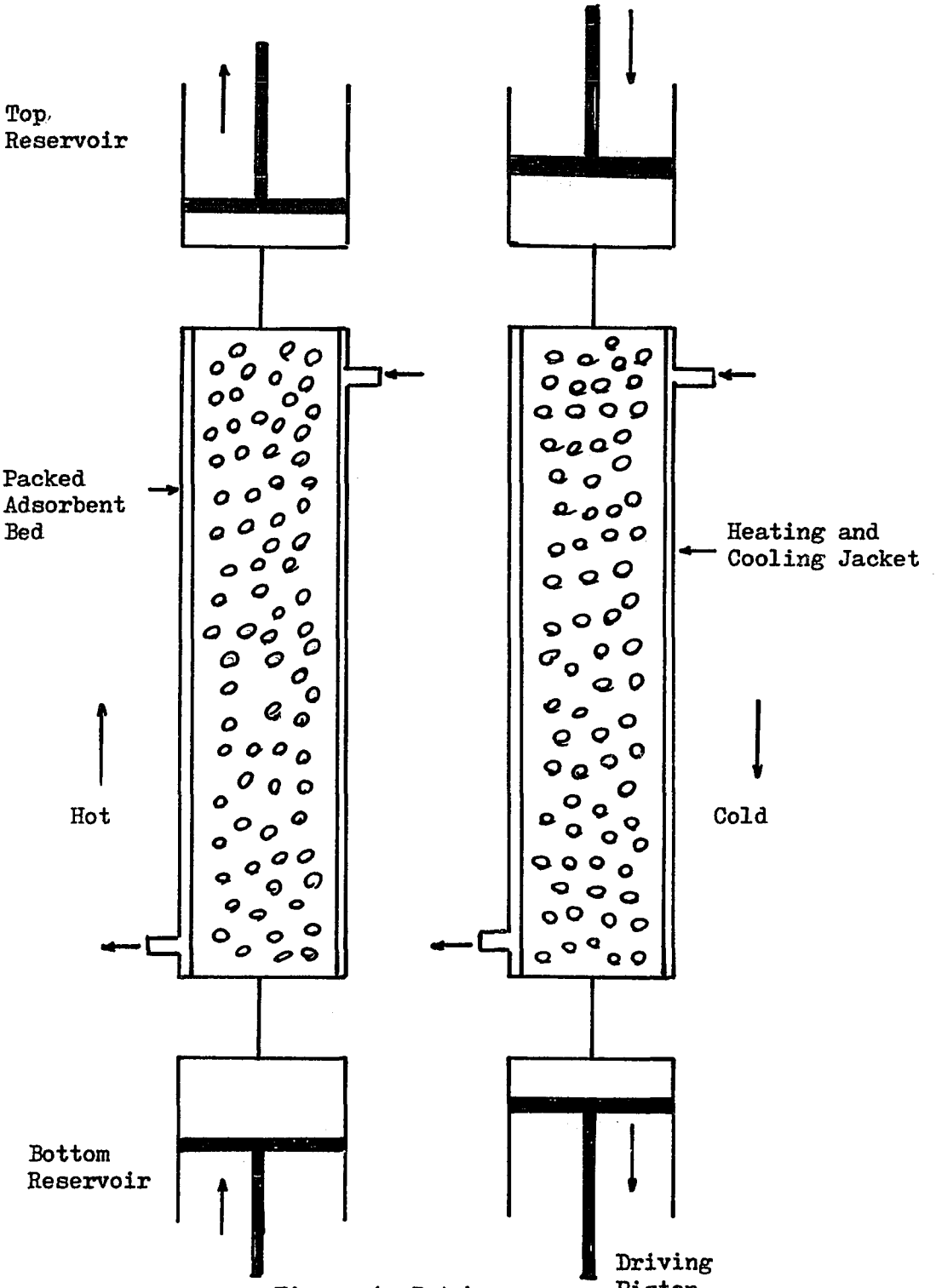


Figure 1. Batch
Parametric Pump

Since industrial production prefers continuous or semi-continuous type of operation, the batch modes were soon followed by open modes, (Gregory and Sweed (1970)[21] and Chen and Hill (1972, 1973).[12] Sabadell and Sweed (1970) were able to use pH as the thermodynamic variable in the separation of Na^+ and K^+ ions in aqueous solution. Shendalman and Mitchell (1972)[38] separated carbon dioxide and helium on silica gel using pressure as the intensive variable and deriving force for separation.

The first true theoretical analysis for the mechanism behind the separation was given by Pigford, et al (1969)[32] through an equilibrium theory. Aris (1969)[3] later generalized the equilibrium theory solution. Gregory and Sweed (1970)[53] and Chen and Hill (1971)[8] extended the equilibrium theory model to include reservoir dead volume. The equations for semi-continuous and continuous parametric pumps were also developed and solved by Chen and Hill (1971, 1972).[8] Sweed and Wilhelm (1969)[40] developed the STOP-GO algorithm which proved to be a more realistic way to solve the pump partial differential equations numerically. The STOP-GO algorithm is a modification of the method of characteristics. It assumes no equilibrium and is carried out in a numerical integration scheme. The equilibrium theory of parametric pumping developed by Pigford was extended to multicomponent separations by Butts, Gupta and Sweed (1972).[52] In their nonsymmetric direct thermal mode, separation was predicted from algebraic equations derived under the assumptions of linear isotherms. Equilibrium staged models were studied by Wankat (1973),[43] and Grevillot (1976, 1977, 1980).[23,24,22] The staged models are more convenient for continuous systems. Grevillot, et al, (1976, 1977, 1980) and Chen, et al (1980)[13] used graphical procedures to predict the separation in direct

and recuperative modes.

Few of the non-equilibrium models were reported; included are Wilhelm and Sweed (1969),[40] Gupta and Sweed (1973)[25] and Chen, et al, (1976).[11] Extraction parametric pumping with reversible reaction were investigated by Goto and Matsubara (1977)[30] and was found quite effective in obtaining higher conversion if the system parameters are chosen properly.

Application of parametric pumping involving the separation of valuable materials such as proteins may be very attractive and profitable. Many proteins are processed batchwise. Parametric pumping offers the possibility of continuous processing, thereby tending to minimize both processing time and degradation. Shaffer and Hamrin (1975)[37] combined affinity chromatography and parametric pumping to reduce trypsin concentration in an aqueous solution. Their results indicate that parametric pumping was a useful technique for enzyme separation. Recently, Chen, et al (1977, 1979)[9,14] have applied parametric pumping and ion-exchange chromatography to the separation of hemoglobin and albumin. It was shown that under certain conditions the para pump had the capacity for removal of protein components from one product fraction and large enrichment in the other fraction. Moreover, the para pump system was shown to be capable of continuous operation with a high throughput. Later, Chen, et al (1980)[15] investigated the fractionation of protein mixtures via multicolumn pH parametric pumping by using series of columns packed alternately with cation and anion exchangers. They showed that the multicolumn system has much higher separating capability than the single column unit.

The separation here depends on the fact that proteins carry both positively and negatively charged groups at a pH lower or higher than their isoelectric point respectively; hence a protein can be bound to both cation and anion exchangers. A change in pH alone may reverse the selectivity of the ion exchanger. In the meantime a change in the ionic strength of the buffer used may also cause partial or complete change from elution to displacement or vice-versa. Thus, a pH-ionic strength parametric pump that operates with levels of two ionic strengths or two pH levels that are above and below the points of zero net charge (isoelectric points) of the two protein components or a combination of pH and ionic strength should be capable of removing one component from one end of the column and enriching it at the other end.

Alkaline Phosphatase

Isoenzymes of alkaline phosphatase (orthophosphoric monoester phosphohydrolase) are found in liver, placenta, kidney, intestine, etc. The difference among them because of their organic origin or physiological functions, are not yet clear. Human Placental Alkaline Phosphatase (HPAP) is an enzyme that is present mainly in mammalian tissues and associated with intracellular lipoprotein. HPAP is a major catalyst in the hydrolysis of phosphomonoesters. Regan Isozyme, a similar enzyme, is a serum component of cancer protein. Alkaline phosphatase has an isoelectric point of 4.5 and a molecular weight approximately equal to 70,000. The detailed composition of the proteins in the enzyme mixture is not fully known. Some of the proteins are thought to be undesirable. Since the enzyme is extracted from the human placental fluid, one major

undesired protein is albumin.

Doellgast, G.J., and Fishman, W.H. (1974)[17] successfully purified human placental alkaline phosphatase by using decreasing concentrations and changing types of salts on a DEAE - Sephadex chromatography column. Brenna, et al (1975)[4] purified the enzyme using affinity chromatography. Trepanier, J.H., et al (1976)[41] had also purified alkaline phosphatase from human liver to homogeneity by using a procedure that involves the solubilization with butanol followed by fractionation with acetone and then chromatography on concanavalin A - Sepharose, DEAE - cellulose, Sephadex G - 200, and DEAE Sephadex. Komoda, T., and Sakagishi, Y. (1976)[29] reported purification of alkaline phosphatase with concanavalin A - Sepharose and tyraminyl derivative using affinity chromatography.

Most of the above methods (if not all) are batchwise, limited, and complex when applied on an industrial scale. From this the need to look for dynamic continuous processes to separate and purify enzymes was obvious.

In this present work, purification of alkaline phosphatase is experimentally investigated by pH - and ionic strength - driven parametric pumping in a recuperative mode. The optimum operating conditions for this purification process were used to develop a mathematical model for the process. The model developed is based on the assumption that complete equilibrium exists between the fluid phase and the solid phase (adsorbent) at the end of each stage in the cycle.

THEORY

Fundamental Principles

All cyclic separation processes that take place inside an adsorbent column rely upon the fact that the rate at which the solute molecules travel through the column is directly related to the distribution of the solute substance between the immobile phase and the mobile phase. This distribution is governed by interphase equilibrium between the stationary phase and the moving fluid.

$$A \underset{\text{desorption}}{\overset{\text{adsorption}}{\rightleftharpoons}} B \qquad (z)$$

The variation in this distribution can be brought about selectively by changing the thermodynamic variables of the system. This changing will cause the direction of equation (z) to go either to the right or to the left in a way which maintains this equilibrium relationship.

Proteins (including enzymes) are bound to cation or anion exchangers depending upon their isoelectric point together with the pH used. At pH higher than the isoelectric point of the protein molecule, the molecule will carry negative charge, and hence will bind to anion exchanger while at pH lower than the isoelectric point , it will carry positive charge and will bind to cation - exchanger. Separation of proteins which have approximately equal isoelectric points will need another thermodynamic variable beside the pH. Ionic strength has often been used as a driving force for elution of proteins and enzymes.

Changing the ionic strength drastically from the ionic strength used for the feed buffer will change the concentration of the protein molecules on the exchanger due to more competition and less interaction between the molecule and the exchanger. Thus, pH and ionic strength could be favorably used as driving force thermodynamic variables in separation and purification of proteins and enzymes by parametric pumping.

Other factors that determine the operating conditions for the purification parametric pumping process are the type of ion exchanger and buffer needed. Once the fundamental operating conditions were found, (and this was done through a series of breakthrough experiments) the road to the major work was open. In this present work, the choice of operating conditions was followed by cycling zone adsorption experiments to predict the feasibility of using the pH - ionic strength parametric pumping in purification of the enzyme alkaline phosphatase (HPAP). One product scheme was first investigated with the purified enzyme accumulated in the top reservoir; then the three products semi-continuous scheme as described in the next section was used. The major parameters that were thought to influence the separation capability of the pump were studied theoretically and experimentally. It was found that the velocity of the fluid, the displacement inside the column, and the product withdrawal volume have serious effects on the purification process.

Based on the experimental results, a mathematical model that includes the important parameters of the process was then developed. It is shown that the experimental results concerning the product concentrations and the behavior of the system agree reasonably well with the model predictions.

A- Adsorption Isotherms:

The ability of certain solids preferentially to concentrate specific substances from solutions onto their surfaces is called adsorption. Adsorption has for some time been recognized in the chemical industries as an effective process for a wide range of solute solvent separation.

There are two types of adsorption: (a) Physical adsorption which is reversible phenomena and it is a result of intermolecular forces of attraction between molecules of solid and substance adsorbed. (b) Chemisorption which is a result of chemical interaction between the solid and the substances adsorbed.

The amount adsorbed in an adsorption process is a function of many factors, some of which are interrelated. Some of these are: (a) the number of adsorbable molecules (i.e., the concentration in the solution), (b) the number of layers of adsorbed molecules, (c) the total area of solid surface, (d) the thermodynamic variable under which the process is carried out.

One of the oldest attempted explanation of adsorption is that known as the exponential equation by Freundlich and can be expressed as

$$X = KC^{\frac{1}{s}} \qquad (1)$$

where,

X = the amount adsorbed per the amount of adsorbent used

C = the equilibrium concentration of the solute

S and K are constants.

Langmuir produced the first theoretical treatment of an isothermal adsorption equilibrium. There are many forms of the Langmuir isotherms. For solid-liquid adsorption we have

$$q = \frac{Nk\Theta}{1+k\Theta} \tag{2}$$

in which the experimentally measured quantities are q and Θ. k and N are constants that can be evaluated if the experimental data are capable of being described by the previous equation.

Adsorption isotherms have been of great importance in ion exchange separation techniques. The thermodynamic theories of equilibria between the ion exchanger and the solution is the most fundamental base on which predictions of separation and column performance is built. The most common form of this equilibrium relation is the linear one, especially in cases of low concentrations;

$$x = my \tag{3}$$

where,

$$m = m(y_i, \eta_i) \tag{4}$$

and η_i's are the intensive system variables. Since adsorption is a reversible process, one should expect that with each change of the intensive variable , the adsorption process will move in the direction that will satisfy the equilibrium relation (3). It is this principle that led to the idea of the parametric pumping in search of improved adsorption techniques. The coupling of the change in a thermodynamic

variable and the direction of the flow inside the column will affect the solute equilibrium distribution between the fluid and the solid phases and if done in a cyclic fashion will create an axial compositional gradient that will lead to a build-up of separation from cycle to cycle.

B- Parametric Pumping

Very high separation factors have been obtained by many investigators for different systems using cycling flow of a binary or multicomponent mixture upward and downward through a column packed with solid adsorbent and alternately changing one or more thermodynamic variables of the system used. The basis for separation by parametric pumping lies in the ability of the solid adsorbent to retain solute adsorbed on it during one part of the cycle and later to release this solute into the fluid phase stream during another part of the cycle. The extent of separation depends on the operating conditions and the system involved. Prediction of separation is relatively simple if the equilibrium theory applies. However, in many systems separation is retarded by some countereffects as (a) the axial fluid phase difussive losses caused by molecular diffusion and by fluid mixing processes in the spaces between particles, (b)mass transfer resistance to diffusion of material between solid and fluid phases. Also other phenomenon may affect the separation.

A recuperative semi-continuous parametric pump has been used for purification of alkaline phosphatase (an enzyme). The model used here is based on the system shown in Figure 2 . Of the many thermodynamic variables that could be used in the parametric pumping system, those

that have serious effect on heterogeneous equilibria are temperature, pressure, pH and ionic strength. The pH parametric pump has been used successfully in separation of proteins where the difference in the isoelectric points of the components to be separated is apparent. However, in cases where components have very close isoelectric points, another thermodynamic variable beside pH might be required. In the case investigated here, the detailed composition of the proteins in the human placental alkaline phosphatase mixture is unknown. However, one major undesired protein in the mixture is albumin which has an isoelectric point approximately equal to that of alkaline phosphatase. Therefore, a thermodynamic variable other than pH such as ionic strength is needed for the purification process. Based on the experimental results obtained, it was found that the most optimum scheme (Figure 2) for the purification process consists of the following stages:

1- The enzyme mixture at the feed conditions (pH = 7.4, I.S. = 0.1) enters the top of the column, while solution emerging from the other end of the column enters the bottom reservoir. As a result, the pH of the column is changed from 4.0 to 7.4 and ion exchanging took place between the counter ions S^- and the mixture (E^- and U^-).

$$R^+S^- + E^- \underset{0.6}{\overset{0.1}{\rightleftharpoons}} R^+E^- + S^-$$

2- The fluid in the top reservoir (pH = 7.4, I.S. = 0.6) enters the top of the column, at the mean time a bottom product (1) (protein free) is removed from the bottom of the column.

3- Circulate between the top reservoir and the column to ensure that the buffer concentration inside the column is changed to 0.6.

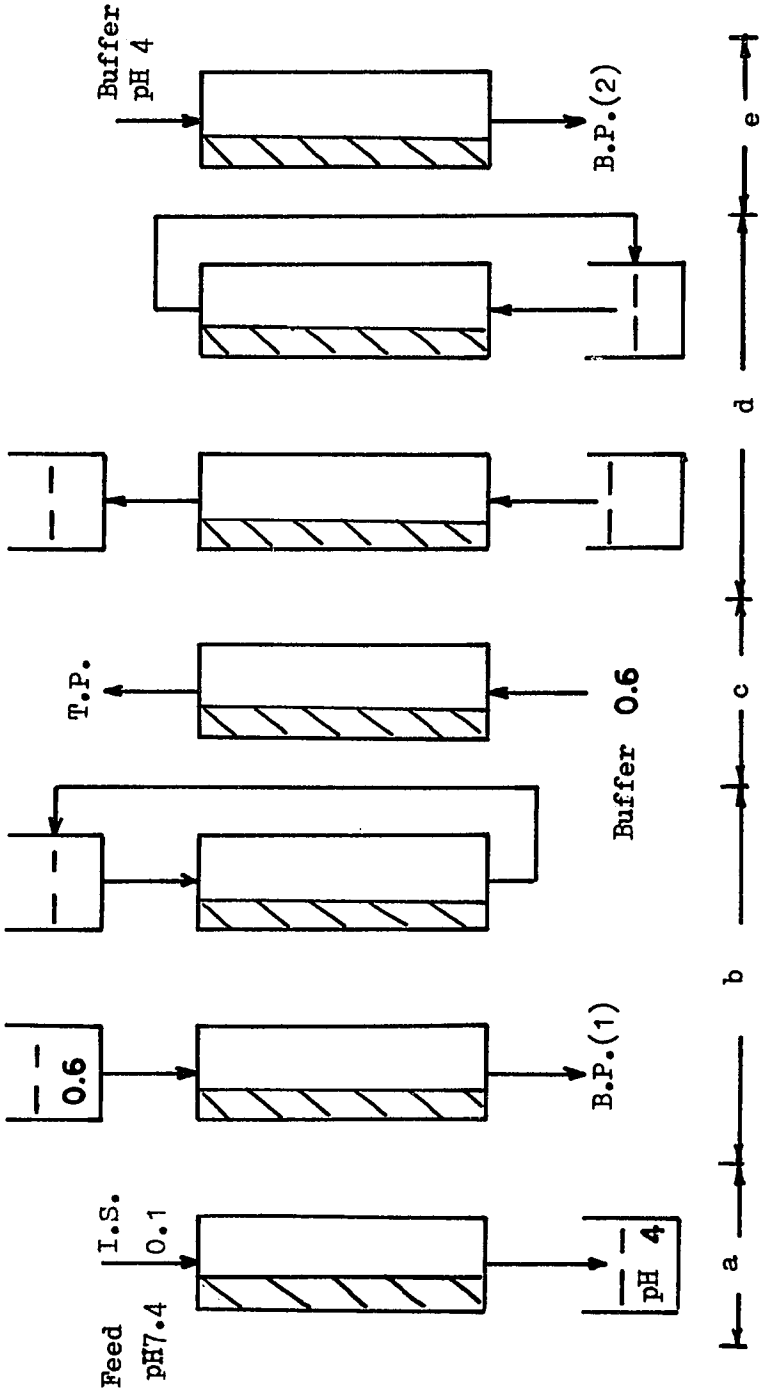


Figure 2. pH-I.S. Parametric Pumping Scheme

Increasing the ionic strength reduces the interaction between the exchanger and E, causing their elution.

4- An elution buffer (pH = 7.4, I.S. = 0.6) is pumped through the bottom of the column and a top product (enzyme enriched) is withdrawn from the column.

5- Pump the bottom reservoir solution (pH = 4.0, I.S. = 0.1) to the bottom of the column, solution emerging from the top enters the top reservoir.

6- Circulate between the bottom reservoir and the column to ensure the shifting of the pH in the column to the value 4.0. Thus, desorption of U^+ occurs and the ion exchanger is regenerated.

$$R^+S^- + \underset{U^+}{U^-} \underset{4.0}{\overset{7.4}{\rightleftharpoons}} R^+U^- + S^-$$

7- Withdraw a bottom product (2) (undesired protein enriched) by pumping to the top of the column an elution buffer of pH 4.0 and I.S. = 0.1.

Mathematical Formulations:

If we assume that, (1) the ion exchanger used has high capacity, (2) the ionic exchange between the counter ion and the protein is essentially complete at the end of stages a, b, d (Figure 2), (3) constant physical properties, (4) dilute system (0.02 wt%) so linear isotherms can be assumed, (5) no axial diffusion takes place. Then a material balance for the solute at the end of each transfer step in the cycle will result in a set of mass balance equations that form the basic equations of the model.

Let

E^J = Free enzyme concentration in mobile phase gm/cc

J = stage number

(F) = feed conditions

T.R. = top reservoir conditions

B.R. = bottom reservoir conditions

x = concentration of enzyme in solid phase gm/cc

ϵ = volume fraction void

V = volume of fluid phase on the stage in cc, (assumed constant)

$\bar{V}$ = volume of solid phase in cc, (assumed constant)

V_D = dead volume in reservoir in cc, (assumed constant)

First Case:

$$V_D = 0$$

To facilitate the mathematics, let us assume that all the mobile phase on each stage of the basic five stages shown in Figure 2 is transferred at each transfer step and that the conditions inside the column at the end of each transfer step is well established.

Mass balance on enzyme at any cycle (n) just before and after reequilibration for the five transfer steps gives:

$$\bar{V}x_n^a + VE_n^a = \bar{V}x_{n-1}^e + VE_F \quad (5)$$

$$\bar{V}x_n^b + VE_n^b = \bar{V}x_n^a + VE_{n-1}^{T.R.} \quad (6)$$

$$\bar{V}x_n^c + VE_n^c = \bar{V}x_n^b + V(\text{buffer } 0.6) \quad (7)$$

but the concentration of the enzyme in the elution buffers are zero, hence equation (7) becomes

$$\bar{V}x_n^c + VE_n^c = \bar{V}x_n^b \quad (8)$$

$$\bar{V}x_n^d + VE_n^d = \bar{V}x_n^c + VE_n^{B.R.} \quad (9)$$

$$\bar{V}x_n^e + VE_n^e = \bar{V}x_n^d + V(\text{buffer pH} = 4.0) \quad (10)$$

and (10) could be written as

$$\bar{V}x_n^e + VE_n^e = \bar{V}x_n^d \quad (11)$$

since the concentration of the mixture used is very low (0.02 wt%) i.e. we can assume an equilibrium relation of the linear form

$$mE = X \quad (12)$$

where m = slope of the distribution isotherm

$$m = m(\text{I.S., pH}) \quad (13)$$

divide equations (5), (6), (8), (9) and (11) by $\bar{V}$ and let $\frac{V}{\bar{V}} = \rho$

Now the set of the conservation of enzyme equations for cycle (n) can be written as follows:

$$m^a E_n^a + \rho E_n^a = m^e E_{n-1}^e + \rho E_F \quad (14)$$

$$m^b E_n^b + \rho E_n^b = m^a E_n^a + \rho E_{n-1}^{T.R.} \quad (15)$$

$$m^c E_n^c + \rho E_n^c = m^b E_n^b \quad (16)$$

$$m^d E_n^d + \rho E_n^d = m^c E_n^c + \rho E_n^{B.R.} \quad (17)$$

$$m^e E_n^e + \rho E_n^e = m^d E_n^d \quad (18)$$

Now, for stage (a) equation (14), concentration of bottom product (1)

is

$$E_n^a = \frac{1}{\rho + m^a}(m^e E_{n-1}^e + \rho E_F) \tag{19}$$

for stage (b), (15) can be written as

$$E_n^b = \frac{1}{\rho + m^b}(m^a E_n^a + \rho E_{n-1}^{T.R.}) \tag{20}$$

since the buffer volume originally presented in the top reservoir = V, then

$$E_{n-1}^{T.R.} = E_{n-1}^c \tag{21}$$

and (20) the equation for the top product is

$$E_n^b = \frac{1}{\rho + m^b}(m^a E_n^a + \rho E_{n-1}^c) \tag{22}$$

from (16) we have

$$E_n^c = \frac{1}{\rho + m^c} m^b E_n^b \tag{23}$$

and (17) could be rewritten as

$$E_n^d = \frac{1}{\rho + m^d}(m^c E_n^c + \rho E_n^{B.R.}) \tag{24}$$

but

$$E_n^{B.R.} = E_{n-1}^e \tag{25}$$

thus,

$$E_n^d = \frac{1}{\rho + m^d}(m^c E_n^c + \rho E_{n-1}^e) \tag{26}$$

which gives the concentration of bottom product (2)

for the concentration in the bottom reservoir at cycle n (18)

$$E_n^e = \frac{1}{\rho + m^e} m^d E_n^d \tag{27}$$

The steady state of the system will be reached when

$E_n^b = E_{n+1}^b$ and the value of E^c, E^d, and E^e at the steady state could be calculated from the steady state value of E_n^b. Since the expected value of E_n^a, the concentration of the enzyme in the bottom product BP(1) at the steady state and in general is trivial for this particular system, it would be improper to calculate it based on the value of E_n^a in the cycle before. Thus the steady state value of E_n^a will be computed from the steady state value of E_n^b.

$$E_n^b = \frac{1}{\rho + m^b} m^a E_n^a + \rho E_{n-1}^c \tag{22}$$

rearrange equation (22), we obtain E_n^a at steady state ,

$$E_n^a = \frac{1}{m^a}((\rho + m^b)E_n^b - \rho E_{n-1}^c) \tag{28}$$

where E_n^b here is the concentration of enzyme in top product at the steady state.

Second Case: $V_D \neq 0$

Let us assume well mixed reservoirs. Furthermore, let us also assume that the conditions in the column at the end of stages b and d after circulation are the same as in the top and bottom reservoir respectively, (see Figure 2).

$$\frac{V + V_D}{V} = \rho + \rho_D \tag{29}$$

where V_D is the dead volume in the reservoirs.

Mass balance on the solute at the end of steps a, b, c, d and e just before and after re-equilibration will result in the following equations:

$$\bar{V}x_n^a + VE_n^a = \bar{V}x_{n-1}^e + VE_F \tag{30}$$

$$\bar{V}x_n^b + (V + V_D)E_n^b = \bar{V}x_n^a + (V + V_D)E_{n-1}^{T.R.} \tag{31}$$

$$\bar{V}x_n^c + VE_n^c = \bar{V}x_n^b + 0 \tag{32}$$

$$\bar{V}x_n^d + (V + V_D)E_n^d = \bar{V}x_n^c + (V + V_D)E_{n-1}^{B.R.} \tag{33}$$

$$\bar{V}x_n^e + VE_n^e = \bar{V}x_n^d + 0 \tag{34}$$

divide by $\bar{V}$ and let $x_n^j = m^j E_n^j$,

equation (30) becomes

$$m^a E_n^a + \rho E_n^a = m^e E_{n-1}^e + \rho E_F \tag{35}$$

and

$$E_n^a = \frac{1}{\rho + m^a}(m^e E_{n-1}^e + \rho E_F) \tag{36}$$

(31) could be written as

$$m^b E_n^b + (\rho + \rho_D)E_n^b = m^a E_n^a + (\rho + \rho_D)E_{n-1}^{T.R.} \tag{37}$$

$$E_n^b = \frac{1}{\rho + \rho_D + m^b}(m^a E_n^a + (\rho + \rho_D)E_{n-1}^{T.R.}) \tag{38}$$

but

$$E^{T.R.}_{n-1} = (V_D E^b_{n-1} + V E^c_{n-1})/(V + V_D) \tag{39}$$

and

$$E^c_{n-1} = \frac{1}{\rho + m^c} m^b E^b_{n-1} \tag{40}$$

i.e.,

$$E^{T.R.}_{n-1} = E^b_{n-1}((V_D + \frac{Vm^b}{\rho + m^c})/(V_D + V)) \tag{41}$$

$\therefore$

$$E^b_n = \frac{1}{m^b + \rho + \rho_D}(m^a E^a_n + (\rho + \rho_D) E^b_{n-1}(V_D + \frac{Vm^b}{+m^c})/(V + V_D)) \tag{42}$$

since

$$(\rho + \rho_D) = \frac{V + V_D}{V} \tag{29}$$

then E^b_n could be expressed as:

$$E^b_n \equiv \frac{1}{m^b + \rho + \rho_D}(m^a E^a_n + \frac{1}{V} E^b_{n-1}(v_D + \frac{Vm^b}{\rho + m^c})) \tag{43}$$

or

$$E^b_n = \frac{1}{m^b + \rho + \rho_D}(m^a E^a_n + E^b_{n-1}(\rho_D + \frac{\rho m^b}{\rho + m^c})) \tag{44}$$

and finally

$$E_n^b = \frac{1}{m^b+\rho+\rho_D}\left(\frac{m^a}{\rho+m^a}\,(m^e E_{n-1}^e+\rho E_F)+E_{n-1}^b\left(\rho_D+\frac{\rho m^b}{\rho+m^c}\right)\right) \tag{45}$$

From (32) we have

$$E_n^c = \frac{1}{\rho+m^c}\,m^b E_n^b \tag{46}$$

and from (33)

$$E_n^d = \frac{1}{\rho+\rho_D+m^d}\,(m^c E_n^c + (\rho+\rho_D)E_{n-1}^{B.R.}) \tag{47}$$

but

$$E_{n-1}^{B.R.} = (V_D E_{n-1}^d + VE_{n-1}^e)/(V+V_D) \tag{48}$$

i.e.,

$$(\rho+\rho_D)E_{n-1}^{B.R.} = \rho_D E_{n-1}^d + \rho E_{n-1}^e \tag{49}$$

but from (34) we have

$$E_{n-1}^e = \frac{1}{\rho+m^e}\,m^d E_{n-1}^d \tag{50}$$

by substitution

$$E_n^d = \frac{1}{m^d+\rho+\rho_D}\left(m^c E_n^c + \rho_D E_{n-1}^e + \frac{\rho m^d}{\rho+m^e}\,E_{n-1}^d\right) \tag{51}$$

or

$$E_n^d = \frac{1}{m^d+\rho+\rho_D}\left(m^c E_n^c + E_{n-1}^d\left(\rho_D + \frac{\rho m^d}{\rho+m^e}\right)\right) \qquad (52)$$

From (34) the expression for E_n^e is

$$E_n^e = \frac{1}{\rho+m^e} m^d E_n^d \qquad (53)$$

The steady state values are computed using the same procedure as mentioned before. The same equations could be used to calculate the concentration of the total protein using the proper notations and the proper values for the slope of the isotherms, (total protein isotherms).

It is clear from the resulting equations that the concentrations in the product streams depend only on the initial concentration E_F (unless the concentration is normalized, $\frac{E}{E_F}$) and the slope of the isotherms together with the ratio $\frac{V}{\nabla}$ or $\frac{V+V_D}{\nabla}$. It is important to determine the isotherms (slope of the equilibrium relation) under the same conditions of the step to be used in.

Unequal Volumes of Fluid Phases

For the case where the volume of the fluid phase is different for each stage, a very simple modification of the equations taking into account this difference can be made, providing these volumes are constant for the specific stage in question during the entire run. Thus instead of using a single ρ as the ratio of the fluid volume to the solid one, we would have different ρ's accounting for each stage,

i.e.,

$$\rho_j = \frac{V_j}{\bar{V}} \tag{54}$$

and the equations for the conservation of the solute should be adjusted. However, the same procedure applies.

A detailed procedure for the general case is given in Appendix (A).

Calculation of m:

From the breakthrough data at the equilibrium state we can relate the amount of solute in the fluid phase to the total amount in the column as:

$$\frac{\text{amount of solute in fluid phase}}{\text{total amount of solute in column}} = \frac{VE}{VE + \bar{V}X} \tag{55}$$

Let us assume that the solid adsorbent and the fluid inside the column are everywhere in equilibrium and that the equilibrium relation is of the linear form,

i.e., $$X = mE \tag{12}$$

Substitute (12) into (55) and rearrange

$$\therefore \frac{\text{amount of solute in fluid phase}}{\text{total amount of solute in column}} = \frac{E}{E + \frac{\bar{V}}{V} mE} \tag{56}$$

but $$\frac{\bar{V}}{V} = \frac{1-e}{e} \tag{57}$$

where

e = the porosity of the adsorbent.

Equation (56) can be approximated as:

$$\frac{E}{E_F} = \frac{1}{1 + \frac{1-\epsilon}{\epsilon} m} \tag{58}$$

where E_F is the concentration of the feed expressed in moles/cc. From (58) m could easily be obtained.

$$m = \left(\frac{E_F}{E} - 1\right)\frac{\epsilon}{1-\epsilon} \tag{59}$$

$\frac{E_F}{E}$ is the reciprocal of the ratio of the concentration in the fluid phase to that of the feed and can be determined by relating the the activity in the sample product at the equilibrium state to that of the feed.

The average solute velocity can be expressed in relation to the constant fluid velocity as:

$$\text{solute velocity} = u\ (pH,\ I.S.) = v\ \frac{\text{amount of solute in mobile phase}}{\text{total amount of solute in column}}$$

$$\text{or} \quad u = \frac{v\,E}{E + \frac{1-\epsilon}{\epsilon} mE} \tag{60}$$

i.e.,

$$u = \frac{v}{1 + \frac{1-\epsilon}{\epsilon} m} \tag{61}$$

This shows that as v decreases, u decreases and more mass transfer

takes place. Equation (61) tells us that the average solute molecule velocity is function of the fluid velocity and the thermodynamic conditions imposing on the system (which determines the value of m). Increasing the ionic strength will reduce the interaction between the ion exchanger and the solute molecules, thus increasing their velocity and as a result their concentration in the fluid phase must increase. This could be illustrated by writing a mass balance on the solute over stages a and b on a section of the column of height L and cross-sectional area equal to A when the ionic strength changes from 0.1 to 0.6 at the pH = 7.4;

$$LA\ eE^b + LA(1-e)x^b = LAeE^a + LA(1-e)x^a \tag{62}$$

assume a linear local equilibrium (x = mE) and rearrange

$$\therefore$$

$$e(E^b - E^a) = (1-e)(m^aE^a - m^bE^b) \tag{63}$$

$$E^b(e + (1-e)m^b = E^a(m^a(1-e) + e) \tag{64}$$

or

$$\frac{E^a}{E^b} = \frac{e + (1-e)m^b}{e + (1-e)m^a} = \frac{u^a}{u^b} \tag{65}$$

since $m^a > m^b$, then it is clear that

$$E^b > E^a$$

and every time the ionic strength, I.S., is increased (from 0.1 to 0.6), the solute concentration in the 0.6 stream (top product) increases.

Application of the Model:

The scheme of calculations is as follows:

1- Compute the slopes of the distribution isotherms, (m)

2- Calculate the concentrations for the cycle n+1, noticing that the product concentrations for the cycle (n-1) where n=1 are all known (initial conditions) using Equations 36, 45, 46, 52 and 53.

3- After the computation for the first cycle is carried out, the calculations for any cycle could be determined by successive substitutions.

4- The steady state concentrations are obtained at the cycle where $E^j(n) = E^j(n+1)$.

It is interesting to notice that calculations based on this simple equilibrium relation facilitate the mathematics and will be shown to predict the product concentrations for this particular system reasonably well.

This simple model shows that the separation is controlled by the differences in equilibrium adsorption at the two ionic strengths. It also shows the effect of the total reservoir displacement on the fraction of the bed storage capacity hence on separation. Slow fluid velocity makes the process close to equilibrium and increases separation. However, very low fluid velocity might cause axial diffusion and thus decrease separation.

PROCESS DESCRIPTION AND EXPERIMENTAL

A- The Enzyme System

A human alkaline-phosphatase mixture (obtained from Sigma Biochemicals of U.S.A.) was chosen to examine experimentally the feasibility of the parametric pumping separation scheme. Alkaline-phosphstase has an iso-electric point of 4.5 and a molecular weight approximately equal to 70,000. The detailed composition of the proteins in the mixture is unknown. Some of the proteins are thought to be undesirable. Since the enzyme is extracted from the human placental fluid, one major undesired protein is albumin, which unfortunately has an isoelectric point approximately equal to that of alkaline-phosphatase. Therefore, a thermodynamic variable other than pH such as ionic strength is needed for the purification process.

B- The Experimental System

A series of experiments for determing the operating conditions which will establish the most suitable buffer pH and ionic strength for the parametric pumping process was carried out. For these experiments a chromatographic column was packed with anionic exchanger (DEAE Sepharose) supplied by Pharmacia Find Chemicals of Sweden. Initially, the exchanger was allowed to reach equilibrium with the feed buffer. For pH equal or above 7.4, the buffer was a mixture of Tris (Hydroxymethyl) Aminomethane and HCL. For pH below 5.2, it was a mixture of acetic acid and sodium acetate (Cclowick and Kaplan, 1955). At $t = 0$, a feed containing a

0.02 wt % of alkaline phosphatase mixture (alkaline phosphatase plus undesired proteins) was introduced at the top of the column. Product samples were collected from the bottom of the column at equal time intervals. Several conditions of pH and ionic strength were investigated. The results are shown and discussed in the next chapter. The enzyme activity, a, was determined by measuring the increase in absorbance resulting from the hydrolysis of p-nitrophenylphosphate (Worthington, 1977). The Bio-Rad protein assay was used to determine the total protein concentration, y. The details of the reagent preparation and method of measurements are given in Appendix D.

Parametric Pumping

Based on the results obtained from the previous experiments, a parametric pumping separation scheme was developed. This scheme has three major steps: (I) addition and binding of all proteins in the mixture at pH 7.4 and I.S. = 0.1 , (II) selective desorption of the desired enzyme at pH 7.4 and I.S. = 0.6 , and (III) regeneration of the ion exchanger at pH 4.0 and I.S. 0.1 .

Figure 3 shows schematically the experimental apparatus used. It consists of a jacketed chromatographic glass column (0.016 m, ID and 0.08 m, height) manufactured by Pharmacia Fine Chemicals, reversible flow direction peristaltic pumps and two jacketed glass reservoirs of size 50. ml, each equipped with magnetic stirrers to assure homogenous mixing. The source of keeping the column and the reservoirs at a temperature of 288 K^{o} was a constant temperature water bath that circulated water through the jackets constantly. The top reservoir was kept at pH 7.4 by an automatic titrator, while a second titrator was used to keep the bottom reservoir at pH 4.0. NaOH and HCL were used to maintain the high and low pH levels. The buffer ionic strengths in the top and bottom reservoirs were maintained, respectively, at 0.6 and 0.1 , by means of two hollow-fiber dialyzers manufactured by Amicon. Product streams were taken off by means of micrometer capillary valves. The feed, containing 0.02 % alkaline phosphatase mixture at pH 7.4 and I.S. = 0.1 was directed to the top of the column. Two elution buffers, one with pH 7.4, I.S. = 0.6 , and the other with pH 4.0 and I.S. = 0.1 , were fed to the bottom and top of the column, respectively. Three product streams were withdrawn from the column; top product (enzyme enriched), bottom product (2) (undesired proteins enriched), and bottom product (1) (protein free).

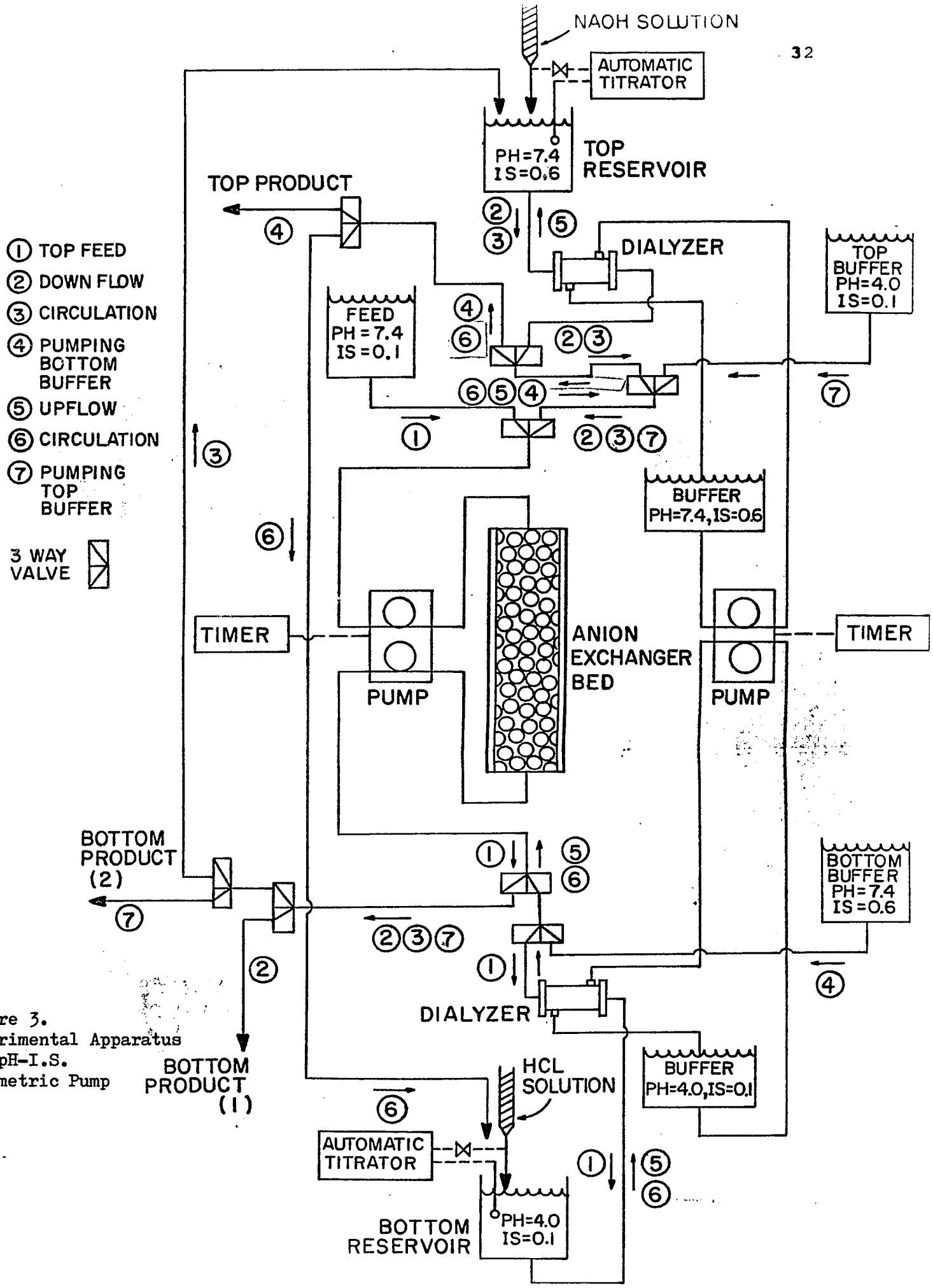


Figure 3.
Experimental Apparatus
for pH-I.S.
Parametric Pump

Prior to each run, the DEAE Sepharose (Registrated Trademark, Pharmacia Fine Chemicals of Sweden) anion exchanger was mixed with the 0.1 I.S., pH 7.4 Tris-HCL buffer and left for several hours. The column was then packed with the swollen ion exchanger and adjusted to 8.0 cm height. A quantity of the buffer (I.S. = 0.1 , pH 7.4) was then passed through the column and the lines to assure removal of the air from the system. At t = 0, the top reservoir is filled with a buffer of pH 7.4 and I.S. = 0.6 . It should be mentioned that except for the first cycle where the solution emerging from the bottom of the column during stage 1 is discarded, the flow system has seven distinct stages in each cycle when circulation is used and five stages if no circulation is introduced (stages 3 and 6 are the circulation stages). The seven stages as shown in Figure 4 are:

1- The enzyme mixture at pH 7.4 and I.S. = 0.1 enters the top of the column for t, time units, while the solution emerging from the other end of the column enters the bottom reservoir. The volume of the feed Qt_1 is set equal to the void volume of the column Ve, that is $Qt_1 = Ve$. As a result of this replacement, the pH of the fluid in the column is changed from 4.0 to 7.4, and the counter ions, S^- are exchanged for both E^- (enzyme) and U^- (undesired protein) present in the feed.

2- The fluid at pH 7.4 and I.S. = 0.6 in the top reservoir enters the top of the column for t_2 time units and simultaneously, a bottom product (1) free of E and U is removed from the column at the same rate.

3- Circulate the fluid between the top reservoir and the column for t_3 time units. This will allow the buffer concentration, I.S., in the column to change from 0.1 to 0.6 . Increasing the ionic strength reduces

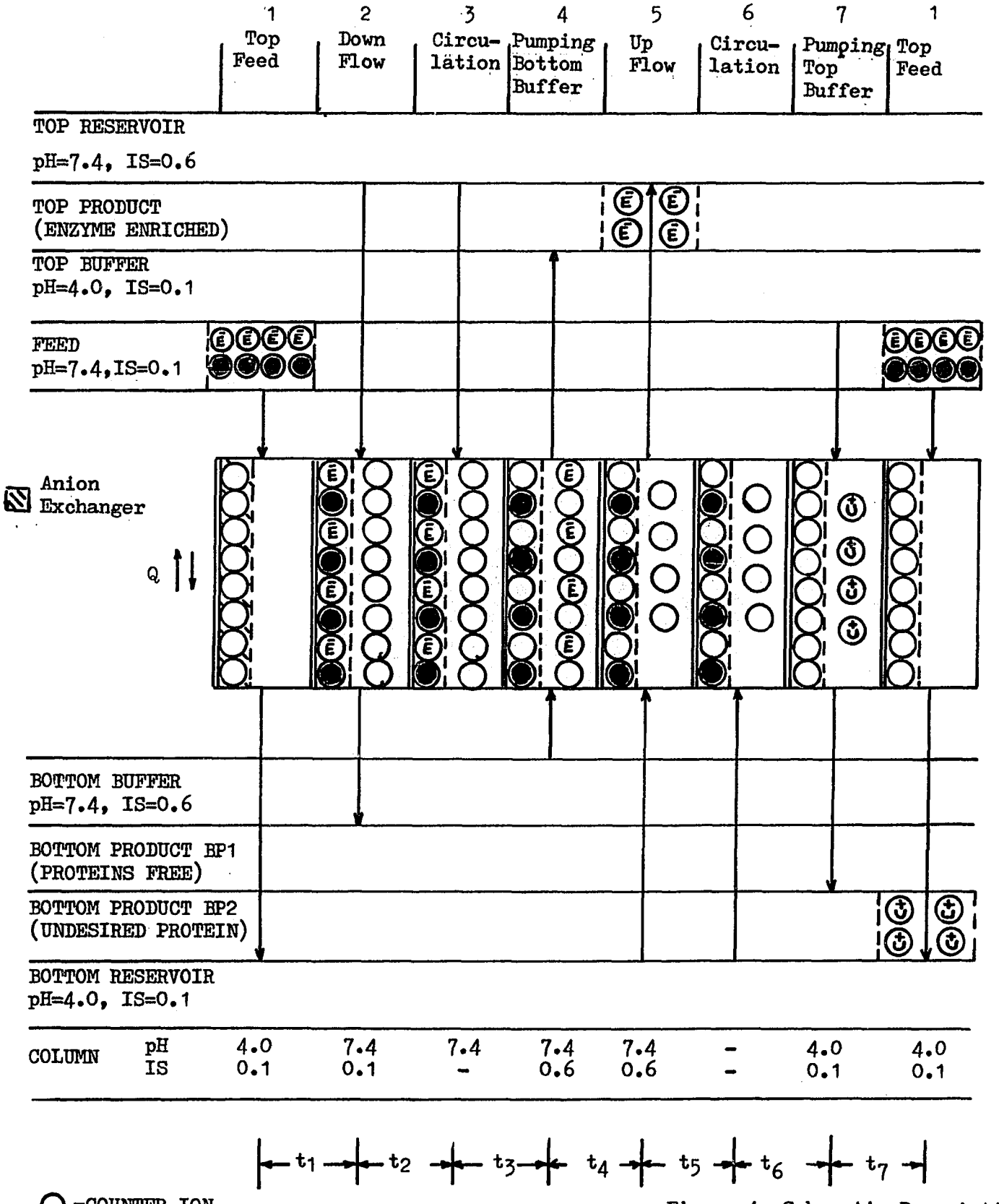


Figure 4. Schematic Description Parametric Pumping Principle

the interaction between the exchanger and E, causing their elution.

4- Pump the bottom buffer (pH 7.4 and I.S. = 0.6) to the bottom of the column for time t_4, while a top product containing only pure E^- is removed from the column.

5- Pump the bottom reservoir solution at pH 4.0 and I.S. = 0.1 to the bottom of the column during time interval t_5, at the same time the solution emerges from the other end enters the top reservoir.

6- Circulate the fluid between the reservoir and the column for t_6 time units. This will ensure that the pH and I.S. in the column are shifted back to 4.0 and 0.1 , respectively. Thus, desorption of U^+ occurs and S^- shifts back to the bed. The ion exchanger is then regenerated.

7- Pump the top buffer at pH 4.0 and I.S. = 0.1 to the top of the column during t_7. A bottom product, BP(2), containing only U^+ is withdrawn from the bottom. One whole cycle is thus completed.

From Figure 4 , one can see that all E and U supplied by the feed moves toward the top and bottom product streams, respectively. Complete split of E and U is achieved during one single complete cycle. This result is based on the following assumptions:
the ion exchanger used has high capacity, and the ionic exchange between the counter ion and the protein (either E or U) is essentially complete at the end of the stages 1, 3, and 6 (Figure 4). In practice it may not be possible to implement the operating conditions that perfectly satisfy the above assumptions. However, a good separation is attainable by repeating the process illustrated in Figure 4 in succeeding cycles.

Some cycling-zone adsorption experiments have been carried out for

comparison with parametric pumping. The difference between the cycling-zone adsorption system and parametric pumping is only in the method of operation. The cycling-zone adsorption flows in a single direction. The basic apparatus consists of a chromatographic column with an inlet stream arranged so that its buffer pH and I.S. and enzyme concentration are varied periodically as shown in Figure 11 . A product stream is withdrawn from the bottom of the column. The product is time-dependent in that the concentrations (a_p, Y_p) vary continuously. However, the apparatus will eventually reach a repeating state where the product concentrations (a_p, Y_p) repeat from cycle to cycle.

RESULTS AND DISCUSSION

A- Determination of Operating Conditions

1- Cation Exchanger

A series of experiments to determine the most suitable operating conditions for the purification process were carried out and the results were plotted as concentration of the solute in the product exiting from the bottom of the column versus volume displaced. The first set of experiments was executed to investigate the feasibility of using cation exchanger as adsorbent inside the column. Tris + NaOH buffer with pH ranging from 5.2 to 8.0 and ionic strengths as low as 0.05 and as high as 0.4 was used on CM - Sepharose cation exchanger. The experimental results shown on Tables 1, 2, and 3, indicate that, except in the case of 0.05M ionic strength at pH 5.2, the protein mixture (enzyme + undesired proteins did not show any binding on the exchanger. This could be easily attributed to the fact that for pH higher than the isoelectric point of the enzyme mixture (4.5), the mixture carries negative charge. Hence, it will not bind to the cation, and the little adsorption that took place at the lower ionic strength showed the adsorbent with no preferential capability; hence, there is no possibility of separation. The same was observed for pH 7.0 and 8.0.

In the next series of experiments, the buffer acetate (acetic acid + sodium acetate) was used. Three pH levels, 4.5, 5.0 and 5.5 at low ionic strengths (0.05 - 0.1) were investigated. The results shown on Table 4 pointed to some adsorption for the enzyme at pH 4.5 and 5.0. Nevertheless, pH 4.5 is not suitable because it is the isoelectric

point of the mixture (the zero charge point). And once we go to higher ionic strenghts, the enzyme mixture will not bind to the exchanger and no separation should be expected. For pH 5.0, the adsorption was close to 50% with regard to the enzyme which means that only 50% of the enzyme activity would be recoved when we go to the desorption step, an achievement that does not meet our goal. For pH 5.5 the same conclusion holds true. In addition it is safer to start with pH that is at least 1.0 pH unit above or below the isoelectric point to facilitate the binding. Using a very low pH is not good with alkaline phosphatase since its activity is unstable below pH 3.0 and it is more stable above its isoelectric point. For proteins which are most stable above their isoelectric points, it is more convenient to use an anion exchanger (Sephades Ion exchanger manual, Pharmacia Fine Chemicals of Sweden).

TABLE 1

BREAKTHROUGH DATA, CATION EXCHANGER

Ion Exchanger	=	Cation (CM - Sepharose)
Feed	=	0.02% Alkaline Phosphatase
Buffer	=	Tris + NaOH
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	5.2

Volume Displaced (cc)	Time t (min)	I.S. = .05		0.2		0.4	
		$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
6	6	0.0000	0.0000	0.1138	0.0854	0.1216	0.2142
12	12	0.0000	0.0000	0.2276	0.4045	0.3161	0.3921
18	18	0.0161	0.1202	0.2966	0.6180	0.4889	0.5912
24	24	0.0161	0.1586	0.4759	0.6809	0.5614	0.7421
30	30	0.0161	0.1586	0.4759	0.6989	0.7841	0.9001
36	36	0.0161	0.1611	0.5931	0.6202	0.9321	1.0211
42	42	0.0201	0.1635	0.5448	0.6831	1.0021	1.0621
48	48	0.0201	0.1923	0.6276	0.6719	0.9948	1.0000
54	54	0.0161	0.1778	0.6414	0.6764	0.9214	0.9841
60	60	0.0201	0.1778	0.6414	0.6652	0.9721	1.0021

TABLE 2

BREAKTHROUGH DATA, CATION EXCHANGER

Ion Exchanger	=	Cation (CM - Sepharose)
Feed	=	0.02% Alkaline Phosphatase
Buffer	=	Tris + NaOH
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	7.0

Volume Displaced (cc)	Time t (min)	I.S. = 0.05		I.S. = 0.1		I.S. = 0.4	
		$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
6	6	0.0123	0.0065	0.0117	0.0546	0.0099	0.0112
12	12	0.7284	0.7778	0.5088	0.5355	0.4106	0.3090
18	18	0.8580	0.8562	0.8246	0.6995	0.9338	0.8371
24	24	0.8951	1.0980	0.8596	0.9399	0.9669	0.9551
30	30	0.8704	1.0719	0.9006	1.1038	0.9735	0.8764
36	36	0.9012	1.0980	0.7719	0.9945	0.9669	0.9157
42	42	0.9259	1.0523	0.8479	0.9945	0.9669	0.7303
48	48	0.9259	1.0131	0.8713	1.0492	0.9669	0.9045
54	54	0.9321	1.0131	0.8187	1.0710	0.9272	0.9101
60	60	0.9198	1.0327	0.8304	1.0493	0.9603	0.9213

TABLE 3

BREAKTHROUGH DATA, CATION EXCHANGER

Ion Exchanger	=	Cation (CM - Sepharose)
Feed	=	0.02% Alkaline Phosphatase
Buffer	=	Tris + NaOH
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	8.0

Volume Displaced (cc)	Time t(min)	I.S. = 0.2		I.S. = 0.4	
		$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
6	6	0.0062	0.0095	0.0203	0.0495
12	12	0.3704	0.1279	0.2770	0.4231
18	18	0.6234	1.0663	0.9797	1.0110
24	24	0.8148	0.8531	1.0878	1.1263
27	27	0.7816	0.7204	1.1621	1.1211
30	30	0.7778	0.7286	0.9979	1.0329
36	36	0.7840	0.6811	1.002	1.0000
39	39	0.7840	0.6778	1.0000	1.0121
42	42	0.8889	0.6161	1.0021	0.9725
45	45	0.8827	0.6919	1.0311	0.9921

TABLE 4

BREAKTHROUGH DATA, CATION EXCHANGER

Ion Exchanger	=	Cation (CM - Sepharose)
Feed	=	0.02% Alkaline Phosphatase
h	=	8 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
Buffer	=	Acetic Acid + Sodium Acetate

Volume Displaced (cc)	Time t(min)	pH 4.5 I.S. = 0.05		pH 5.0 I.S. = 0.1		pH 5.5 I.S. = 0.1	
		$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
6	6	0.0000	0.0000	0.0059	0.0243	0.0214	0.1811
12	12	0.0000	0.0488	0.0778	0.2683	0.6143	0.8478
18	18	0.0000	0.0691	0.4880	0.8719	0.6143	1.0000
24	24	0.0000	0.0691	0.4940	0.9085	0.6143	1.0001
27	27	0.0000	0.0694	0.4790	0.9085	0.6412	1.0001
30	30	0.0000	0.0662	0.4461	0.9085	0.6500	1.0000
36	36	0.0000	0.0813	0.4701	0.9146	0.5929	0.9994
39	39	0.0000	0.0732	0.4580	0.9146	0.5929	1.0011
42	42	0.0000	0.0813	0.4820	0.9146	0.6286	1.0131
45	45	0.0000	0.0772	0.4721	0.9112	0.5714	1.0281

2- Anion Exchanger

The following set of breakthrough experiments was designed to determine the most feasible operating conditions using an anion exchanger (DEAE Sepharose) through the study of the influence of the pH and the ionic strength on the adsorption and desorption of the enzyme mixture components. Four different pH's (4.0, 6.6, 7.4, and 8.4) with buffer concentrations (I.S.) ranging from 0.05 to 0.7M were selected. At pH 4.0 (buffer acetate), alkaline phosphatase and most of the undesired proteins carried positive charges, hence, the exit concentrations a and y rose sharply to steady values as shown on Table 5. The amount of the enzyme and undesired proteins desorbed increased with the increasing of ionic strength, and at I.S. = 0.2 almost all the enzyme and the undesired proteins desorbed. As expected, there was no significant selectivity between the components desorbed.

When the pH rose to 6.6 (Tris + NaOH buffer) less desorption at lower ionic strength (0.1) was observed. The steady values of the exit concentrations were around 47% for the enzyme and 27% for undesired proteins of the feed concentrations. Increasing the ionic strength increased the competition and reduced the interaction between the ion exchanger and the protein molecules for both the enzyme and the undesired proteins resulting in very high to almost complete desorption of the mixture at buffer concentration (I.S.) of 0.6 and 0.7, respectively. Unexpectedly, close to 50% of the enzyme was desorbed at the 0.1 buffer ionic strength. This could be attributed to the type of buffer used (Tris + NaOH). It is possible that denaturation of proteins took place in the presence of NaCl. It is also possible that side reaction might

have occurred since DEAE is known to have more than one charged group. Table 6 summarizes the experimental results obtained.

At pH 7.4 with Tris + HCl used as buffer, all proteins in the mixture including the enzyme carried negative charges. The product had essentially zero concentrations for I.S. = 0.1. However, increasing the ionic strength increases the total number of ions present in solution and decreases the strength of binding of each individual ion by increasing competition between ions for binding sites. This will decrease the interaction between the protein molecules and the adsorbent which leads to less capacity for the ion exchanger. Thus, elution will take place. As shown on Table 8 at I.S. = 0.6, $(\frac{a}{a}_{F,t=\tau}) = 0.66$ and $(\frac{Y}{Y}_{F,t=\tau}) = 0.33$ which means that the majority of alkaline phosphatase proteins were unbound to the exchanger while undesired proteins were bound relatively more strongly. Therefore, the enzyme activity per unit weight of total protein in the product stream was higher than that in the feed, i.e.

$$(a/Y)p/(a/Y)_F > 1.0$$

For I.S. = 0.7 the ratio is 1.37 while for I.S. = 0.6 the ratio is 2.0 which indicates that I.S. = 0.6 is the most favorable condition for elution of enzyme.

Finally a much higher pH (8.4) was investigated. The results (Table 9) showed that the enzyme was adsorbed at levels as low as 0.1 strength and as high as 0.7. When the ionic strength increased to 0.7, the enzyme did not elute to a satisfactory level (15% compared to 25% for undesired proteins). The results for all conditions investigated for the anion exchanger are shown in Figures 5, 6, and 7. It is obvious that a system based on adsorption at pH 7.4 and I.S. = 0.1 followed by

elution of enzyme at I.S. = 0.6 and the same pH is the most promising. To make sure of this conclusion, adsorption-desorption experiments for the cases 0.1, 0.6 and 0.1, 0.7 at pH 7.4 were conducted. As pointed out in Tables 10 and 11 and Figure 8, the case with 0.1, 0.6 is the most suitable one for purifying the enzyme. To desorb the undesired proteins and regenerate the column, a lower pH (4.0) was preferable to a higher ionic strength at pH 7.4.

TABLE 5

BREAKTHROUGH DATA, ANION EXCHANGER

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Sodium Acetate + Acetic Acid
Feed	=	0.02% Alkaline Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	4.0

Volume Displaced(cc)	Time t(min)	I.S. = 0.05 a/a_F	I.S. = 0.05 y/y_F	I.S. = 0.1 a/a_F	I.S. = 0.1 y/y_F	I.S. = 0.2 a/a_F	I.S. = 0.2 y/y_F
3	3	0.0000	0.0100	0.0000	0.0200	0.0000	0.0045
9	9	0.2692	0.5000	0.1622	0.6839	0.3315	0.3545
15	15	0.5385	0.7885	0.5946	0.7677	0.5674	0.5545
21	21	0.5154	0.8221	0.6486	0.7935	0.8876	0.7091
27	27	0.5346	0.8173	0.6847	0.8194	0.9157	0.8273
33	33	0.5654	0.8221	0.6847	0.7968	0.9831	0.8500
36	36	0.5808	0.8654	0.7117	0.8129	0.9324	0.7455
39	39	0.6038	0.8317	0.6712	0.8290	0.9382	0.8455
42	42	0.6115	0.8798	0.7252	0.8290	0.9438	0.8773
45	45	0.6269	0.8221	0.7117	0.8324	0.9438	0.8727
48	48	0.6615	0.8558	0.7072	0.8258	0.9213	0.8227
51	51	0.6692	0.8077	0.7793	0.8419	0.9551	0.8455
57	57	0.6692	0.8462	0.8198	0.8484	0.9831	0.8682
60	60	0.6701	0.8464	0.8198	0.8489		

TABLE 6

BREAKTHROUGH DATA, ANION EXCHANGER

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + NaOH
Feed	=	0.02% Alkaline Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	6.6

Volume Displaced(cc)	Time t(min)	I.S. = 0.1 a/a_F	I.S. = 0.1 Y/Y_F	I.S. = 0.6 a/a_F	I.S. = 0.6 Y/Y_F	I.S. = 0.7 a/a_F	I.S. = 0.7 Y/Y_F
3	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	6	0.0063	0.0009	0.0039	0.0097	0.0070	0.0128
9	9	0.0281	0.0030	0.0787	0.0552	0.0246	0.0230
15	15	0.0156	0.0060	0.4094	0.3052	0.5986	0.4373
21	21	0.0219	0.0729	0.7244	0.6331	0.8838	0.8517
27	27	0.0719	0.2188	0.8504	0.9513	0.9049	0.8542
33	33	0.2063	0.2492	0.8465	0.9448	0.9085	0.8798
36	36	0.2813	0.2553	0.8740	0.9935	0.9120	0.8747
42	42	0.3934	0.2644	0.8858	0.9968	0.9120	0.8696
45	45	0.4469	0.2644	0.8858	1.0000	0.9225	0.8875
48	48	0.4219	0.2827	0.8898	1.0000	0.9437	0.8977
51	51	0.5250	0.2675	0.8661	0.9578	0.9401	0.8849
57	57	0.4938	0.2736	0.8819	0.9740	0.9296	0.9003

TABLE 7

BREAKTHROUGH DATA, ANION EXCHANGER

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
Feed	=	0.02% Alkaline Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	7.4

Volume Displaced(cc)	Time t(min)	I.S. = 0.1		I.S. = 0.2		I.S. = 0.4	
		a/a_F	Y/Y_F	a/a_F	Y/Y_F	a/a_F	Y/Y_F
3	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0123
12	12	0.0000	0.0000	0.0000	0.0000	0.0000	0.0173
18	18	0.0000	0.0000	0.0000	0.0000	0.0152	0.0519
24	24	0.0000	0.0000	0.0000	0.0000	0.0061	0.0346
30	30	0.0000	0.0000	0.0000	0.0000	0.0545	0.2099
36	36	0.0000	0.0000	0.0000	0.0000	0.0545	0.2148
42	42	0.0000	0.0253	0.0000	0.0000	0.0485	0.2247
48	48	0.0000	0.0207	0.0000	0.0000	0.0364	0.1951
54	54	0.0000	0.0415	0.0000	0.0000	0.0424	0.1901
60	60	0.0000	0.0253	0.0000	0.0000	0.0424	0.1951

TABLE 8

BREAKTHROUGH DATA, ANION EXCHANGER

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
Feed	=	0.02% Alkaline Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	7.4

Volume Displaced(cc)	Time t(min)	I.S. = 0.5 a/a_F	I.S. = 0.5 Y/Y_F	I.S. = 0.6 a/a_F	I.S. = 0.6 Y/Y_F	I.S. = 0.7 a/a_F	I.S. = 0.7 Y/Y_F
6	6	0.0000	0.0021	0.0000	0.0000	0.0000	0.0000
12	12	0.0239	0.1745	0.2632	0.1218	0.0304	0.0247
15	15	0.0239	0.1771	0.3841	0.1923	0.2264	0.1071
21	21	0.0287	0.1589	0.5559	0.2308	0.5405	0.2088
27	27	0.0239	0.1899	0.6112	0.2821	0.7939	0.3681
33	33	0.0335	0.2031	0.6613	0.3462	0.8378	0.3984
36	36	0.0431	0.2031	0.6504	0.3589	0.8953	0.5330
42	42	0.0431	0.2188	0.6712	0.3333	1.0135	0.6538
45	45	0.0478	0.2240	0.6524	0.3077	0.9527	0.7115
48	48	0.0670	0.2370	0.6321	0.3205	1.0405	0.7225
51	51	0.0909	0.2266	0.6943	0.3462	0.9932	0.7115
57	57	0.1866	0.2344	0.6518	0.3333	1.0338	0.7473
60	60	0.1866	0.2370	0.6216	0.3561	1.0270	0.7720

TABLE 9

BREAKTHROUGH DATA, ANION EXCHANGER

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + Hcl
Feed	=	0.02% Alkaline Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}
pH	=	8.4

Volume Displaced(cc)	Time t(min)	I.S. = 0.1 a/a_F	I.S. = 0.1 Y/Y_F	I.S. = 0.6 a/a_F	I.S. = 0.6 Y/Y_F	I.S. = 0.7 a/a_F	I.S. = 0.7 Y/Y_F
6	6	0.0000	0.0000	0.0000	0.0321	0.0031	0.0550
12	12	0.0000	0.0113	0.0000	0.0408	0.0031	0.0183
18	18	0.0000	0.0254	0.0000	0.0671	0.0031	0.0245
24	24	0.0000	0.0198	0.0025	0.0875	0.0061	0.0367
30	30	0.0000	0.0311	0.0000	0.1137cc	0.0061	0.0459
36	36	0.0000	0.0424	0.0000	0.1137	0.0061	0.0398
42	42	0.0000	0.0480	0.0000	0.1254	0.0092	0.1529
48	48	0.0025	0.0565	0.0025	0.1166	0.0123	0.1865
51	51	0.0000	0.0565	0.0000	0.1137	0.0124	0.2171
54	54	0.0000	0.0537	0.0000	0.1137	0.0153	0.2477
60	60	0.0000	0.0565	0.0000	0.1195	0.0123	0.2446

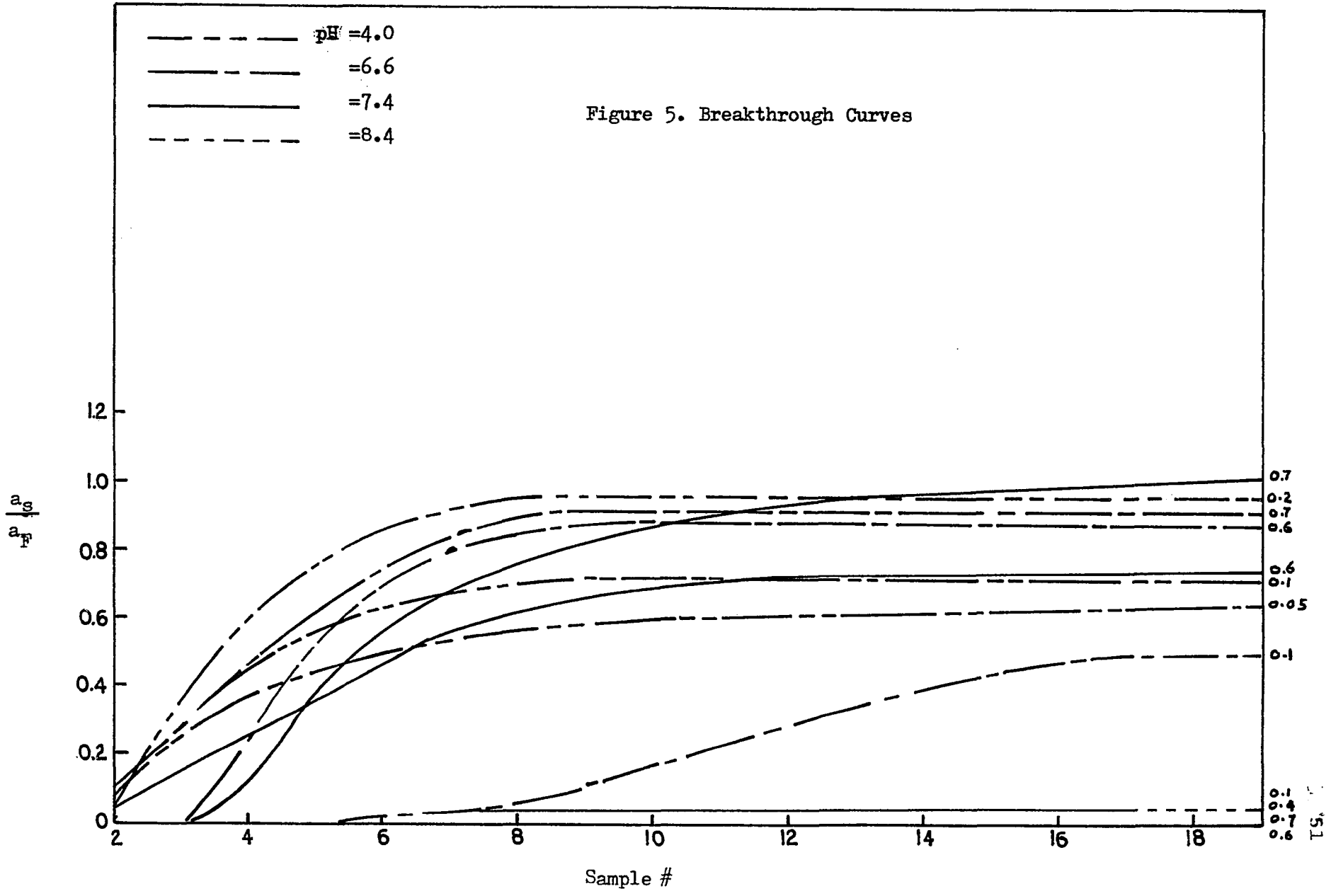


Figure 5. Breakthrough Curves

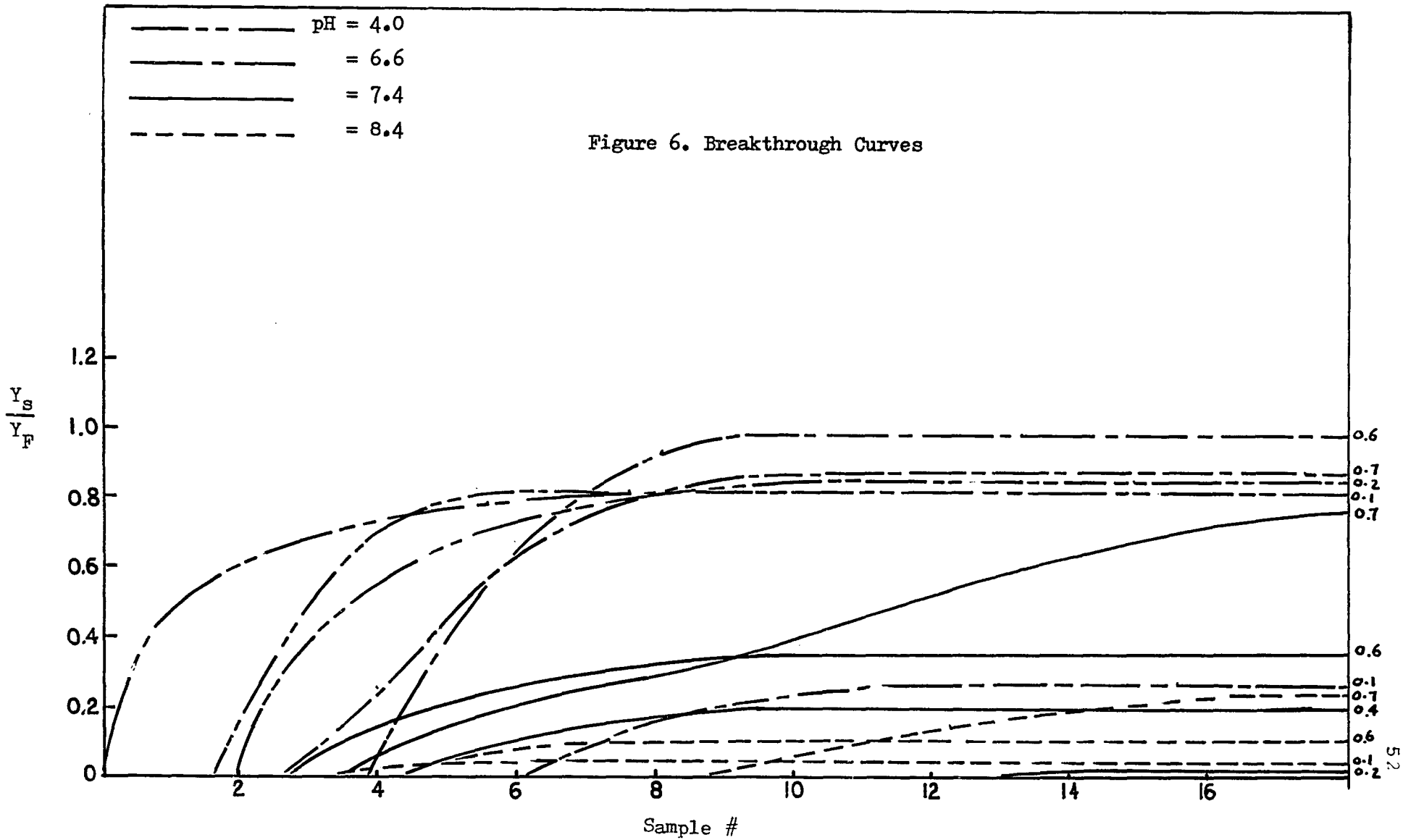


Figure 6. Breakthrough Curves

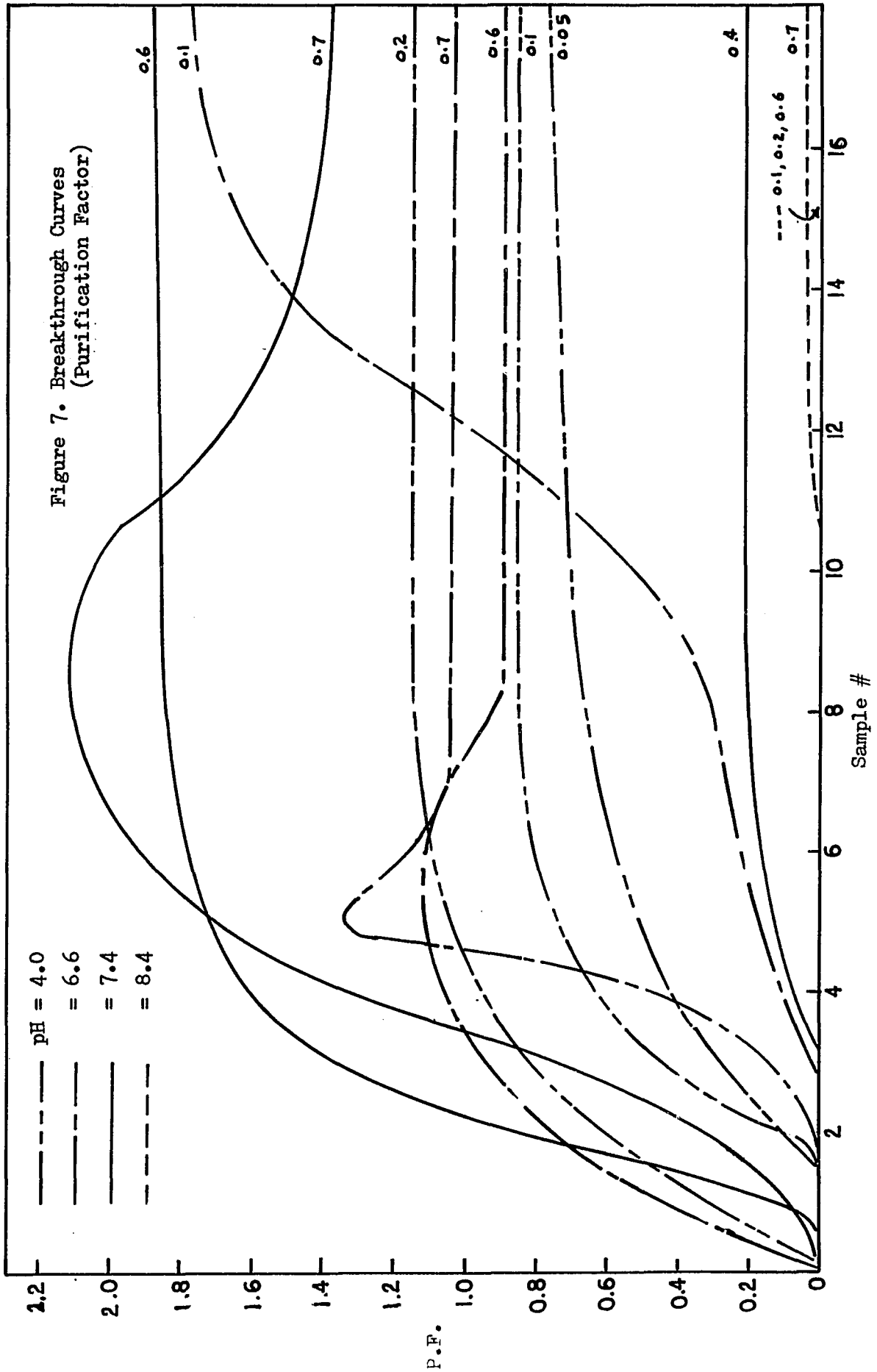


Figure 7. Breakthrough Curves
(Purification Factor)
pH = 4.0
= 6.6
= 7.4
= 8.4
0.6
0.1
0.7
0.2
0.7
0.6
0.1
0.05
0.4
0.1, 0.2, 0.6
0.7
P.F.
Sample #
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2
4
6
8
10
12
14
16

TABLE 10

ADSORPTION - DESORPTION BREAKTHROUGH DATA

AT pH 7.4

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
Feed	=	0.02% Alkaline Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{O}

Volume Displaced(cc)	Feed I.S.	$\frac{a}{a_F}$	$\frac{Y}{Y}$	Feed I.S.	$\frac{a}{a_F}$	$\frac{Y}{Y}$
3	0.1	0.0000	0.0000	0.1	0.0000	0.0000
12	0.1	0.0000	0.0000	0.1	0.0000	0.0000
15	0.1	0.0000	0.0000	0.1	0.0000	0.0000
21	0.1	0.0000	0.0000	0.1	0.0000	0.0000
27	0.1	0.0000	0.0000	0.1	0.0000	0.0000
30	0.1	0.0000	0.0000	0.1	0.0000	0.0000
33	0.6	0.0000	0.0000	0.7	0.0000	0.0000
42	0.6	0.0029	0.0000	0.7	0.0000	0.0000
48	0.6	0.0116	0.0000	0.7	0.6536	0.4719
54	0.6	0.0173	0.0601	0.7	3.2680	1.5303
60	0.6	1.0636	0.7933	0.7	1.6667	0.8090
66	0.6	1.8295	0.3365	0.7	1.4445	0.8090
72	0.6	1.7514	0.2404	0.7	1.1503	0.6404
78	0.6	1.5087	0.2091			
84	0.6	1.3353	0.2163			

TABLE 11

ADSORPTION - DESORPTION BREAKTHROUGH DATA

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
h	=	8.0 cm
Q	=	1.0 cc/min
T	=	288 K^{o}

Feed	pH	I.S.	Volume Displaced (cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02%	8.4	0.4	3	0.0000	0.0000
Alk.Ph.			6	0.0000	0.0000
			12	0.0000	0.0000
			18	0.0000	0.0356
			24	0.0000	0.0110
			30	0.0000	0.0466
Pure	7.4	0.6	36	0.0000	0.0247
Buffer			42	0.0000	0.0521
			48	0.0000	0.0712
			54	0.3984	0.6630
			57	3.8486	1.0301
			60	1.4104	0.3836
			63	0.4223	0.1918
			69	0.1275	0.1507
			75	0.0876	0.2219
			81	0.0478	0.0521

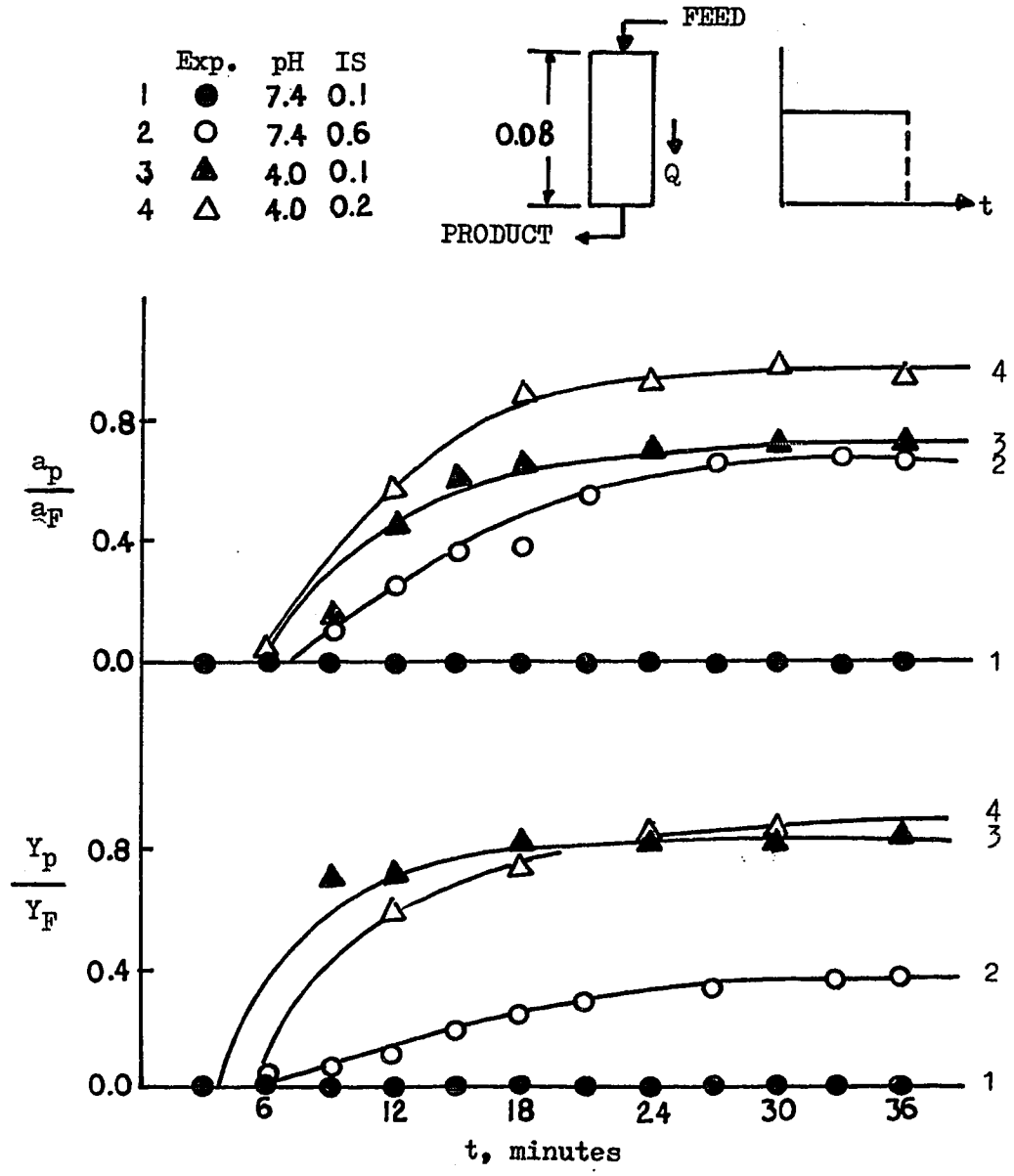


Figure 8. Effect of Buffer pH and Ionic Strength on Adsorption

B- Cycling Zone Adsorption

Cycling zone adsorption is similar to parametric pumping in using a thermodynamic vaiable as driving force for separation. However, the flow is an undirectional one. The basic apparatus consists of a chromatographic column with an inlet stream arranged so that its buffer pH and I.S., and enzyme concentrations are varied periodically as shown in Figure 9. A product stream is withdrawn from the bottom of the column. The product is time-dependent in that the concentrations (a_p,Y_p) vary continuously. However, the apparatus will eventually reach a repeating state where the product concentrations a_p,Y_p, repeat from cycle to cycle. The system used is a traveling wave mode system, i.e., the entering fluid pH or ionic strength is changed. And when these waves of fluid reach a spot in the column, the solid adsorbent will change its pH or ionic strength and will adsorb or release solute.

Several experiments were done for two reasons: first, to examine the feasibility of the cycling separation and hence form an idea about the operating conditions when applied to the parametric pumping; and second, for matter of comparison with parametric pumping later. Two cases were studied. In the first case (one pH, 7.4) the ionic strength was employed as the cyclic variable. Different displacements were examined. As shown on Tables 12, 13, 14 and 15 and Figures 9 and 10, the protein mixture including the enzyme was adsorbed on the solid phase at lower ionic strength (0.1) and released in more purified form at higher ionic strength (o.5M). The high ionic strength forces the solute to move faster because it is more concentrated, while the lower ionic strength will tend to slow the solute velocity because it is less con-

centrated. The slowly moving solute will be overtaken by the ionic strength wave. Hence, the ionic strength will decrease, and when this occurs, the adsorbent releases more solute into the moving fluid phase. The 0.5M was used instead of 0.6 because of fear that mixing might take place if difference between the two largest ionic strengths is not apparent. If this happens, it will cause the enzyme to drag with it more undesired proteins. At ionic strength equal 0.8M, the column will be regenerated by expelling the undesired proteins out of it. In the second case pH 4.0, which is less than the isoelectric point of the mixture, the undesired proteins were desorped because of their net positive charge at this pH. For pH 4.0, two cases for the enzyme elution buffer were studied, 0.6 ionic strength and 0.8. As shown in Tables 16 and 17, purification with 0.6 is higher than 0.8. This is because 0.8M is relatively strong and this causes more undesired proteins to desorb with the major purified stream.

It was found that a displacement of 15.0 cc for a column of height 8.0 cm would be adequate for minimizing the intermixing and reaching suitable separation. This shows that the cycling zone adsorption process has an optimum displacement which depends on the time the wave concentration needs to pass through the column.

Another set of experiments was carried out using the multiple zone mode. The results for the two column system shown on Tables 18 and 19 and Figure 12 indicate higher purification and more enzyme recovery.

TABLE 12

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

VARIABLE DISPLACEMENT

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
h	=	8.0 cm
O	=	1.0 cc/min
Displacement	=	9.0 cc
Feed	=	0.02% Alkaline Phosphatase
T	=	288 K^{O}
pH	=	7.4

Volume Displaced (cc)	Feed I.S.=o.1		Feed 0.5		Buffer 0.8	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
3	0.0000	0.0006	0.0471	0.2653	2.0706	1.1667
6	0.0002	0.0012	0.4667	0.5612	0.9961	1.2891
9	0.0011	0.0016	1.5137	0.5272	0.2275	0.6871
3	0.0235	0.2177	0.2824	0.4218	2.2588	1.2585
6	0.0078	0.1122	0.7608	0.4966	0.9176	1.2109
9	0.0000	0.1122	1.1020	0.4082	0.2431	0.7109
3	0.0235	0.1837	0.3529	0.4456	1.9871	1.1146
6	0.0000	0.0986	0.7647	0.4592	0.9321	1.0008
9	0.0000	0.1327	1.1843	0.4082	0.2614	0.7325

TABLE 13

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

VARIABLE DISPLACEMENT

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
h	=	8.0 cm
Q	=	1.0 cc/min
Displacement	=	12 cc
Feed	=	0.02% Alk. Phosphatase
T	=	288 K^{o}
pH	=	7.4

Volume Displaced(cc)	Feed I.S. = 0.1		Feed I.S. = 0.5		Buffer I.S. = 0.8	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
3	0.1820	0.3792	0.0000	0.0283	0.8159	0.2302
6	0.0293	0.0623	0.0147	0.1057	1.1004	0.5095
9	0.0084	0.0264	0.3306	0.3113	1.1297	1.1340
12	0.0000	0.0000	0.5251	0.2359	0.3808	0.7906
3	0.1799	0.3151	0.0021	0.0057	0.6820	0.2132
6	0.0356	0.1113	0.0565	0.1000	1.0335	0.3113
9	0.0063	0.0227	0.3306	0.2774	0.8494	0.9378
12	0.0021	0.0472	0.4373	0.2434	0.3431	0.6604

TABLE 14

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

VARIABLE DISPLACEMENT

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
h	=	8.0 cm
Q	=	1.0 cc/min
Displacement	=	15.0 cc
Feed	=	0.02% Alk. Phosphatase
T	=	288 K^{o}
pH	=	7.4

Volume Displaced (cc)	Feed I.S. = 0.1		Feed I.S. = 0.5		Buffer I.S. = 0.8	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
3	0.0000	0.0000	0.0013	0.0040	0.752	0.2889
6	0.0000	0.0000	0.0079	0.0286	0.6874	0.2381
9	0.0000	0.0000	0.1544	0.1270	0.5304	0.8842
12	0.0000	0.0000	0.8945	0.3857	0.1926	1.1030
15	0.0000	0.0000	0.8232	0.4080	0.0858	0.6746
3	0.0343	0.4492	0.0026	0.0222	0.7718	0.2349
6	0.0040	0.1016	0.0093	0.0334	1.0436	0.2222
9	0.0000	0.0000	0.2652	0.0476	0.5502	0.8000
12	0.0000	0.0000	0.5765	0.1920	0.2269	1.0572
15	0.0000	0.0159	0.7718	0.3127	0.0937	0.6826

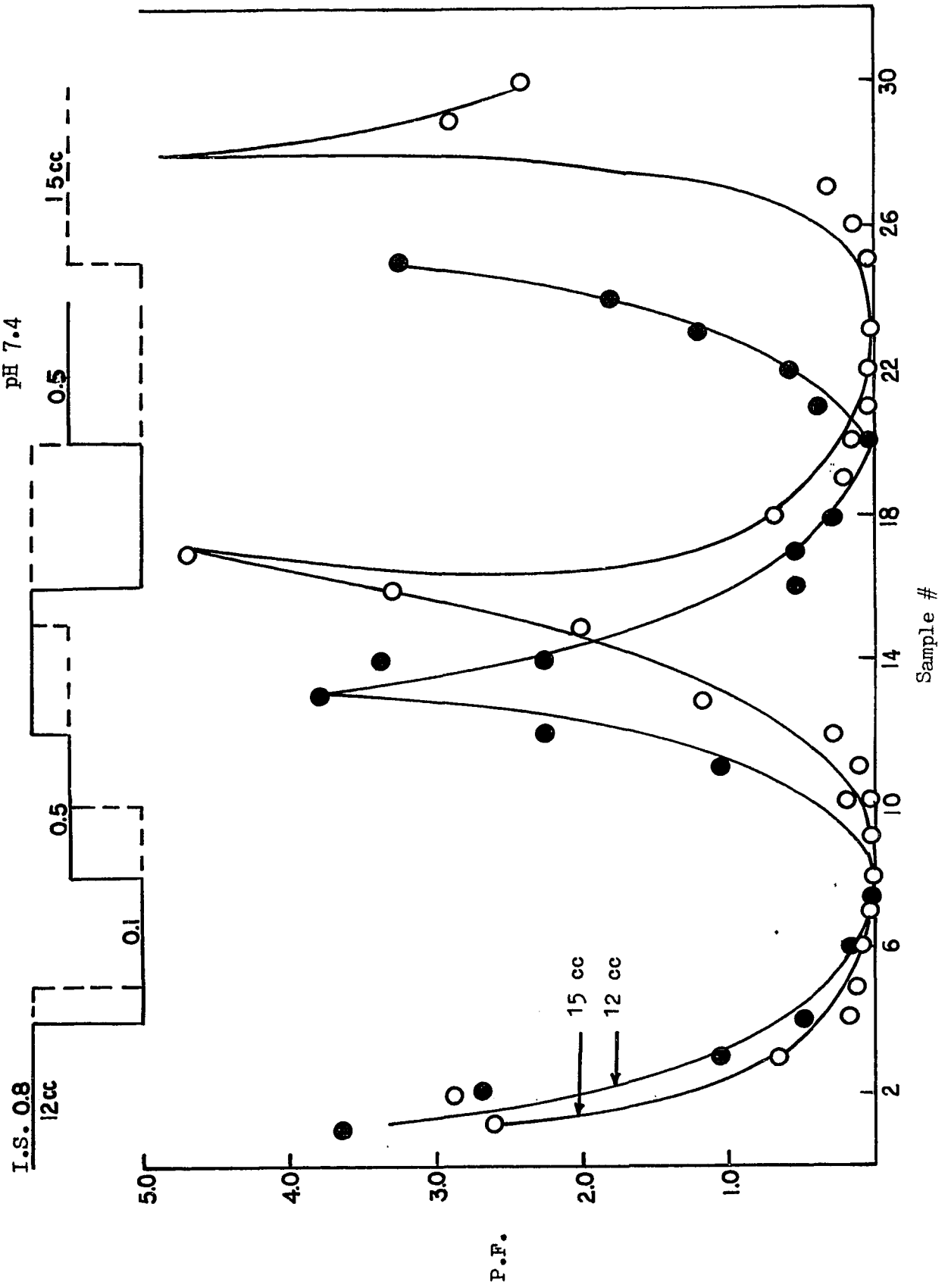


Figure 9. Cycling Zone Adsorption, Effect of Displacement

TABLE 15

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

VARIABLE DISPLACEMENT

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HC l
h	=	8.0 cm
Q	=	1.0 cc/min
Displacement	=	24 cc
Feed	=	0.02% Alk. Phosphatase
T	=	288 K^{o}
pH	=	7.4

Volume Displaced (cc)	Feed I.S. = 0.1		Feed I.S. = 0.5		Buffer I.S. = 0.8	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
3	0.0000	0.0000	0.8953	0.1755	0.3143	0.5994
6	0.0000	0.0000	0.7429	0.1573	0.1857	0.2980
9	0.0000	0.0000	0.6143	0.1325	0.1238	0.1275
12	0.0000	0.0000	0.4881	0.0977	0.1000	0.0812
15	0.0000	0.0000	0.4262	0.0944	0.1191	0.0646
18	0.0262	0.0199	0.6619	0.0861	0.0643	0.0315
21	0.4192	0.2533	1.3619	1.2699	0.0000	0.0000
24	0.6572	0.2351	0.5238	0.8510	0.0000	0.0000
3	0.0000	0.0000	0.8857	0.2053	0.1072	0.1076
6	0.0000	0.0000	0.7619	0.1490	0.1000	0.0530

TABLE 15 (CONTINUED)

9	0.0000	0.0000	0.6476	0.0878	0.1048	0.0563
12	0.0000	0.0000	0.5476	0.0961	0.0691	0.0331
15	0.0000	0.0000	0.4619	0.0812	0.0000	0.0000
18	0.0262	0.0364	0.7238	0.0997	0.0000	0.0000
21	0.3357	0.2980	1.3334	1.0696	0.0000	0.0000
24	0.6048	0.2219	0.5762	0.8440	0.0000	0.0000

Figure 10. Cycling Zone Adsorption, Effect of Displacement

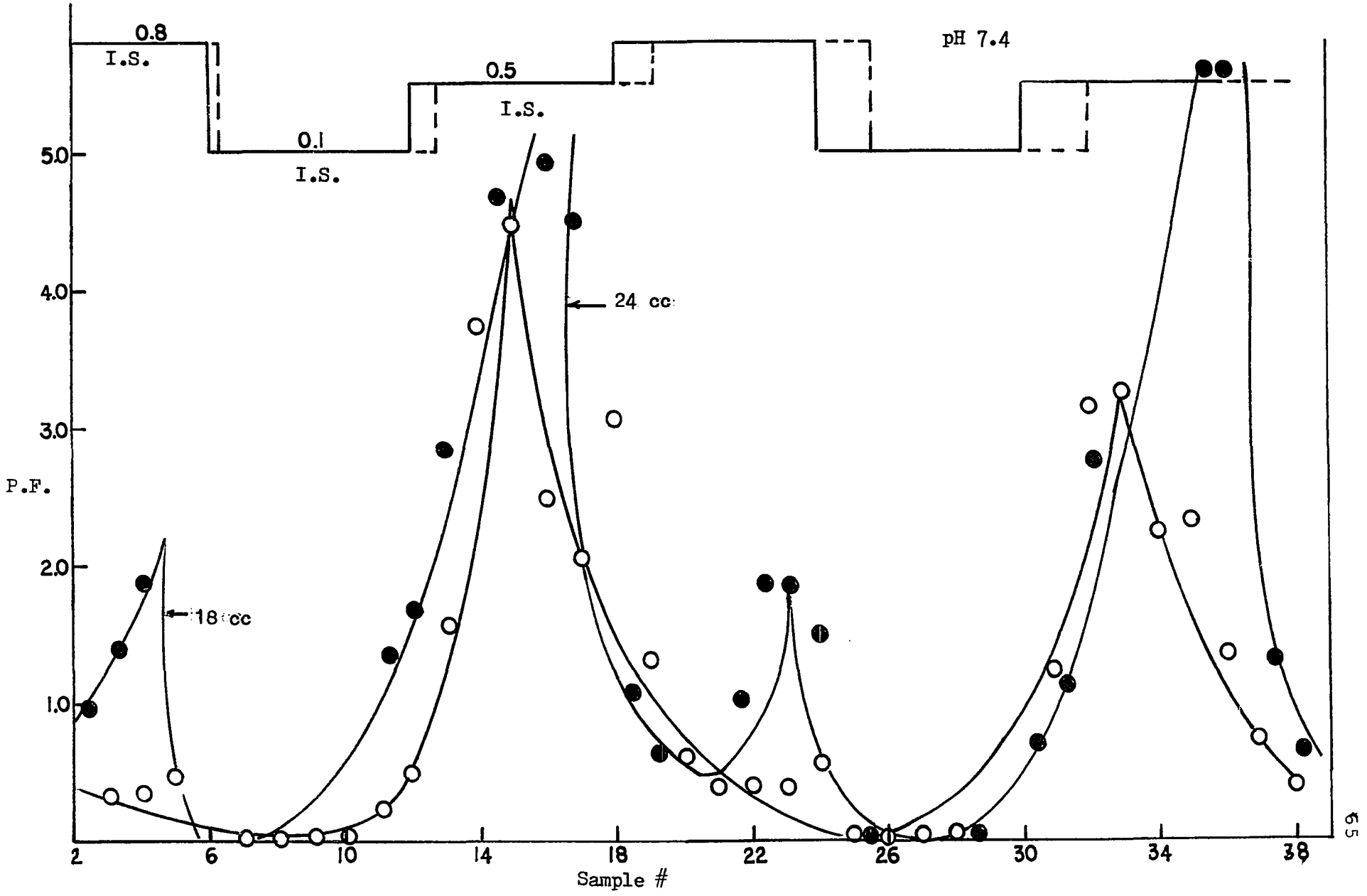


TABLE 16

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

Ion Exchanger	=	Anion (DEAE)
h	=	8.0 cm
Q	=	1.0 cc/min
Displacement	=	15.0 cc
T	=	288 K^{o}
Feed	=	0.02% Alk. Phosphatase, pH 7.4 , I.S. = 0.1

Volume Displaced (cc)	pH	I.S.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
3	7.4	0.1	0.0000	0.0400	0.0000
6			0.0000	0.0300	0.0000
9			0.0000	0.0200	0.0000
12			0.0000	0.0200	0.0000
15			0.0000	0.0200	0.0000
3	7.4	0.6	0.0200	0.1100	0.1500
6			0.4900	0.4500	1.0900
9			0.8300	0.4900	1.7200
12			1.3900	0.4400	3.1600
15			0.9600	0.2600	3.6300
3	4.0	0.1	0.3900	0.2200	3.2700
6			0.0200	0.0300	0.50
9			0.0000	0.0400	0.0000
12			0.0000	0.0100	0.0000
15			0.0000	0.0300	0.0000

TABLE 16 (CONTINUED)

3	7.4	0.1	0.0000	0.0060	0.0000
6			0.0000	0.1200	0.0000
9			0.0900	0.9400	0.1000
12			0.1600	1.1000	0.1500
15			0.0800	0.5500	0.1600
3	7.4	0.6	0.1500	0.6300	0.2400
6			1.0800	0.8700	1.2500
9			1.1600	0.5800	2.0200
12			0.7100	0.3400	2.0800
15			0.4500	0.2300	1.9100
3	4.0	0.1	0.0900	0.1300	0.7000
6			0.0100	0.0400	0.1250
9			0.0000	0.0300	0.1000
12			0.0000	0.0300	0.0000
15			0.0000	0.0500	0.0000
3	7.4	0.1	0.0000	0.0600	0.0000
6			0.0000	0.0600	0.0000
9			0.0000	0.0400	0.0000
12			0.0000	0.0500	0.0000
15			0.0300	0.2100	0.1600
3	7.4	0.6	0.1300	0.2200	0.5900
6			0.9000	0.5000	1.8000
9			1.3500	0.5900	2.3200
12			0.7600	0.3500	2.1900
15			0.3900	0.2300	1.700

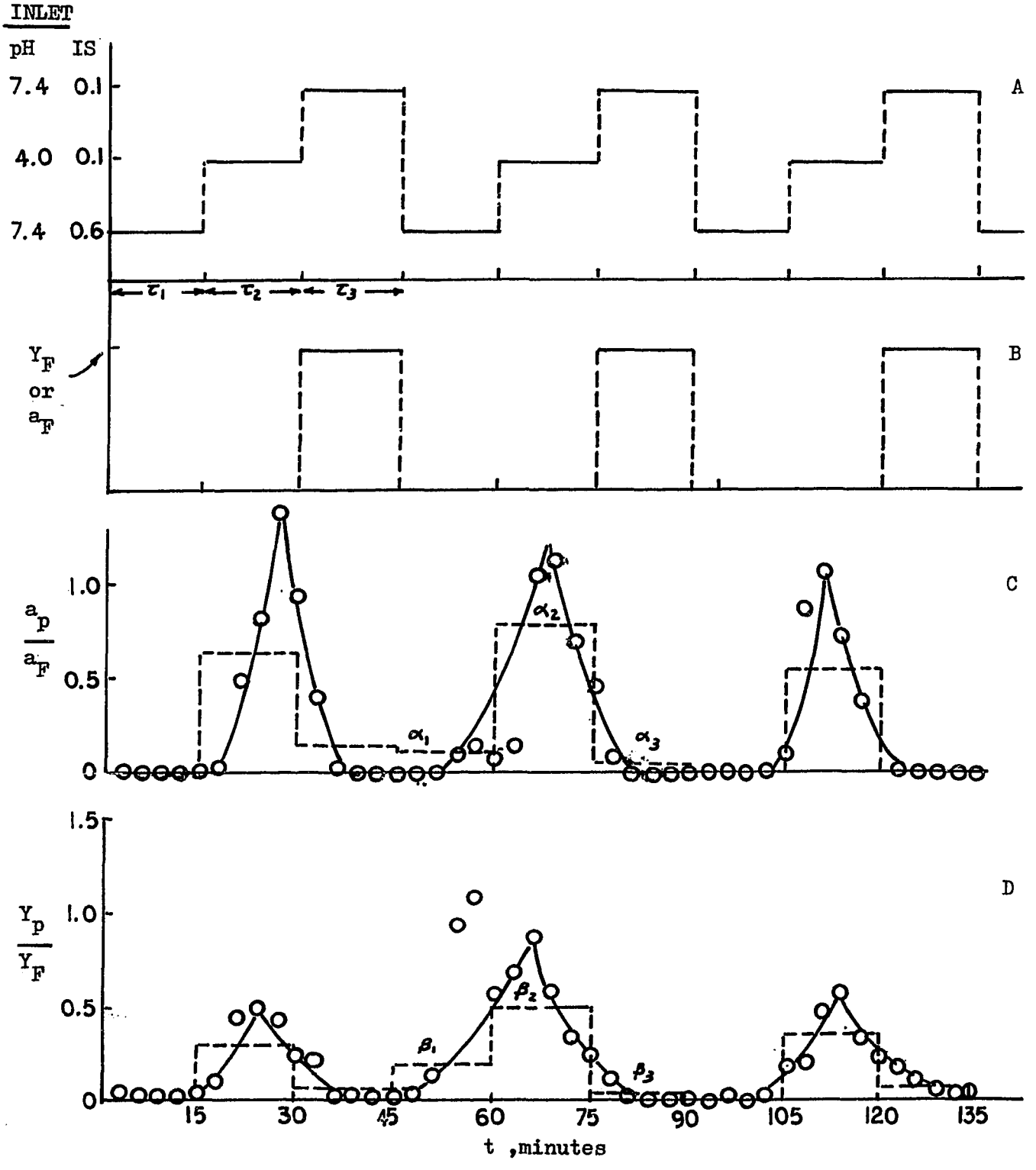


Figure 11. Cycling Zone Adsorption

TABLE 17

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

Ion Exchanger	=	Anion (DEAE)
h	=	8.0 cm
Q	=	1.0 cc/min
Displacement	=	15.0 cc
T	=	288 K^{o}
Feed	=	0.02% Alk. Ph. pH 7.4, I.S. 0.1

Product Stream

Volume Displaced (cc)	pH 7.4, I.S.=0.1		I.S.=0.8		pH 4.0, I.S.=0.1	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
3	0.0000	0.0075	0.0214	0.0224	0.2429	0.2575
6	0.0000	0.0000	1.1643	0.4291	0.0643	0.1604
9	0.0000	0.0112	1.5214	0.5746	0.0179	0.0970
12	0.0000	0.0112	0.8536	0.5373	0.0179	0.0784
15	0.0000	0.0000	0.4786	0.2686	0.0036	0.0224
3	0.0393	0.1455	0.0393	0.0336	0.2036	0.1791
6	0.0143	0.0336	1.0321	0.4440	0.1143	0.3582
9	0.0000	0.0224	1.3786	0.5336	0.0321	0.1455
12	0.0000	0.0000	0.7429	0.3657	0.0107	0.1157
15	0.0000	0.0000	0.4607	0.3955	0.0071	0.0448

TABLE 18

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

TWO COLUMNS

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
Q	=	1.0 cc/min
Displacement	=	15.0 cc
Feed	=	0.02% Alkaline Phosphatase
pH		7.4

I.S.:		0.1		0.5		0.8	
Volume Displaced (cc)	Time t (min)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
3	3	0.0000	0.0000	0.1602	0.2560	1.4574	0.9814
6	6	0.0000	0.0000	0.2481	0.2913	1.1318	1.2820
9	9	0.0000	0.0000	0.5031	0.3247	0.5840	1.0297
12	12	0.0000	0.0000	1.5194	0.4230	0.3075	0.4787
15	15	0.0000	0.0000	2.5581	0.7273	0.1111	0.1484
3	3	0.0000	0.0000	0.3152	0.2115	1.3437	1.0241
6	6	0.0000	0.0000	0.3103	0.2078	0.4134	0.8275
9	9	0.0000	0.1262	0.5271	0.2208	0.4806	0.9907
12	12	0.0000	0.0315	1.4729	0.3432	0.2817	0.8256
15	15	0.0000	0.0000	1.9069	0.6586	0.1602	0.5250

Figure 12. Cycling Zone Adsorption, Two Column System

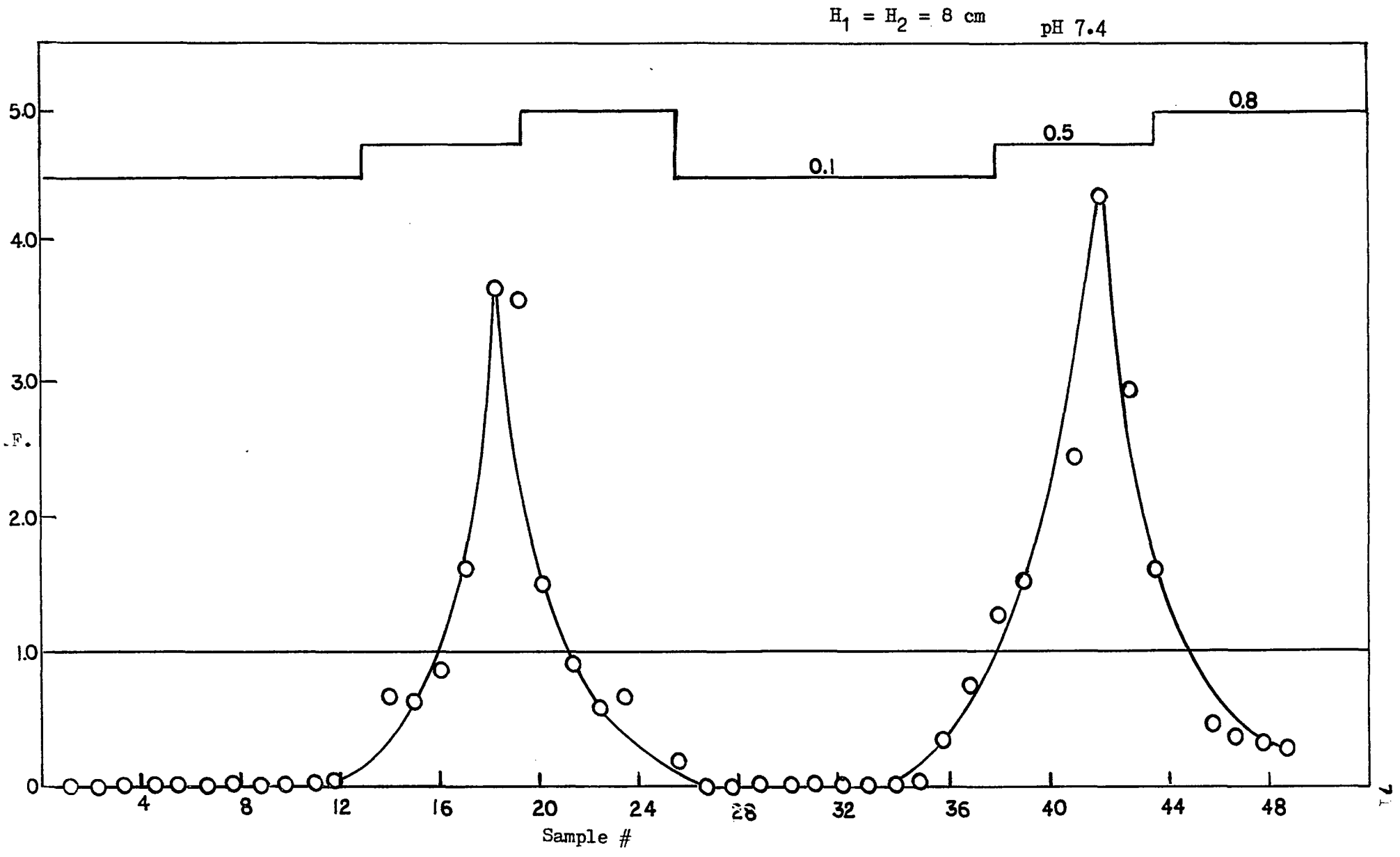


TABLE 19

EXPERIMENTAL RESULTS FOR CYCLING ZONE ADSORPTION

TWO COLUMNS

Ion Exchanger	=	Anion (DEAE)	
Buffer	=	Tris + HCl, pH 7.4	
Q	=	1.0 cc/min	
Displacement	=	15.0 cc	
Feed	=	0.02% Alkaline Phosphatase	
I.S.:	0.1	0.6	pH 4.0

Volume Displaced (cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
3	0.0000	0.0000	0.0128	0.0190	0.0090	0.2184
6	0.0000	0.0000	1.3654	0.3544	0.0321	0.0791
9	0.0000	0.0000	1.9359	0.4842	0.0000	0.0000
12	0.0001	0.0002	0.8590	0.4367	0.0000	0.0094
15	0.0000	0.0000	0.3462	0.4557	0.0000	0.0000
3	0.0321	0.1297	0.3782	0.2215	0.0641	0.1239
6	0.0256	0.0917	1.8330	0.6614	0.0192	0.0012
9	0.0256	0.0633	0.9615	0.5380	0.0128	0.0063
12	0.0192	0.0601	0.8590	0.5190	0.0128	0.3924
15	0.0128	0.0475	0.2308	0.2468	0.0134	0.3214

C- Parametric Pumping

1-Preliminary Runs

Series of semi-continuous parametric pumping runs have been carried out to purify the enzyme alkaline phosphatase. The results obtained indicatied that the purification increased with the number of cycles until a steady value was reached. The ionic strength proved to be a valuable thermodynamic driving force for the purification of enzymes. To our knowledge, this is a first in the area of parametric pumping. The results also support and confirm the theory described before. Enzyme (alkaline phosphatase) migrates upward and accumulates at the top of the bed.

The first several runs were carried out in a simple way such that the purified enzyme concentrated in the top reservior while the undesired proteins accumulated in the bottom reservior. A sketch of the system is shown on Figure 13. The typical results obtained for one of those runs are shown of Table 20. It is shown that the a_n/a_f (activity in the product stream/activity in the feed) in the top reservoir increases with the number of cycles, then decreases a little to a steady value as a result of the intermixing between reserviors. A low total protein is observed. The reverse holds true for the bottom reservior, i.e., more concentrated undesired protein is observed. This implies that the enzyme and undesired proteins move in opposite directions and are concentrated in the top and bottom reserviors, respectively.

The purification factor (P.F) is plotted vs n, the number of cycles on Figure 14. The purification factor is defined as the ratio of the activity per unit of total protein in the product to

that in the feed, i.e.

$$P.F. = ({}^{a}n/ {}_{Y}n)/ (a_{F/Y_F})$$

The concentrations of the only product stream coming out of the column is essentially zero, since it is the concentration immediately after adsorption took place.

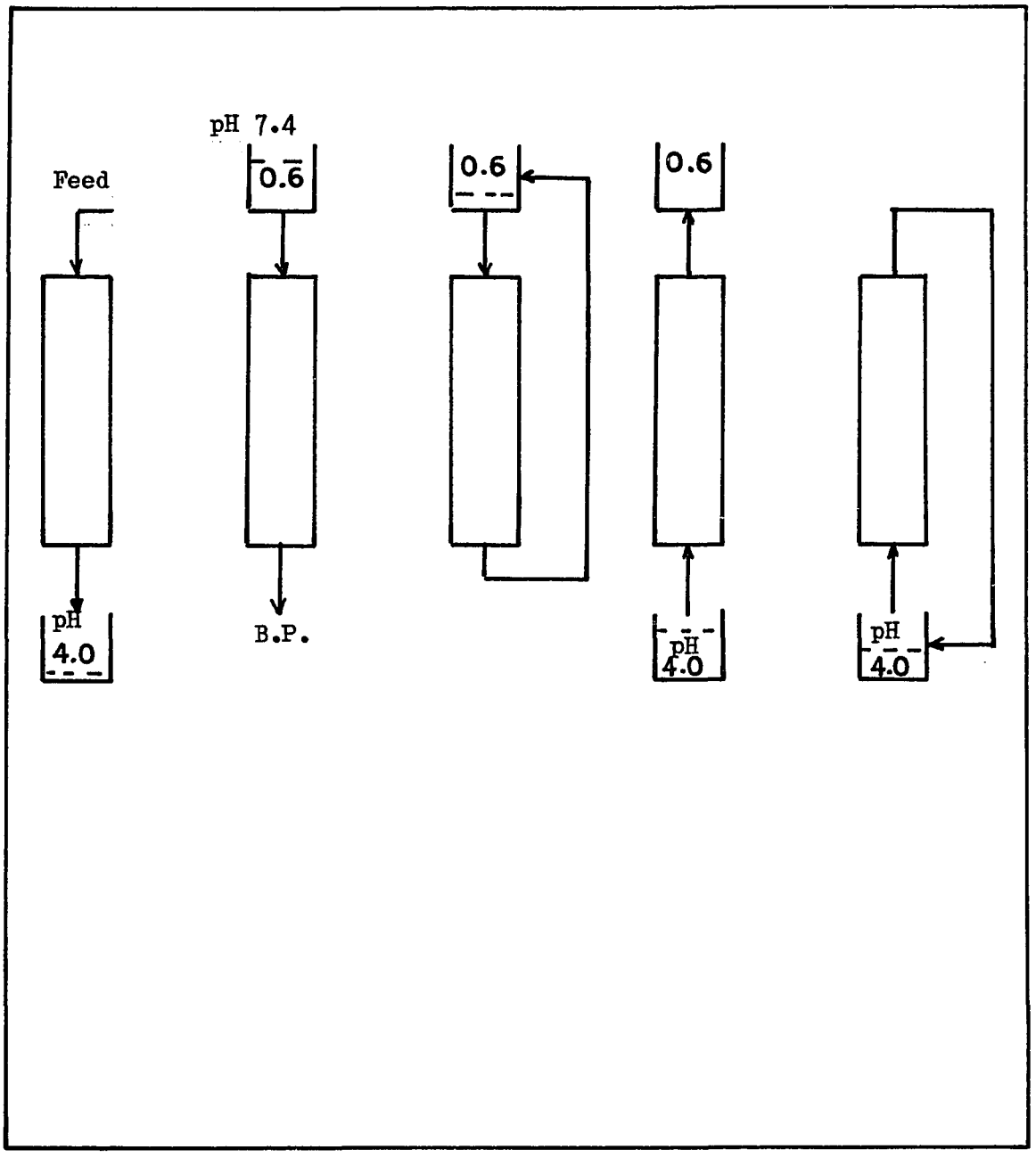


Figure 13. One Product Scheme Parametric Pump

TABLE 20

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, ONE PRODUCT SCHEME

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl for pH=7.4
	=	Sodium Acetate + Acetic Acid for pH=4.0
Feed	=	0.02% Alk.Phosphatase, I.S=0.1 , pH 7.4
h	=	8.0 cm
Q	=	1.0 cc/min
Qt	=	15.0 cc
Dead Volume	=	45 cc

Cycle No.:	0.6 Reservoir			pH=4 Reservoir	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
1	0.2166	0.1133	1.912	0.0481	0.3467
2	0.4599	0.1700	2.705	0.1123	0.6033
3	0.5241	0.1633	3.209	0.1257	0.9000
4	0.6631	0.2100	3.158	0.1390	1.1433
5	0.7914	0.2300	3.441	0.1310	1.2467
6	0.8289	0.3033	2.733	0.1898	1.3333
7	0.8984	0.3100	2.898	0.1604	1.2067
8	0.9626	0.4033	2.387	0.0508	0.3633
9	0.8943	0.3111	2.875	0.1310	0.8800

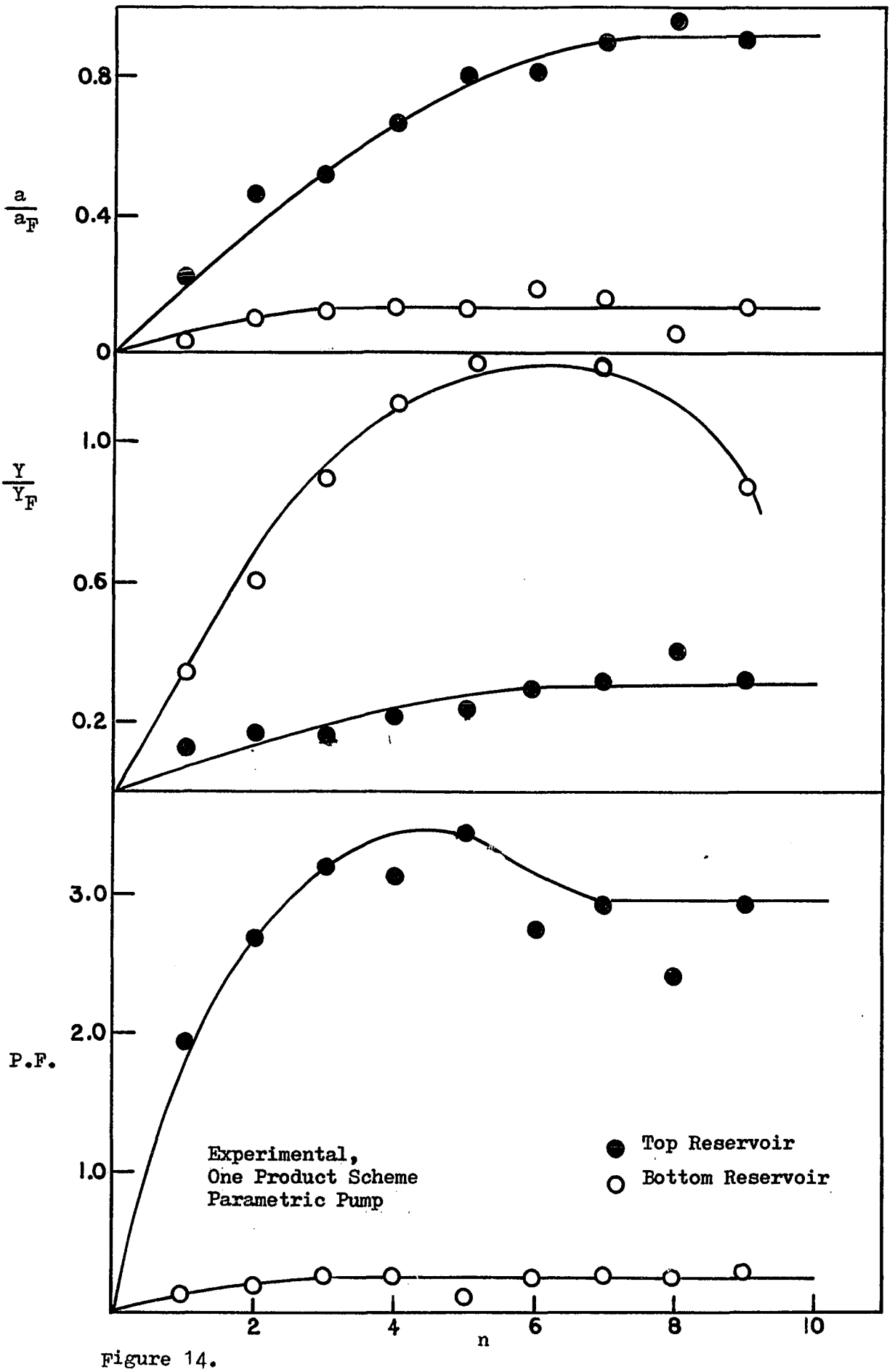

0.8
0.4
0
a/a_F
1.0
0.6
0.2
Y/Y_F
3.0
2.0
1.0
P.F.
Experimental,
One Product Scheme
Parametric Pump
Top Reservoir
Bottom Reservoir
2
4
6
8
10
n

Figure 14.

2- Demonstration

Human alkaline phosphatase from placenta, liver, and bone are glycoproteins that contain sialic acid. Since the enzyme HPAP is extracted from human placental fluid, one major undesired protein that is mixed with it is albumin. Thus, from a developmental point of view, the addition of albumin to the unpurified enzyme poses an interesting question. Can the method we developed purify the enzyme to a satisfactory level after this contamination? Our major interest is to show that the answer is yes. This is best accomplished by carrying out our investigations for this part in three separate steps. First, the albumin would be adsorbed and desorbed under the same conditions used for the enzyme, i.e., pH 7.4 with 0.1 and 0.6 ionic strengths respectively. Second, the pH 4.0 and 0.1 I.S. would be used instead of pH 7.4 and I.S. 0.6 for the desorption process. Finally, a mixture of albumin and enzyme was subjected to a parametric pumping run.

From Table 21, and 22, and Figure 16, it is significant to notice that the steady value of the albumin desorbed by using 0.6M, pH 7.4 buffer (Tris + HCl) amounts to approximately 50% of the original feed compared to 90% in case of enzyme. The failure of the exchanger (DEAE-Sepharose) to desorb the albumin in high quantities at 0.6M makes it more likely to assume relatively stronger bonding between the undesired proteins and the ion exchanger compared with the enzyme. In the case of pH 4.0, the value rose to approximately 80%. For the parametric pumping run (feed is mixture of 0.02% alkaline phosphatase + 0.02% albumin), the steady value of the total proteins including the added albumin amount to 44% of the original feed. In a regular para-

metric pumping run with feed consisting only of 0.02% alkaline phosphatase carried under the same operating conditions, the total protein (including enzyme) exiting with the enriched enzyme product is close to 36% of the original feed. This shows the utility of the method we developed as a useful separation technique. Therefore, we have demonstrated the feasibility and advantages of this semi-continuous purification process.

Based on the experimental results obtained so far, we concluded the significance of the method developed for purification of the enzyme, alkaline phosphatase, and a new chapter for optimizing the process began.

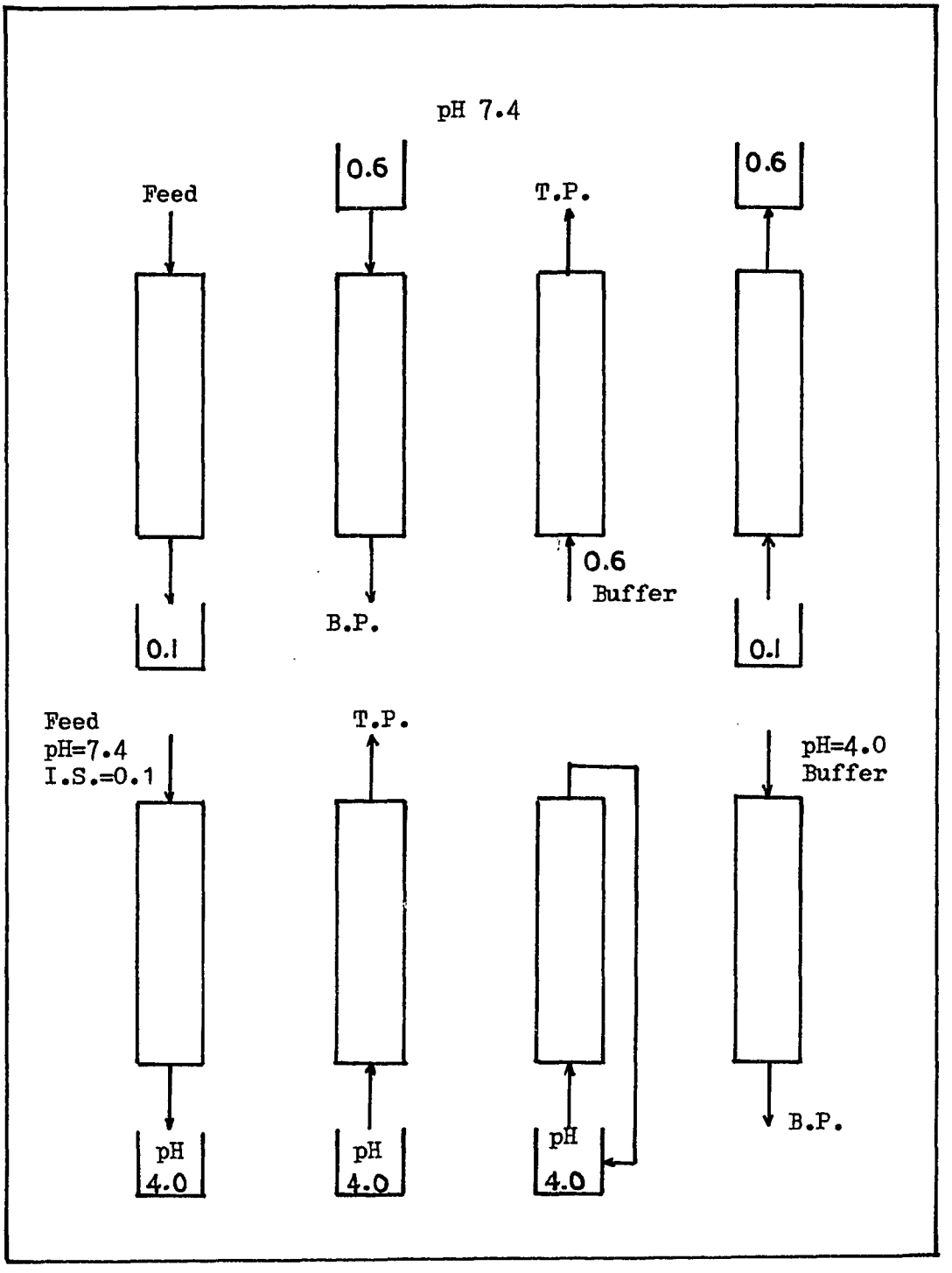


Figure 15. Scheme of Albumin Removal

TABLE 21

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, ALBUMIN

Ion Exchanger	=	Anion (DEAE)
h	=	8 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Feed	=	0.02% Albumin, I.S.=0.1 , pH 7.4
Qt	=	15 cc
Dead Volume	=	45 cc

Elution Buffer:

pH	7.4	4.0
I.S.	0.6	0.1
Cycle No.:	$\frac{Y_{T.P.}}{Y_F}$	$\frac{Y_{T.P.}}{Y_F}$
1	0.2671	0.2169
2	0.3509	0.3905
3	0.4737	0.3781
4	0.4347	0.6736
5	0.4444	0.5868
6	0.4191	0.6591
7	0.4815	0.7789
8	0.5536	0.7727
9	0.5497	0.7355
10	0.4698	0.7872
11	0.4795	

TABLE 22

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VERIFICATION OF ENZYME TAKE-OUT

Ion Exchanger	=	Anion (DEAE)	
Q	=	1.0 cc/min	
$Q_{T.P.}$	=	0.5 cc/min	
Qt	=	15 cc	
Dead Volume	=	45 cc	
h	=	8 cm	
Feed	0.02% Alk. Ph. + 0.01% Albumin		+ 0.02% Albumin

Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{Y}{Y_F}$
1	0.2825	0.2613	1.081	0.1693
2	0.3679	0.2613	1.510	0.2638
3	0.8821	0.2450	2.186	0.3996
4	0.9024	0.4036	2.351	0.5472
5	0.9329	0.3838	2.148	0.4862
6	0.8171	0.4342	2.010	0.4547
7	0.9126	0.3604	2.146	0.4449
8	0.9167	0.4252	2.027	0.4469
9	0.9228	0.3874	2.382	0.4341
10	0.8557	0.3459	2.472	0.4350

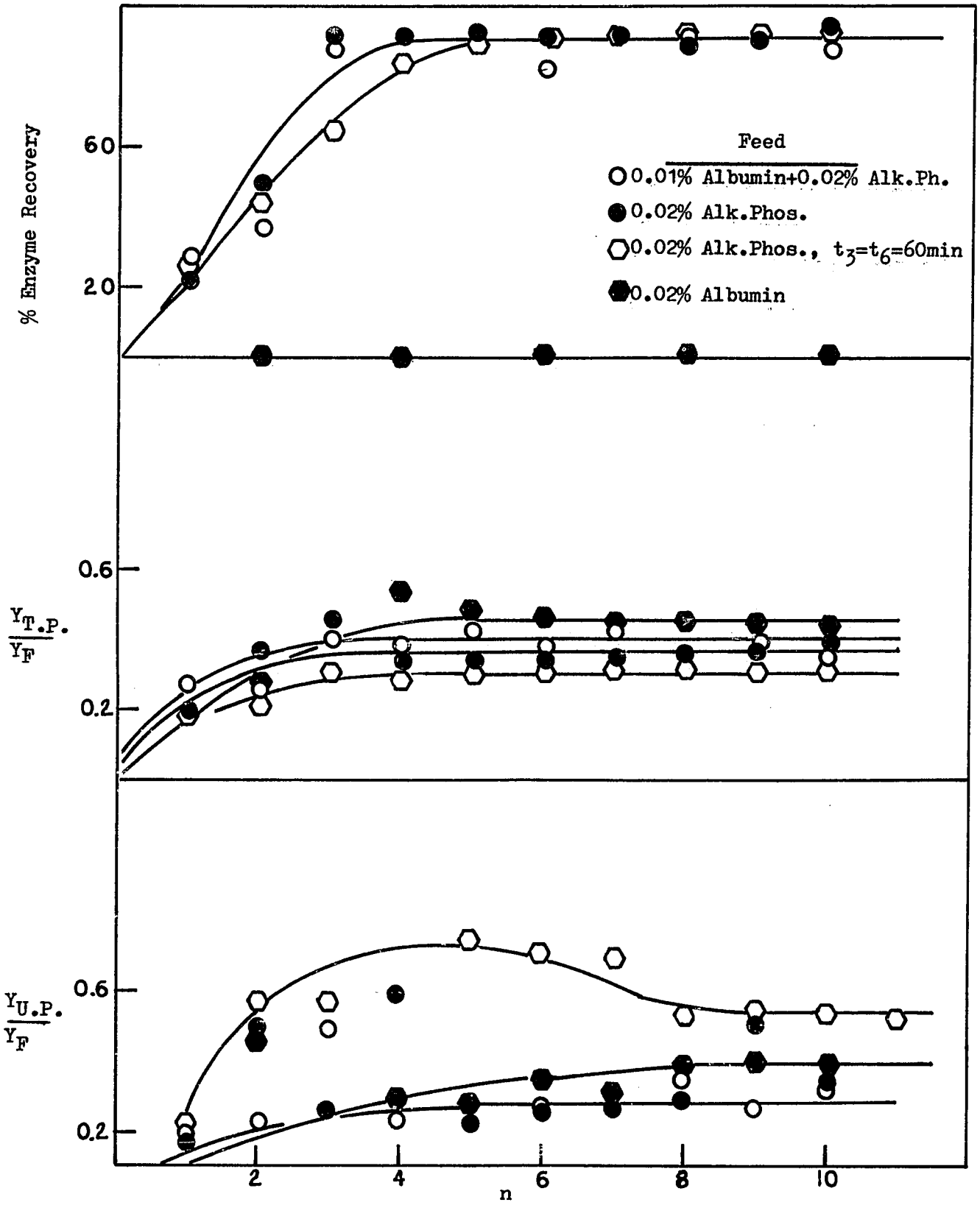


Figure 16. Albumin-Alk.Phos. Experimental Results

3- Effect of Top Product Elution Buffer, I.S.

The dependence of separation, hence purification on the ionic strength of the top product elution buffer, was demonstrated through a series of runs. The results appear on Tables 23, 24, 25, and Figure 18.

The increase in ionic strength will cause an increase in the enzyme velocity. However, a dramatic increase will result in increasing the velocity of the undesired proteins which will cause their elution, too.

An increase in the ionic strength of the top product elution buffer from 0.5M to 0.6 increases the concentration of the enzyme in the top product, therefore increasing the purification. This is due to a velocity increase as mentioned above and an increase in the competition between the enzyme mixture ions as a result of more concentrated Cl^- counter ions in the exchanger. A too strong ionic strength may weaken the adsorption of the whole mixture, and large quantities of the undesired proteins will desorb with enzyme, resulting in a less purified product as is the case with the 0.8 ionic strength buffer. Once the task of choosing the suitable conditions concerning ion exchanger, buffer pH and ionic strength has been solved, we now optimize the process by investigating the response of the para pump to changes of the operating parameters of the process one at a time.

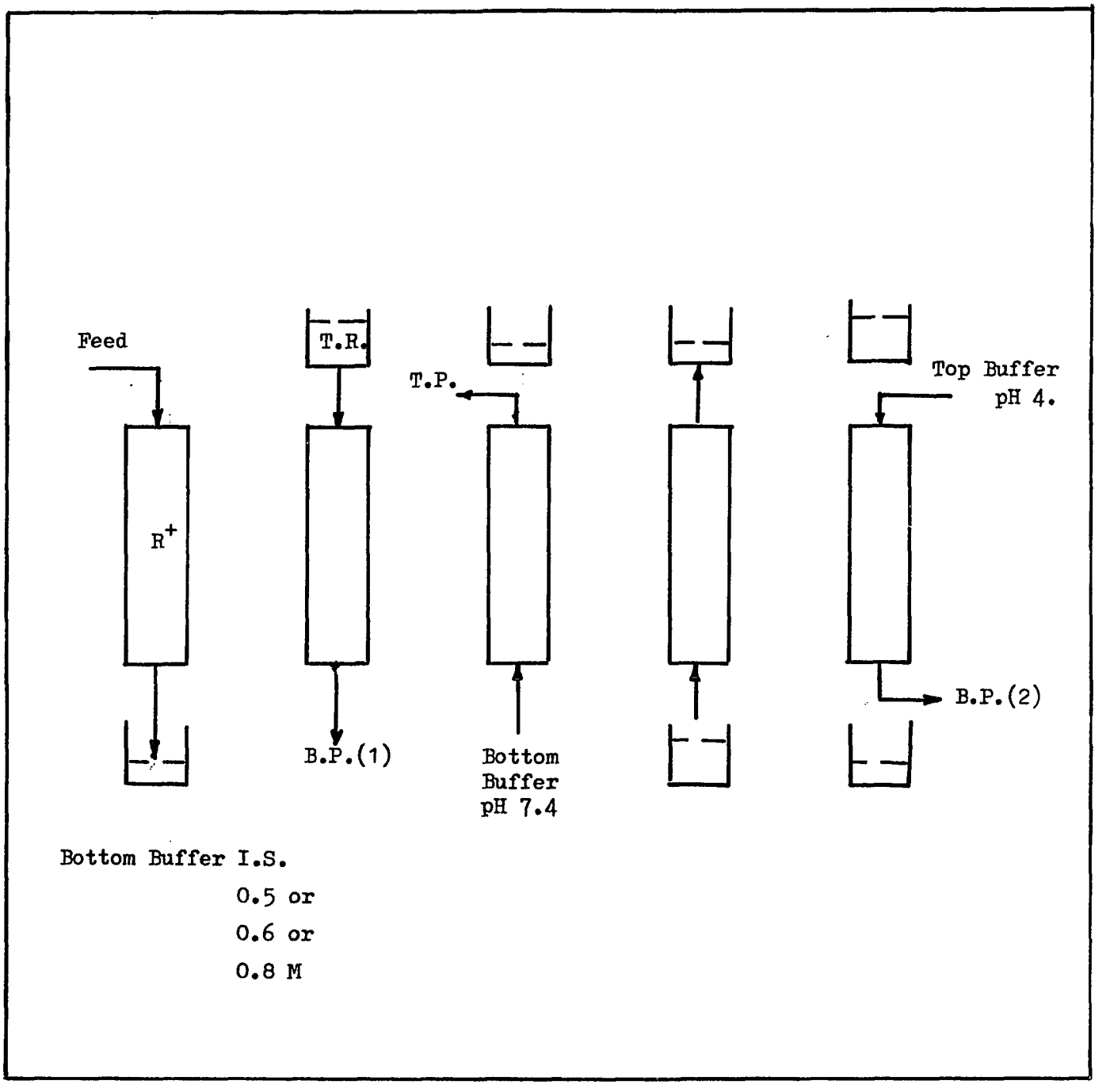


Figure 17. No Circulation Parametric Pump, Scheme of Operation
Effect of Top Product Elution Buffer I.S.

TABLE 23

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, TOP PRODUCT ELUTION

BUFFER IONIC STRENGTH = 0.5

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl For pH=7.4
	=	Acetic Acid + Sodium Acetate for pH=4.0
Feed	=	0.02% Alk. Phosphatase, I.S.=0.1 , pH=7.4
h	=	8.0 cm
Q	=	1.0 cc/min
Dead Volume	=	45 cc
Qt	=	15 cc

	pH 7.4			pH 4.0	
Cycle No.:	Top Product			Bottom Product B.P.(2)	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
1	0.1549	0.0789	1.963	0.0113	0.1170
2	0.2592	0.1725	1.503	0.0141	0.1287
3	0.3493	0.1842	1.896	0.0254	0.2573
4	0.2986	0.1696	1.761	0.0056	0.2368
5	0.3268	0.1842	1.774	0.0141	0.2749
6	0.5380	0.2310	2.329	0.0338	0.3216
7	0.4732	0.2719	1.740	0.0169	0.3655
8	0.3944	0.2456	1.606	0.0113	0.2515
9	0.4817	0.3041	1.585	0.0338	0.3684

TABLE 23 (CONTINUED)

10	0.5070	0.2602	1.949	0.0085	0.3684
11	0.6113	0.3509	1.741	0.0423	0.3947
12	0.5021	0.2800	1.793	0.0420	0.3591

TABLE 24

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, TOP PRODUCT ELUTION

BUFFER IONIC STRENGTH = 0.6

Ion Exchange	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
Qt	=	15.0 cc
Dead Volume	=	45.0 cc

Top Product

Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2138	0.2886	0.741
2	0.6449	0.4362	1.478
3	0.8080	0.4262	1.896
4	0.6377	0.3624	1.760
5	0.6413	0.2987	2.147
6	0.6558	0.3322	1.974
7	0.6630	0.3087	2.148
8	0.6014	0.3121	1.927
9	0.6051	0.2785	2.172
10	0.6450	0.3221	2.002

TABLE 25

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, TOP PRODUCT ELUTION

BUFFER IONIC STRENGTH = 0.8

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
Qt	=	15.0 cc
Dead Volume	=	45 cc

	pH 7.4			pH 4.0	
Cycle No.:	Top Product			Bottom Product B.P.(2)	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$		$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
1	0.1560	0.3090	0.505	0.0000	0.0000
2	0.4987	0.3174	1.571	0.0077	0.0337
3	0.5473	0.3933	1.392	0.0051	0.0365
4	0.7340	0.3680	1.995	0.0077	0.0169
5	0.7391	0.4775	1.548	0.0077	0.1039
6	0.7263	0.5702	1.2738	0.0077	0.0938
7	0.7570	0.5702	1.328	0.0102	0.0871
8	0.8261	0.5758	1.435	0.0128	0.0646
9	0.7877	0.6348	1.241	0.0102	0.0534
10	0.8132	0.5871	1.385	0.0102	0.0506
11	0.8159	0.5927	1.378	0.0153	0.0758

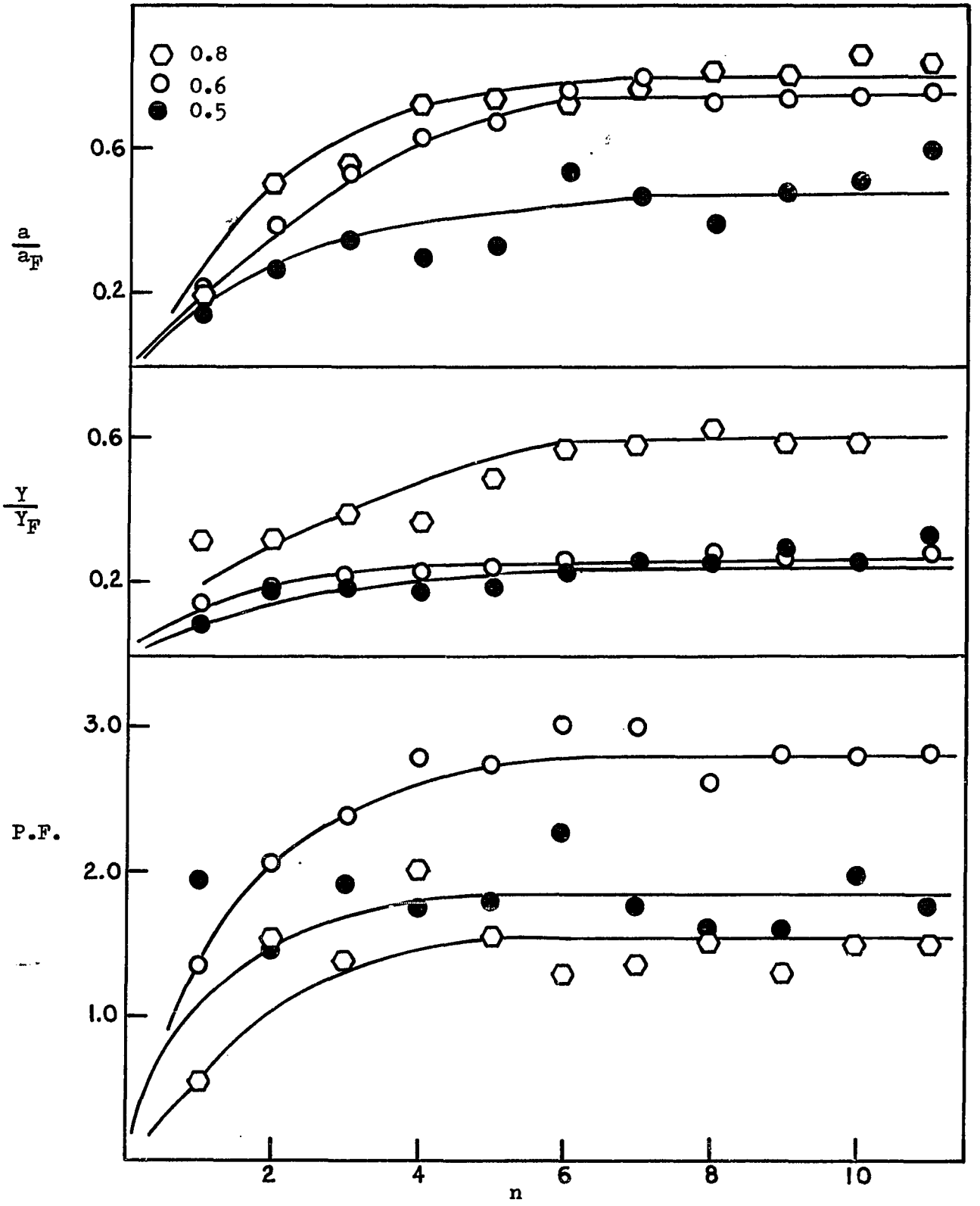


Figure 18. Effect of Top Product Elution Buffer Ionic Strength, Experimental Results

4- Effect of Flow Rate and Displacement

Since the previous parametric pumping runs showed the bottom products with almost zero enzyme concentrations, then from an overall mass balance, it is clear that the enzyme concentration in the top product is a function of different parameters. The most noticable are fluid velocity, displacement, and amount of the withdrawal product.

For this reason a series of experiments has been carried out to examine the effect of velocity and displacement on the separation process. Various flow rates ranging from 0.5 cc/min to 2.0 cc/min with four different displacements were investigated. The displacements are 9.0 cc, 12.0 cc, 15.0 cc and 18.0 cc. The results for the 0.5 cc/min flow rate are shown on Tables 26 and 27 and Figure 19.

It is found that increasing the cycle time, which means decreasing fluid flow rate (velocity), brings the operation close to equilibrium and improves the mass transfer between the fluid and solid phases. However, the fraction of the bed storage capacity is controlled by the fluid displacement. A too large displacement will result in breakthrough and intermixing between the contents of the top and bottom reservoir will take place leading to less separation. It was also found that increasing the volume of the fluid on the stage decreases the binding of the enzyme and causes it to break faster since column capacity is decreased. This could be seen in cases of 15.0 cc and 18.0 cc displacements. For displacements of 9.0 cc and 18.0 cc the enzyme concentration in the top product increased with the number of cycles, then decreased sharply to lower steady values. The decrease in the concentration of the undesired proteins was less. This caused the purification factor to decline in both cases. This could be attributed to the intermixing phenomena as

explained before. For the cases of 12.0 cc and 15.0 cc (the void volume of the column is slightly less than 15.0 cc), the purification factor increased with the number of cycles and then decreased slightly to a steady value. The steady value obtained with 15.0 cc (2.0) is about 1.25 times that of 12.0 cc where a decrease in the enzyme concentration after the sixth cycle was observed.

When the velocity was raised to 1.0 cc/min, the same changes were observed. However, the steady values of the purification factors reached were higher for each case, and no sharp decline for the enzyme concentration in the top product was seen except for the 9.0 cc case. The increase in the undesired proteins concentration of the top product with the number of cycles is more pronounced in the cases of 12.0 and 18.0 cc. This caused the purification factor to decrease as the number of cycles increased. The purification factor reached a steady value much faster in the cases of 12.0 cc and 15.0 cc displacements. This is because operating with a displacement close to the void volume of the column will decrease the possibility of a concentration wave being overtaken by a different wave of different ionic strength. The results also show poorer separation for short cycle time. This is, of course, due to lesser chance for the interphase mass transfer taking place. The longer the time, the more the chance for the enzyme to exit. However, if the time exceeded a limit, the undesired proteins will appear in large quantities too as is the case with 18.0 cc. The results of this set of runs are given on Tables 28 and 29 and Figure 20.

For the case of higher velocity (2.0 cc/min), the purification factor is less for almost every case except the 18.0 cc displacement case where it shows a slim increase over the 0.5 cc/min run. The little

separation observed indicates that higher velocities will not give the bed enough time to experience the change in the driving force thermodynamic variable (ionic strength), and as a result, the difference observed by the adsorbent is less than adequate; hence, less separation occurs. The results given on Table 30 and 31 and sketched on Figure 21 show that higher velocity will result in higher desorption for both the enzyme and the undesired proteins.

Based on the above results, another set of experiments was conducted to examine the effect of allowing more time (i.e., slower velocity, 0.5 cc/min) for the enzyme to exit into the top product (enzyme enriched stream). In the meantime, all other velocities were kept constant at 1.0 cc/min. A higher enzyme recovery and a purification factor of 2.6 were obtained in the case of 15.0 cc. This could be attributed to the improvement in mass transfer due to longer time, and also to the fact that a bottom product concentration close to zero with top reflux larger than the bottom reflux (1.0 and 0.5 respectively), as is the case here, would enhance the top product concentration.

In general, the previous results point out the importance of operating the parametric pumping porcess at an optimum velocity and displacement. Enough time should be allowed for the mass transfer between phases to take place, and a reasonable displacement that will minimize the possibility of intermixing should be used in order to achieve good separation. (Table 33 and 34 and Figure 22)

TABLE 26

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	0.5 cc/min
T	=	288 K^{o}
Dead Volume	=	45 cc

Qt(cc)	9.0			12.0		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2327	0.2581	0.902	0.3186	0.2853	1.117
2	0.4286	0.3516	1.219	0.4709	0.3037	1.551
3	0.5408	0.3000	1.803	0.6232	0.3681	1.693
4	0.5347	0.3161	1.691	0.5972	0.3650	1.636
5	0.4776	0.3290	1.451	0.6192	0.3344	1.852
6	0.3429	0.2806	1.222	0.6794	0.3926	1.730
7	0.3551	0.2871	1.237	0.5371	0.3067	1.751
8	0.3490	0.3226	1.082	0.4870	0.3037	1.604
9	0.3551	0.3161	1.123	0.4790	0.3129	1.531
10	0.3388	0.3097	1.094	0.4689	0.2975	1.576

TABLE 27

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	0.5 cc/min
Dead Volume	=	45 cc

	Qt(cc) 15.0			Qt(cc) 18.0		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.7092	0.4385	1.617	0.3055	0.2621	1.166
2	0.7092	0.2839	2.498	0.6354	0.5069	1.254
3	0.9220	0.2713	3.399	0.6517	0.4828	1.350
4	0.8227	0.3659	2.248	0.7189	0.6276	1.146
5	0.7979	0.3659	2.180	0.4929	0.2586	1.906
6	1.0000	0.4385	2.281	0.7189	0.5897	1.219
7	0.7908	0.3975	1.990	0.6314	0.5310	1.189
8	0.7989	0.4132	1.931	0.4868	0.5138	0.947
9	0.9681	0.4984	1.942	0.5316	0.5034	1.056
10	0.9574	0.4795	1.997	0.5275	0.5138	1.027
11	0.7730	0.3438	1.965	0.5310	0.5001	1.062

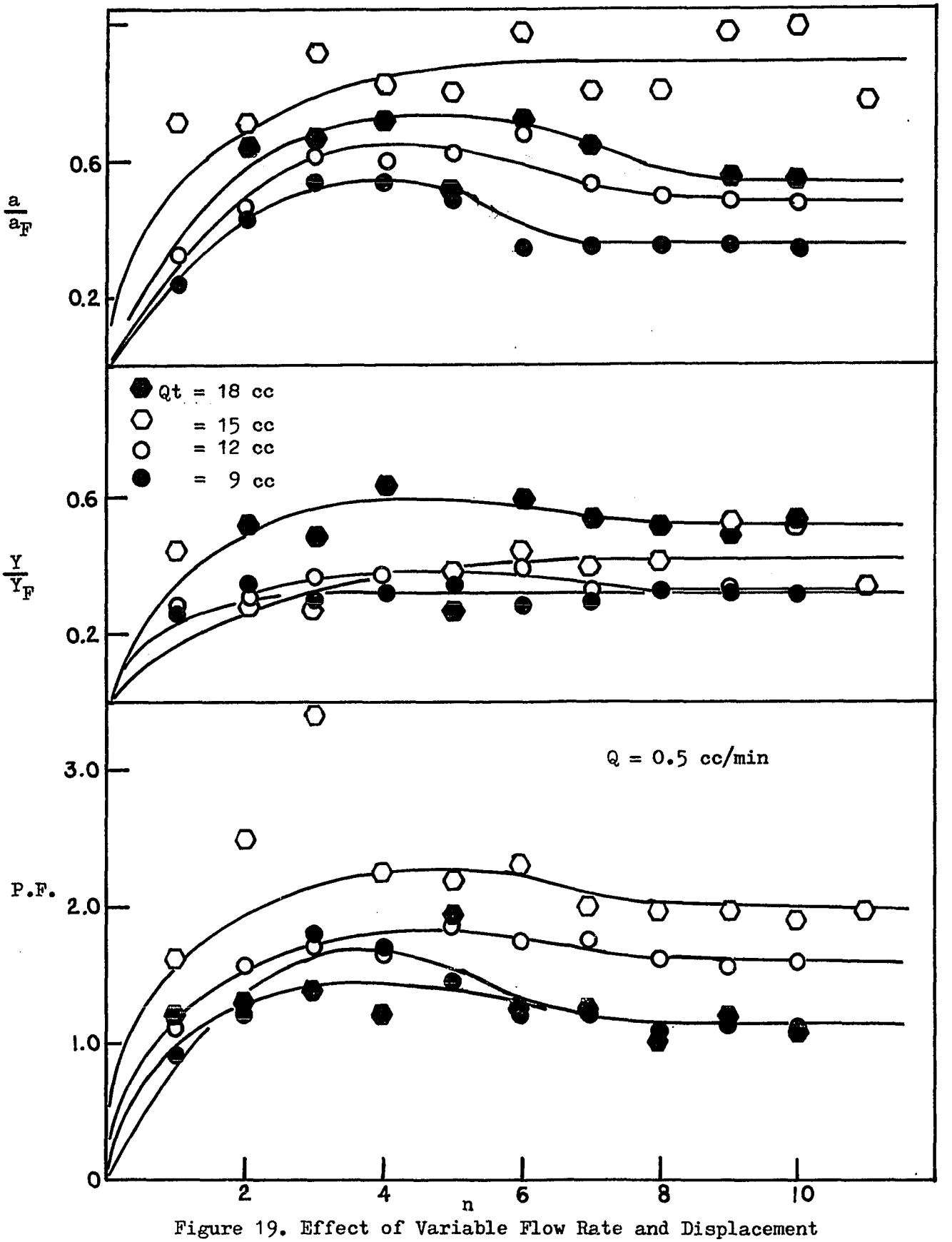


Figure 19. Effect of Variable Flow Rate and Displacement Experimental

TABLE 28

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
Dead Volume	=	45 cc

Qt(cc)	9.0			12.0		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.3274	0.2124	1.542	0.3310	0.1308	2.530
2	0.3477	0.1917	1.814	0.4828	0.1449	3.333
3	0.3249	0.1917	1.694	0.5172	0.1682	3.075
4	0.3071	0.1770	1.735	0.5379	0.1822	2.952
5	0.3071	0.1740	1.765	0.5931	0.2383	2.489
6	0.2843	0.1504	1.890	0.4966	0.2477	2.005
7	0.2893	0.2212	1.308	0.5966	0.3037	1.964
8	0.2563	0.1622	1.580	0.6207	0.3271	1.898
9	0.2411	0.1740	1.385	0.6069	0.3084	1.968
10	0.2716	0.1534	1.770	0.6069	0.3037	1.998
11	0.2639	0.1947	1.356			

TABLE 29

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchange	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
Dead Volume	=	45 cc

Qt(cc)	15.0			18.0		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2138	0.2886	0.741	0.3892	0.3151	1.983
2	0.6449	0.4362	1.478	0.6270	0.3562	2.827
3	0.8080	0.4262	1.896	0.6432	0.4452	2.320
4	0.6377	0.3624	1.760	0.6973	0.5445	2.056
5	0.6413	0.2987	2.147	0.6703	0.5993	1.796
6	0.6558	0.3322	1.974	0.6703	0.4897	2.198
7	0.6630	0.3087	2.148	0.7020	0.6986	1.615
8	0.6014	0.3121	1.927	0.6378	0.6130	1.671
9	0.6051	0.2785	2.172	0.6549	0.7397	1.431
10	0.6450	0.3221	2.002	0.6324	0.6918	1.468

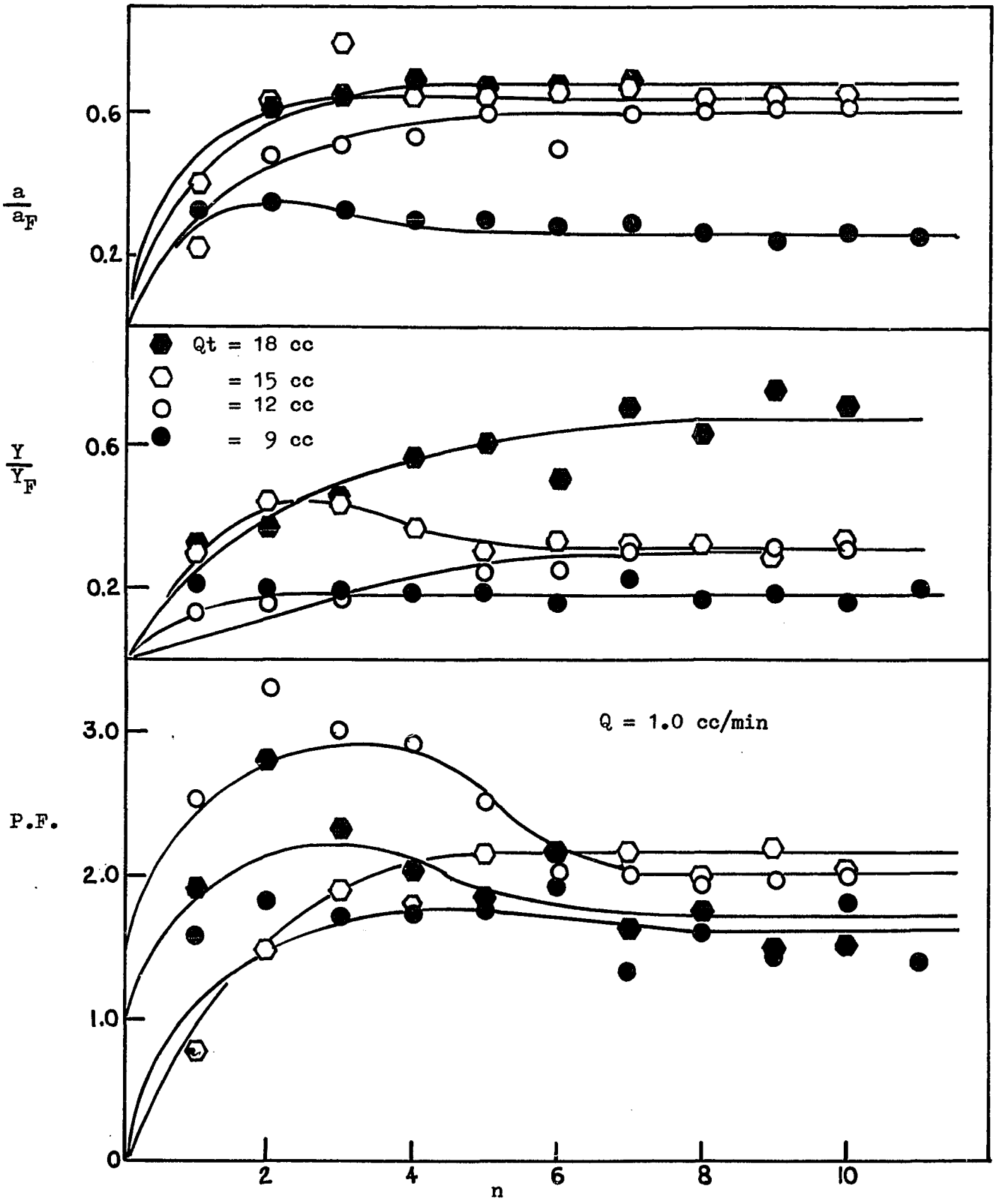


Figure 20. Effect of Variable Flow Rate and Displacement Experimental

TABLE 30

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk Phosphatase
h	=	8.0 cm
Q	=	2.0 cc/min
Dead Volume	=	45 cc

Qt(cc)	9.0			12.0		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2355	0.0582	4.046	0.2923	0.2068	1.413
2	0.2934	0.1199	2.447	0.5519	0.2284	2.417
3	0.3368	0.2397	1.405	0.4139	0.2438	1.698
4	0.2913	0.1986	1.467	0.4006	0.2593	1.545
5	0.2665	0.2192	1.216	0.4466	0.2469	1.809
6	0.2417	0.2089	1.157	0.4555	0.2963	1.537
7	0.2335	0.1781	1.311	0.5697	0.2377	2.397
8	0.2479	0.2466	1.006	0.5252	0.4012	1.309
9	0.2190	0.2500	0.876	0.4407	0.4259	1.035
10	0.1983	0.2226	0.891	0.5727	0.3778	1.516
11	0.1880	0.2055	0.915	0.5742	0.4043	1.420

TABLE 31

SEMI-CONTINUOUS PARAMETRIC-PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	2.0 cc/min
Dead Volume	=	45 cc

Qt(cc)	15.0			18.0		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2713	0.2815	0.964	0.3049	0.3919	0.7781
2	0.7666	0.4663	1.644	0.5987	0.3851	1.554
3	1.0126	0.8680	1.167	0.6143	0.4865	1.263
4	0.7666	0.4809	1.594	0.5493	0.4865	1.129
5	1.0631	0.7126	1.492	0.5269	0.6250	0.8430
6	0.9590	0.8035	1.194	0.5359	0.5405	0.9914
7	0.9621	0.8211	1.172	0.5404	0.5169	1.045
8	0.9495	0.8094	1.173	0.6166	0.4932	1.259
9	0.9180	0.7390	1.242	0.5336	0.4561	1.170
10	0.9180	0.6950	1.321	0.5897	0.5270	1.119
11	0.9243	0.7067	1.308	0.6076	0.5068	1.199

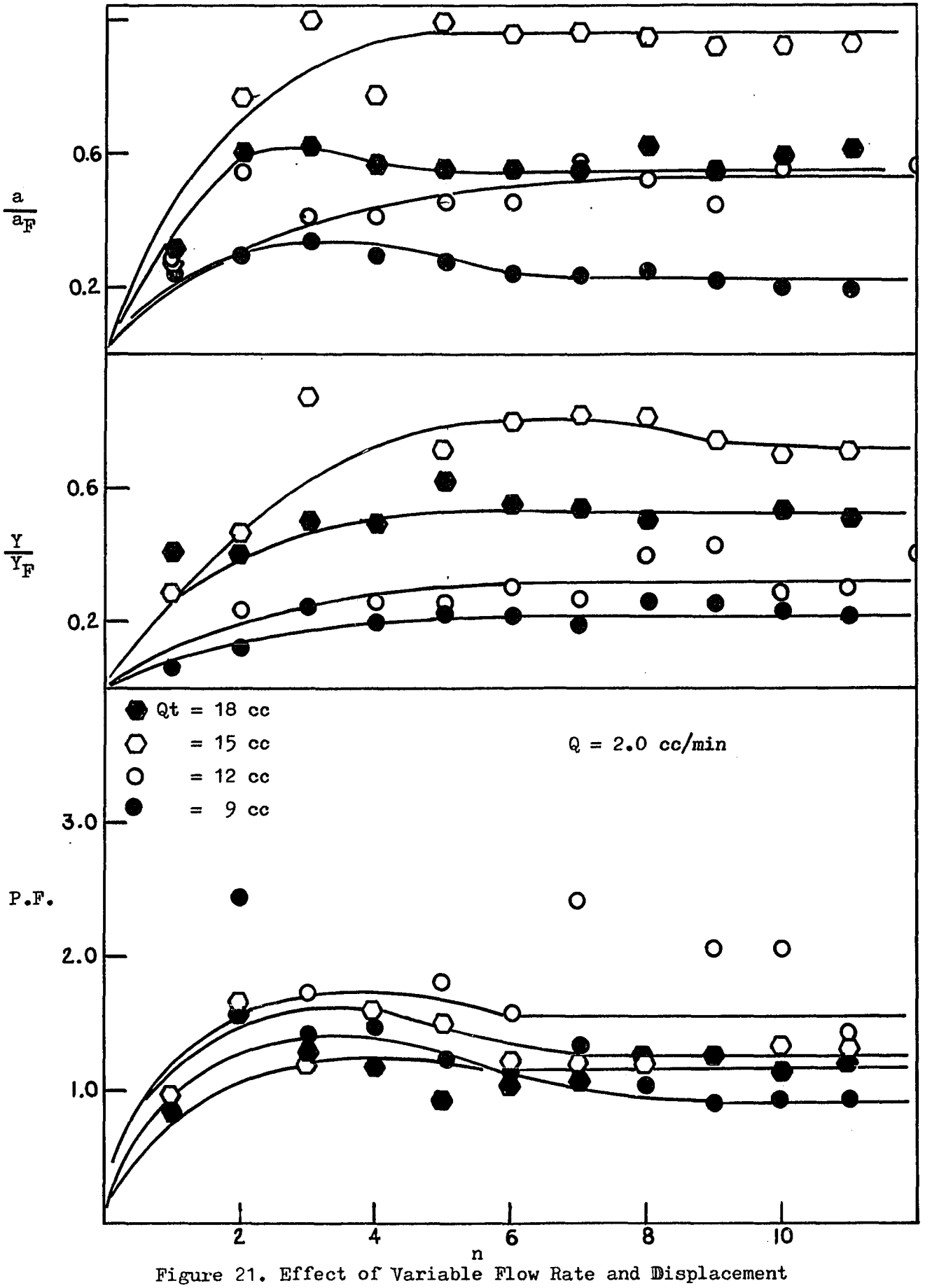


Figure 21. Effect of Variable Flow Rate and Displacement Experimental

TABLE 32

BREAKTHROUGH DATA AT pH 7.4

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
Feed	=	8.0 cm
Q	=	0.5 cc/min
T	=	288 K^{o}
I.S.	=	0.6 M

Volume Displaced (cc):	Time (min)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
6	12	0.0002	0.0000
12	24	0.2529	0.1217
15	30	0.3529	0.2047
27	54	0.5558	0.3294
33	66	0.6559	0.3472
36	72	0.6706	0.3561
42	84	0.6647	0.3731
45	90	0.7265	0.3591
48	96	0.7382	0.3680
51	102	0.7765	0.3680
57	114	0.7941	0.3650
60	120	0.7929	0.3511

TABLE 33

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Qi	=	1.0 cc/min
$Q_{T.r.}$	=	0.5 cc/min
Dead Volume	=	45 cc

Qt(cc)	9.0			12.0		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.4231	0.2658	1.592	0.4519	0.4800	0.9414
2	0.4349	0.2492	1.745	0.6111	0.4933	1.239
3	0.4053	0.2093	1.937	0.5741	0.6222	0.923
4	0.4379	0.2957	1.481	0.5259	0.5644	0.932
5	0.4438	0.2658	1.670	0.5926	0.5333	1.111
6	0.4497	0.2824	1.593	0.6667	0.4444	1.5000
7	0.4704	0.2757	1.706	0.6370	0.5200	1.225
8	0.5000	0.3621	1.381	0.6962	0.5022	1.386
9	0.4911	0.3588	1.369	0.6519	0.4667	1.397
10	0.4704	0.3621	1.299	0.6741	0.4933	1.366

TABLE 34

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE FLOW RATE AND DISPLACEMENTS

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q_i	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45 cc
Q_t(cc)	15.0	18.0

	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1.	0.2231	0.1886	1.183	0.5844	0.4036	1.448
2	0.4872	0.3686	1.322	0.7031	0.4629	1.519
3	0.9154	0.4486	2.041	0.6344	0.3561	1.782
4	0.9077	0.3371	2.692	0.6656	0.5371	1.239
5	0.8849	0.3314	2.700	0.9250	0.7181	1.288
6	0.9077	0.3429	2.647	0.8000	0.6320	1.266
7	0.9128	0.3457	2.640	0.7719	0.4955	1.558
8	0.8744	0.3600	2.429	0.7156	0.5460	1.311
9	0.9333	0.3629	2.572	0.7406	0.5519	1.342
10	0.9410	0.3943	2.387	0.7313	0.5668	1.290

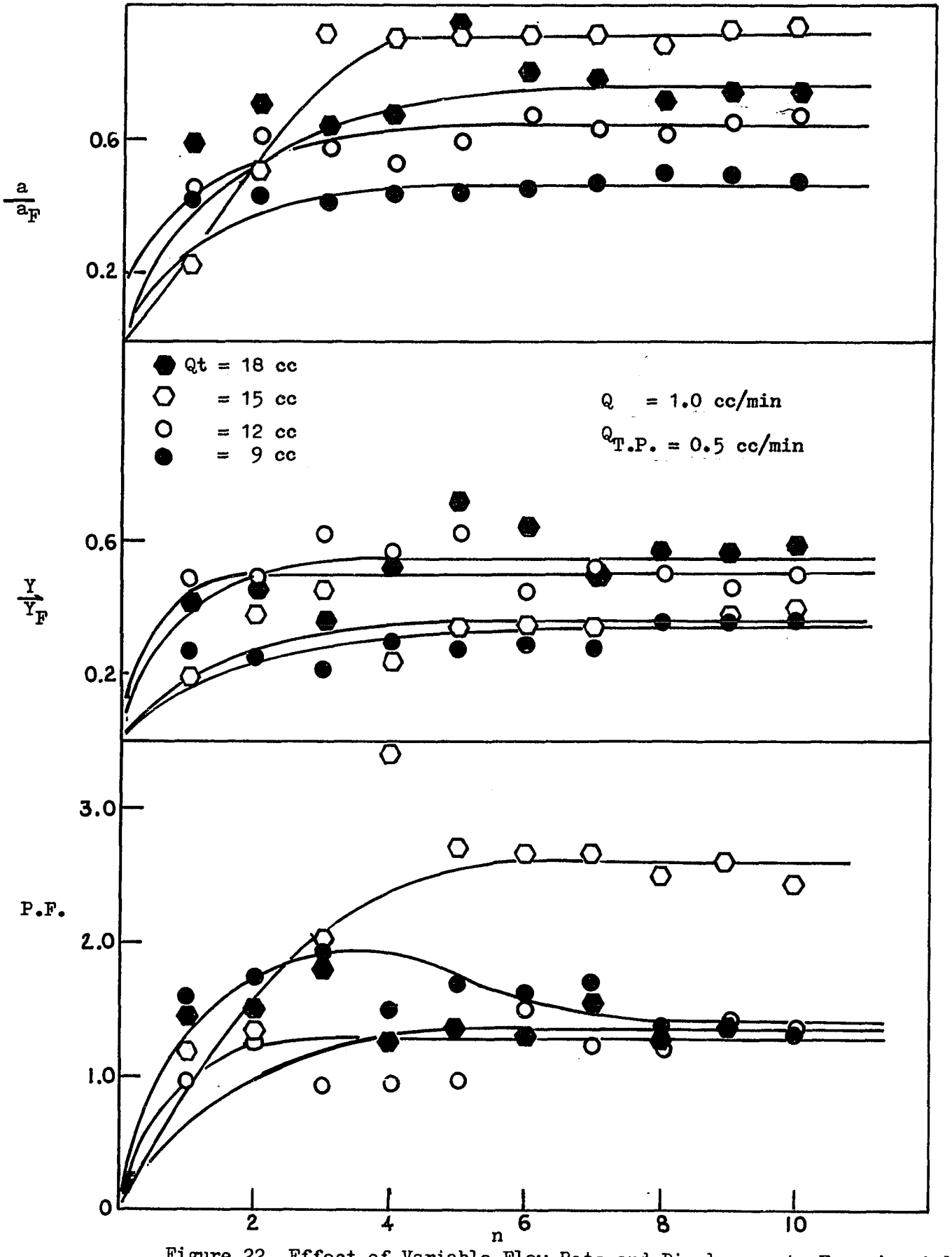


Figure 22. Effect of Variable Flow Rate and Displacement, Experimental

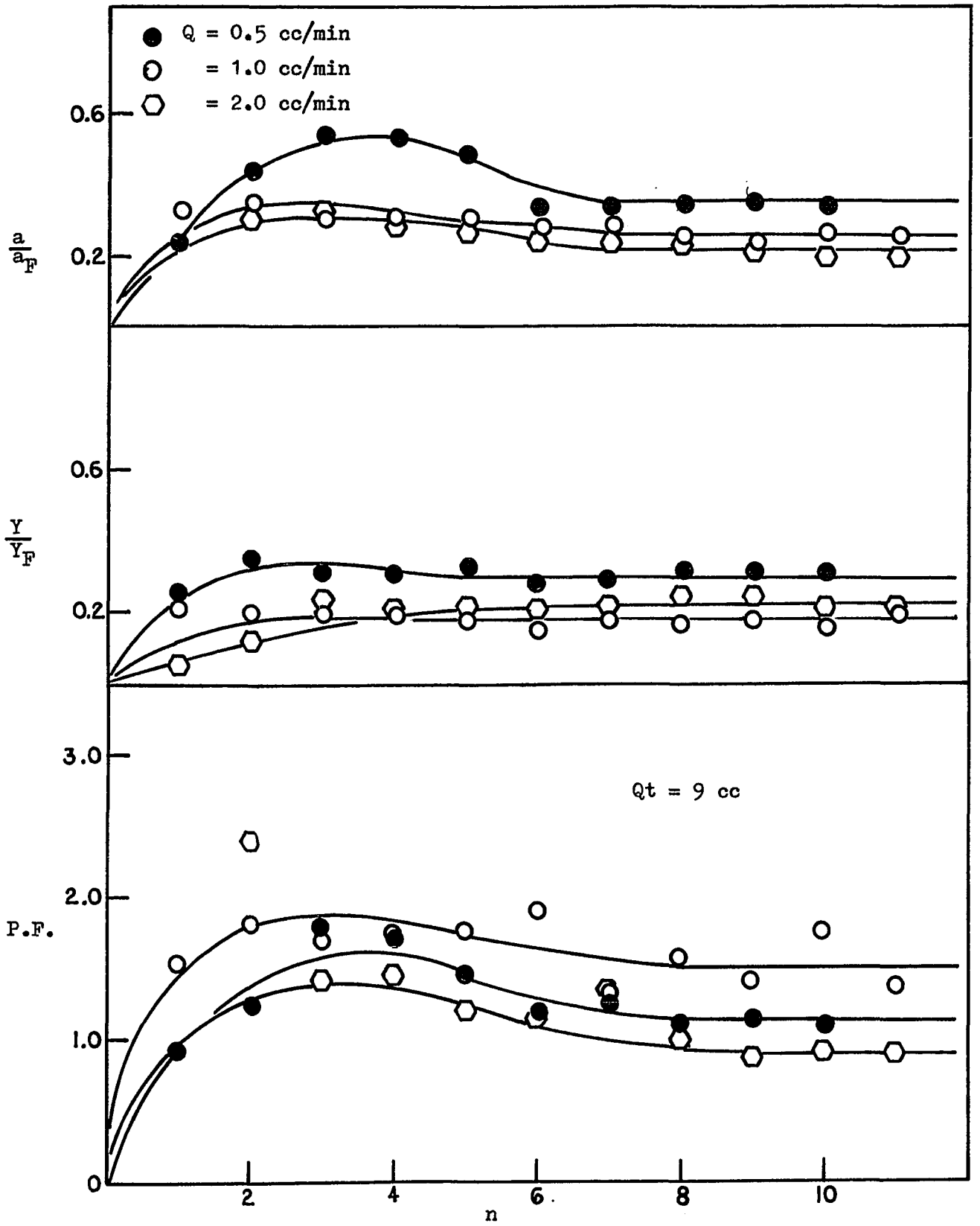


Figure 23. Effect of Variable Flow Rate, Experimental

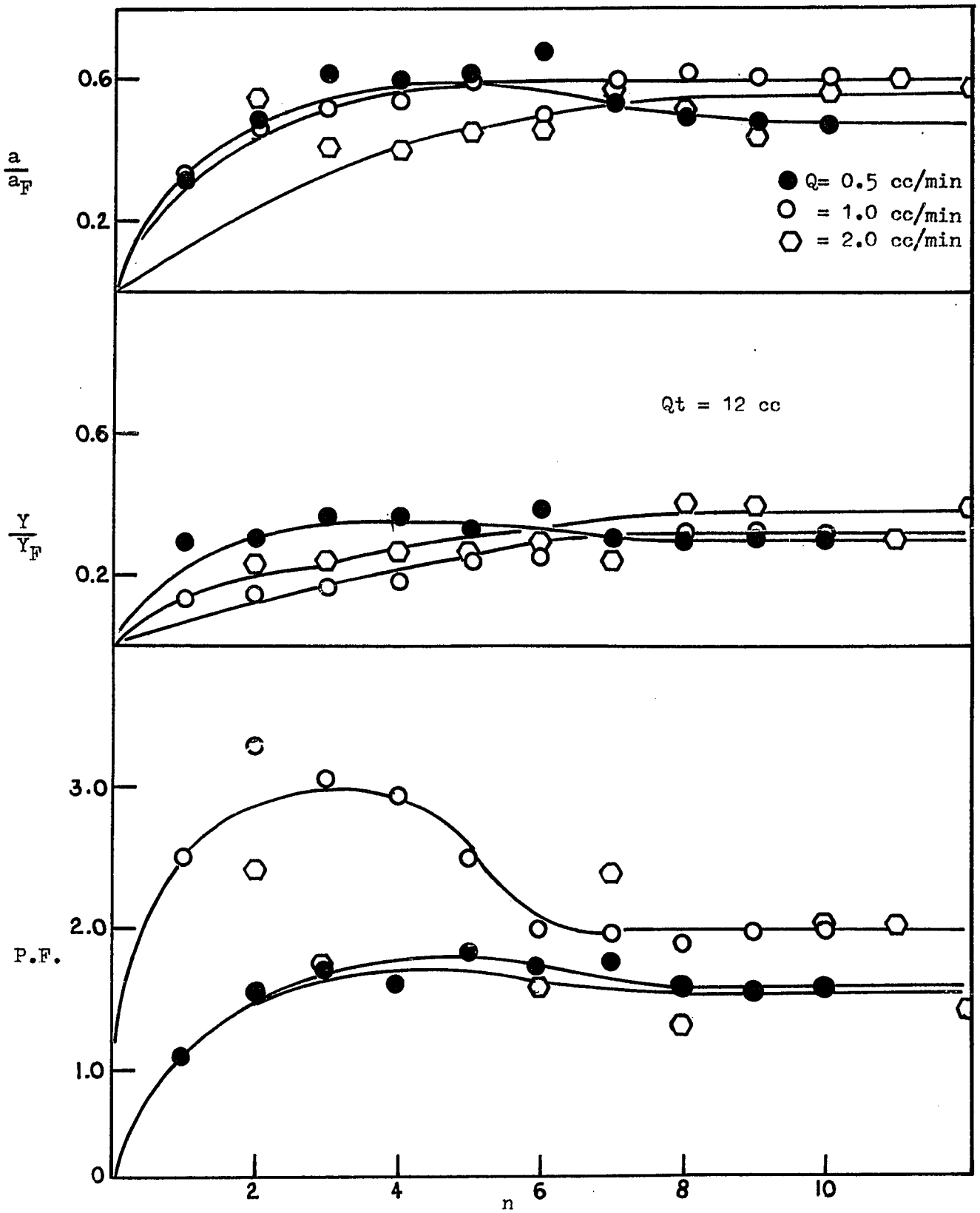


Figure 24. Effect of Variable Flow Rate, Experimental

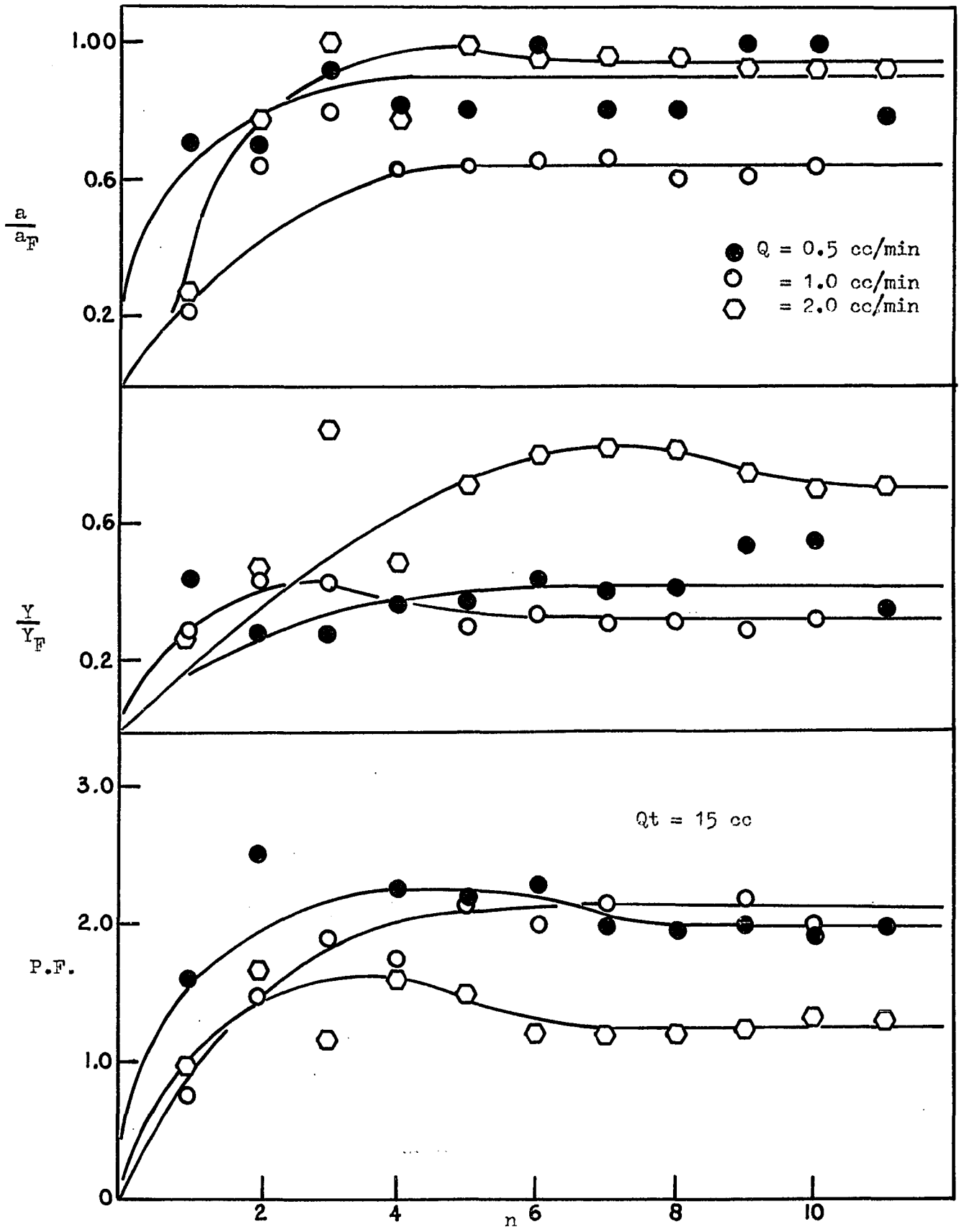


Figure 25. Effect of Variable Flow Rate, Experimental

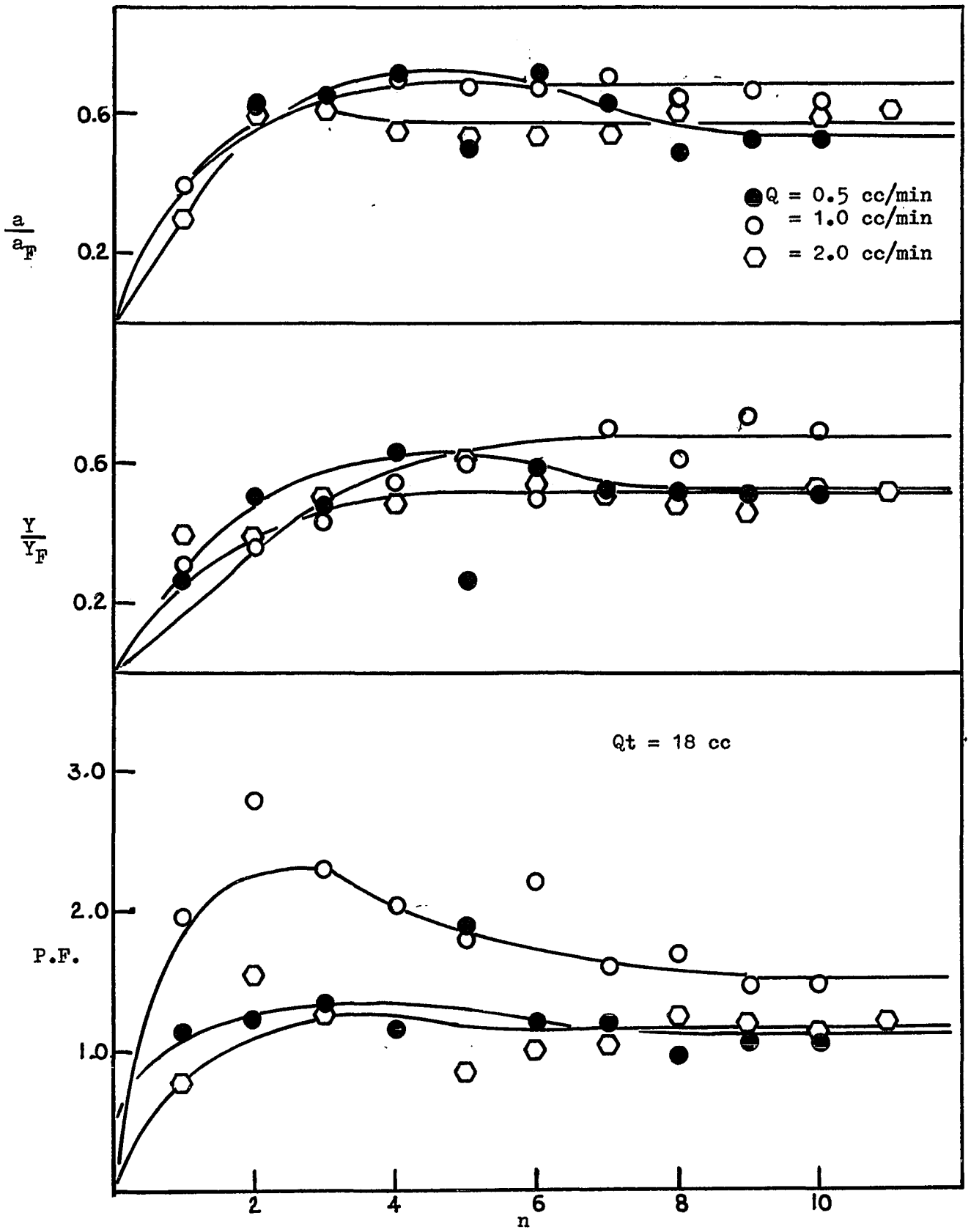


Figure 26. Effect of Variable Flow Rate, Experimental

5- Effect of Product Withdrawal Volume

The theoretical analysis and the experimental results indicated that the enzyme concentration in the bottom product is very small. This also shows that the enzyme concentration in the top product depends on some factors, among them is the volume of the product withdrawn. Therefore, the following series of experimental runs were designed to investigate this effect. Two groups of experiments, each with three runs were carried out. The first group was run with variable top product volumes and the other with variable bottom product, (BP(2)). The bottom product volumes used were 10, 15, and 20 cc respectively. All other conditions including bottom product (1) remained unchanged.

From the results given on Table 35 and 36 and sketched on Figures 27 and 28, it is clear that the enzyme concentration in the enriched enzyme product stream does depend on the amount of product withdrawn or, in other words, on the top and bottom reflux (defined here as the ratio of the fluid volume entering a reservoir at one end of the column to the total volume of the products withdrawn at this end). For top product of 10 cc (less than the void volume of the column), the recovery of the enzyme increased with cycle number until it reached a steady value of 60% at which the purification factor was 2.2 When the volume of the top product was increased to 15.0 cc (slightly higher than the void volume), the same was observed. However, the recovery reached 90%, and a purification factor of 2.6 was obtained. Further increase in top product volume (20 cc) resulted in lower recovery (80%) and less purification (P.F. = 1.2).

The less volume (10 cc) did not provide enough time to elute the

enzyme, and the entire column did not experience the full effect of the change in the thermodynamic variable in a case where the adsorbent capacity is good. The higher volume (20 cc) resulted in intermixing after several cycles. This intermixing sent less concentrated enzyme to the top reservoir in the next stage of the process. This could be seen from the results on Table 35 where the recovery and the purification increased with cycle number, then decreased when the column experienced the effect of the intermixing.

For the group of the variable bottom product, the reverse holds true. In the case of 10 cc, a poor separation was obtained and a purification factor less than 1.0 for the top product was observed because of higher desorption of undesired proteins with the enriched enzyme stream. The results of the 15.0 cc case are the same as that in the top product 15.0 cc case. Those for the 20.0 cc bottom product case showed a top product with 71% enzyme recovery and 1.4 purification factor. This is due to the fact that the increase in bottom product volume caused more undesired proteins to desorb; therfore, less was observed in the top product.

TABLE 35

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE TOP PRODUCT WITHDRAWAL

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase, I.S.=0.1 , pH 7.4
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45 cc

Top Product Withdrawal ($Q_4 t_4$)

	10 CC		15 CC		20 CC	
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
1	0.1872	0.1671	0.2231	0.1886	0.3205	0.2299
2	0.3935	0.2716	0.4872	0.3686	0.5381	0.2667
3	0.4049	0.2412	0.9154	0.4486	0.6728	0.3862
4	0.4183	0.2583	0.9077	0.3371	0.7451	0.4643
5	0.5065	0.2640	0.8949	0.3314	0.7108	0.4321
6	0.5960	0.3305	0.9077	0.3429	0.6372	0.4551
7	0.5570	0.3624	0.9128	0.3457	0.7563	0.5745
8	0.5845	0.2811	0.8744	0.3600	0.7841	0.6068
9	0.5593	0.2679	0.9333	0.3629	0.8214	0.6719
10	0.5972	0.2607	0.9410	0.3943	0.7694	0.6436
11	0.6018	0.2659			0.8135	0.6704

TABLE 35 (CONTINUED)

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE TOP PRODUCT WITHDRAWAL

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45 cc

Top Product

Withdrawal (cc)

	Purification Factor		
Cycle No.:	10	15	20
1	1.120	1.183	1.394
2	1.449	1.322	2.017
3	1.679	2.041	1.742
4	1.620	2.692	1.605
5	1.919	2.700	1.645
6	1.803	2.647	1.400
7	1.537	2.640	1.316
8	2.079	2.429	1.292
9	2.088	2.572	1.223
10	2.291	2.387	1.196
11	2.263		1.214

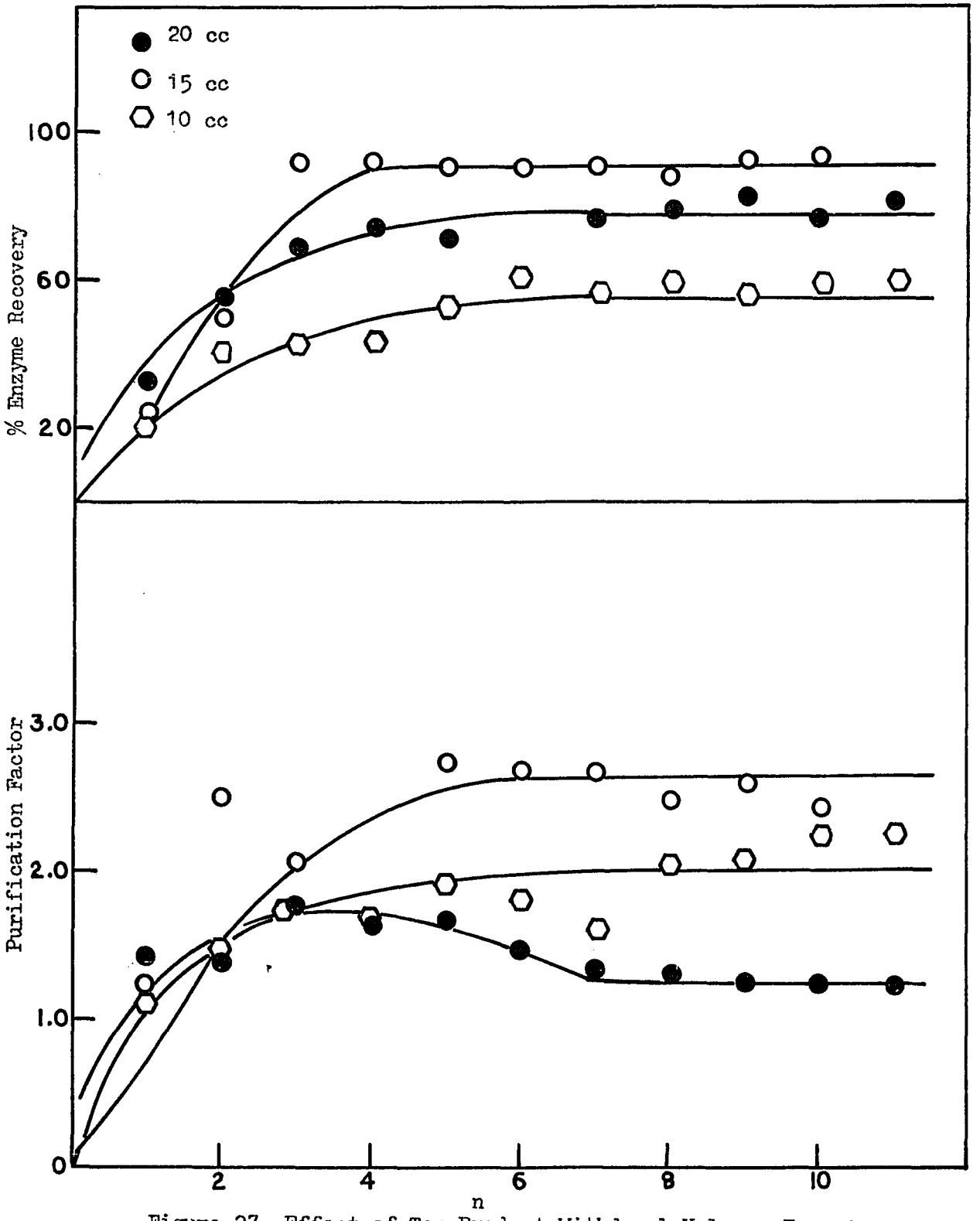


Figure 27. Effect of Top Product Withdrawl Volume, Experimental

TABLE 36

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE BOTTOM PRODUCT B.P.(2) WITHDRAWAL

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45 cc

Bottom Product

Withdrawal $Q_7 t_7$ (cc)

	10		15		20	
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
1	0.2540	0.3252	0.2231	0.1886	0.2018	0.1524
2	0.5143	0.4126	0.4872	0.3686	0.4500	0.3659
3	0.5365	0.5437	0.9154	0.4486	0.4714	0.3933
4	0.6286	0.5825	0.9077	0.3371	0.5946	0.3780
5	0.7079	0.5583	0.8949	0.3314	0.6158	0.5035
6	0.6698	0.6650	0.9077	0.3429	0.7125	0.5330
7	0.6952	0.8107	0.9128	0.3457	0.7220	0.5474
8	0.6921	0.8058	0.8744	0.3600	0.7142	0.5156
9	0.7238	0.8349	0.9333	0.3629	0.6804	0.5005
10	0.7143	0.7476	0.9410	0.3943	0.7058	0.5130
11	0.6889	0.6990				

TABLE 36 (CONTINUED)

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, VARIABLE BOTTOM PRODUCT B.P.(2) WITHDRAWAL.

TOP PRODUCT P.F.

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02 % Alk. Phos.
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45 cc

Bottom Product Withdrawal Q_7t_7 (cc)	10	15	20
Cycle No.:		P.F.	
1	0.781	1.183	1.324
2	1.246	1.322	1.230
3	0.987	2.041	1.370
4	1.079	2.692	1.573
5	1.268	2.700	1.223
6	1.007	2.647	1.337
7	0.858	2.640	1.319
8	0.859	2.429	1.385
9	0.867	2.572	1.359
10	0.956	2.387	1.376
11	0.986		

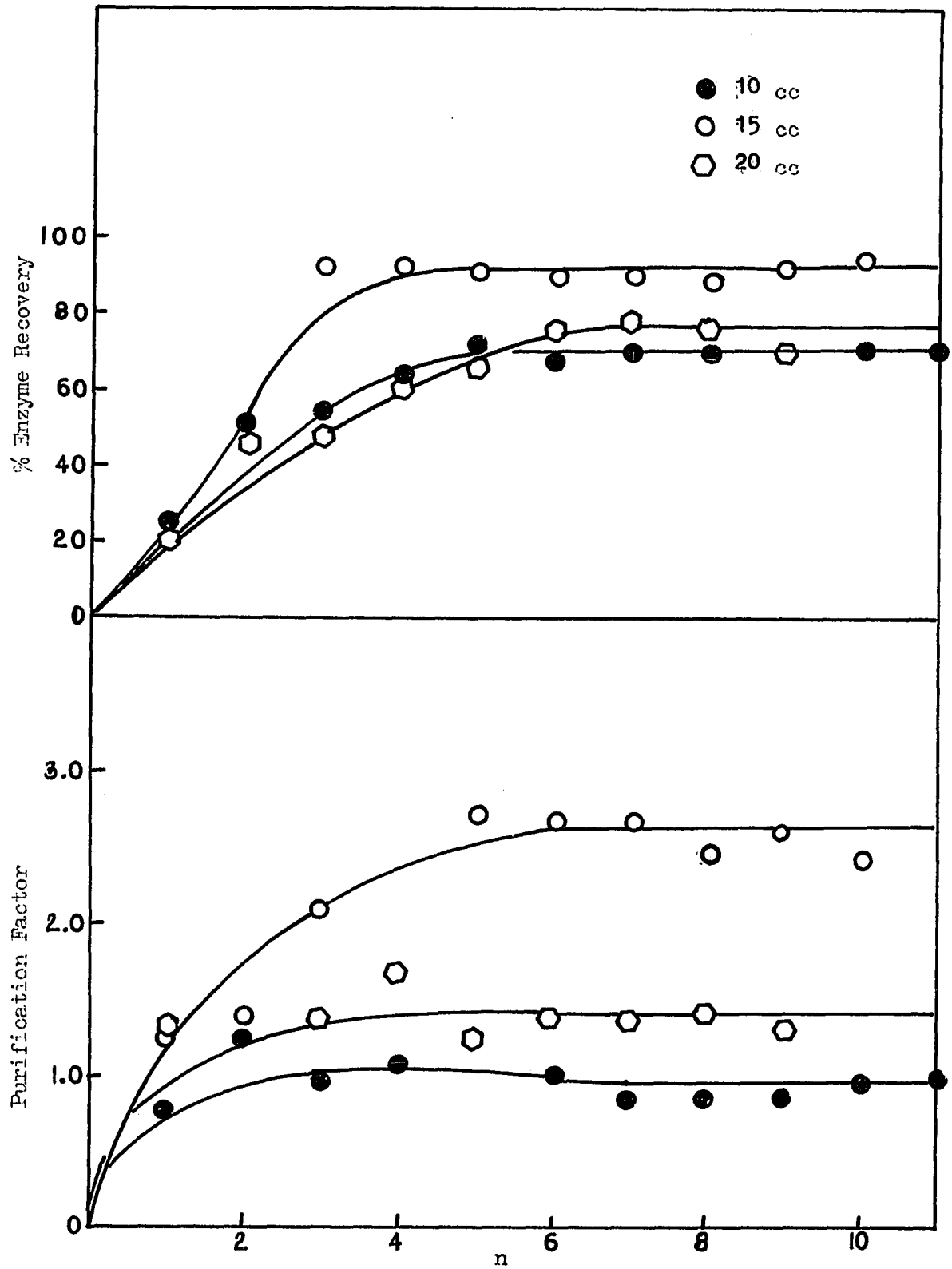


Figure 28. Effect of Bottom Product Withdrawl Volume, Experimental

6- Effect of Feed Concentration

The mathematical analysis and the experimental results show that the concentration of the enzyme in the bottom products is very small, and its effect on the top product concentration is negligible. It was also shown that the top product concentration depends on the fresh feed concentration. To investigate the effect of the feed concentration on the separation, and hence the purification process, a series of experiments using different feed concentrations (0.01, 0.02, and 0.03% alkaline phosphatase) was executed.

The results obtained are given on Table 37 and Figure 29. It is apparent from these results, that lower values of feed concentrations (as is the case with 0.01%) cause the enzyme to hold strongly to the adsorbent due to the column higher capacity at this low concentration. As a result, less enzyme will be desorbed and, therefore, less purification will be observed. The desorption of the undesired proteins was not affected.

When the concentration of the feed increases, the adsorption of the enzyme on the bed is not too strong. As a result, the 0.6M ionic strength wave moves at a velocity less than the enzyme velocity, causing the enzyme to be trapped at the front of ionic strength wave and an increase in the enzyme concentration would result; hence, more separation would take place. Therefore, a higher purification factor will be obtained, as is the case with the 0.02% feed concentration.

For the 0.03% feed concentration, a slight decrease in the concentration of the enzyme in top product in comparison with the 0.02% case was observed. It is possible that the column became saturated

to some extent at this concentration (0.03%) and part of the enzyme which appeared in the top product was probably enzyme that was not retained by the column. However, the difference was not significant.

TABLE 37

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, EFFECT OF FEED CONCENTRATION

Ion Exchanger	=	Anion (DEAE)
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{t.p.}$	=	0.5 cc/min
Qt	=	15 cc
Dead Volume	=	45 cc

Feed Conc.						
Top Product	0.01%		0.02%		0.03%	
Cycle No.:	$\frac{a}{a}$	$\frac{Y}{Y}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
1	0.3667	0.3077	0.2231	0.1886	0.2612	0.3110
2	0.4556	0.3154	0.4872	0.3686	0.4423	0.3214
3	0.5111	0.4308	0.9154	0.4486	0.8422	0.3601
4	0.5278	0.4231	0.9077	0.3371	0.8621	0.3467
5	0.5278	0.3615	0.8949	0.3314	0.8734	0.3601
6	0.5167	0.3538	0.9077	0.3429	0.8920	0.3213
7	0.5889	0.3846	0.9128	0.3457	0.8764	0.3301
8	0.5944	0.3538	0.8744	0.3600	0.9001	0.3712
9	0.5333	0.3538	0.9333	0.3629	0.8811	0.3632
10	0.5722	0.3539	0.9410	0.3943	0.8920	0.3814

TABLE 37 (CONTINUED)

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, EFFECT OF FEED CONCENTRATION

Ion Exchanger	=	Anion (DEAE)
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Qt	=	15 cc
Dead Volume	=	45 cc

Feed Concentration:	0.01% alk.ph.	0.02%	0.03%
Cycle No.:		P.F.	
1	1.192	1.183	0.840
2	1.445	1.322	1.376
3	1.186	2.041	2.339
4	1.247	2.692	2.486
5	1.460	2.700	2.425
6	1.460	2.647	2.776
7	1.531	2.640	2.648
8	1.680	2.429	2.425
9	1.507	2.572	2.371
10	1.617	2.387	2.339

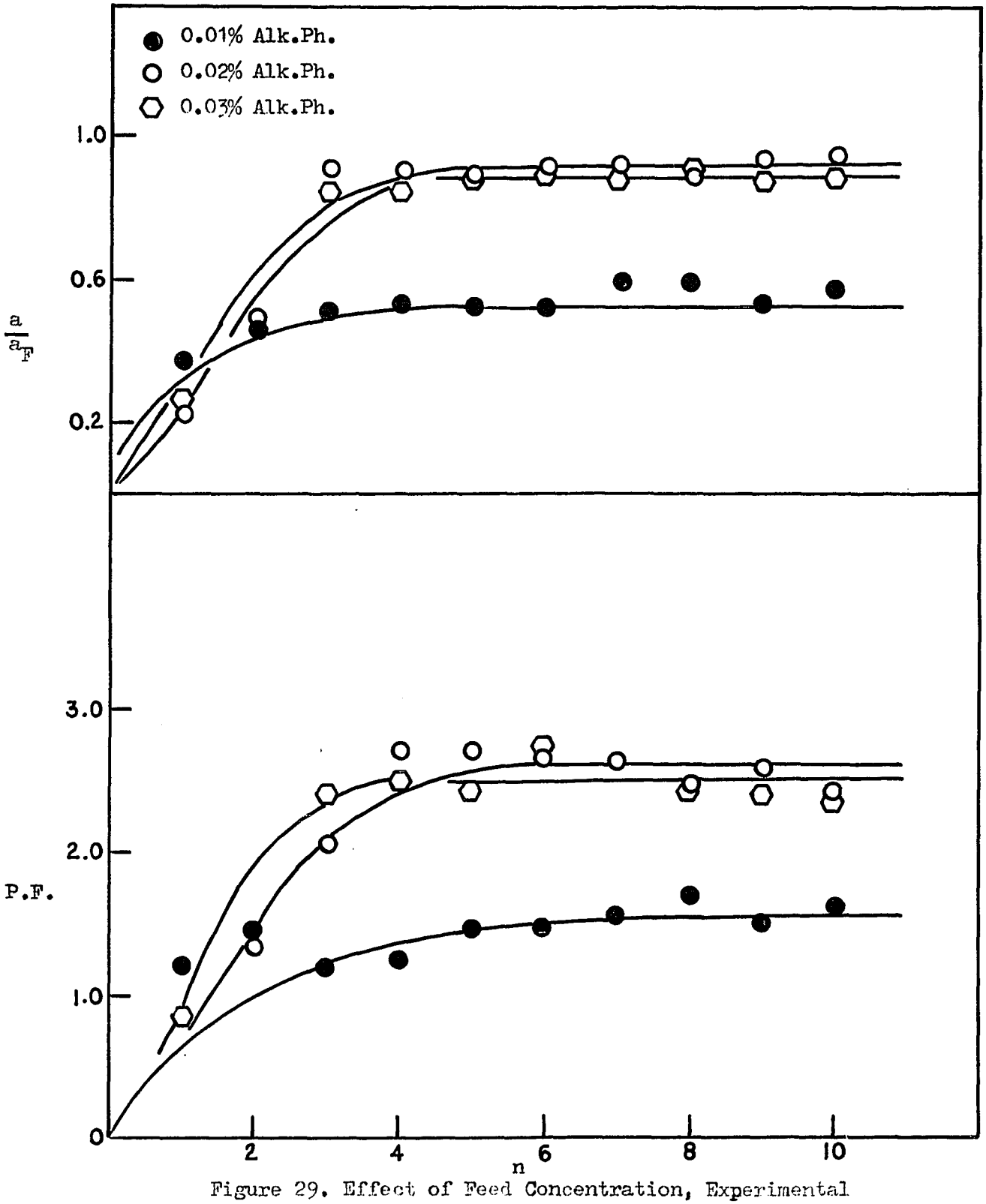


Figure 29. Effect of Feed Concentration, Experimental

7- Other Parameters:

In a series of experiments to complete the optimization process, a number of other parameters that might affect the separation were studied.

a- Top and Bottom Product Elution Buffers

The effect of top and bottom product buffers was studied through four runs. The first two had to do with the top product elution buffer. Two types of buffer were compared. The first was pure buffer with I.S. = 0.6M and pH 7.4, and the second was 0.02% alkaline phosphatase in the same 0.6M buffer. The results indicate high enzyme recovery and better purification when pure buffer is used. A possible explanation is that the feed type elution buffer in the second case saturated the column, and the enzyme exiting out in the top product stream is not the enzyme retained on the adsorbent during the adsorption step. Thus, the change in the concentration of solute in the fluid phase resulting from the desorption process is very small and less separation is observed.

For the bottom product (2) elution buffer, the same technique was used, i.e., a pure buffer of pH 4.0 and I.S. = 0.1, and a buffer with 0.02% alkaline phosphatase at the same pH and ionic strength were compared. As expected, the purification factors were almost the same. However, there was a slight decrease in enzyme recovery in the second case (0.02% alk. ph. in 0.1M buffer at pH 4.0), but this was balanced by a decrease in the concentration of the undesired proteins in the top product. The 0.02% alkaline phosphatase elution buffer coupled with the positive charge carried by the mixture at this pH (4.0) caused the mixture to change its conformation on the exchanger much faster which

led to more desorption at the regeneration step. The results for these runs are shown on Tables 38 and 39, Figures 30 and 31.

TABLE 38

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, EFFECT OF TOP PRODUCT ELUTION SOLUTION

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45 cc
Qt	=	15 cc

	0.6M Pure Buffer Top Product			0.02% Alk. Ph. in 0.6M Buffer Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2231	0.1886	1.183	0.1882	0.1719	1.095
2	0.4872	0.3686	1.322	0.3038	0.2842	1.069
3	0.9154	0.4486	2.041	0.4355	0.3281	1.327
4	0.9077	0.3371	2.692	0.4570	0.3404	1.343
5	0.8949	0.3314	2.700	0.5430	0.2789	1.947
6	0.9077	0.3429	2.647	0.5081	0.3474	1.463
7	0.9128	0.3457	2.640	0.5094	0.3509	1.452
8	0.8744	0.3600	2.429	0.5672	0.4158	1.364
9	0.9333	0.3629	2.572	0.6022	0.4596	1.310
10	0.9410	0.3943	2.387	0.6075	0.4649	1.309
11				0.5349	0.3509	1.525

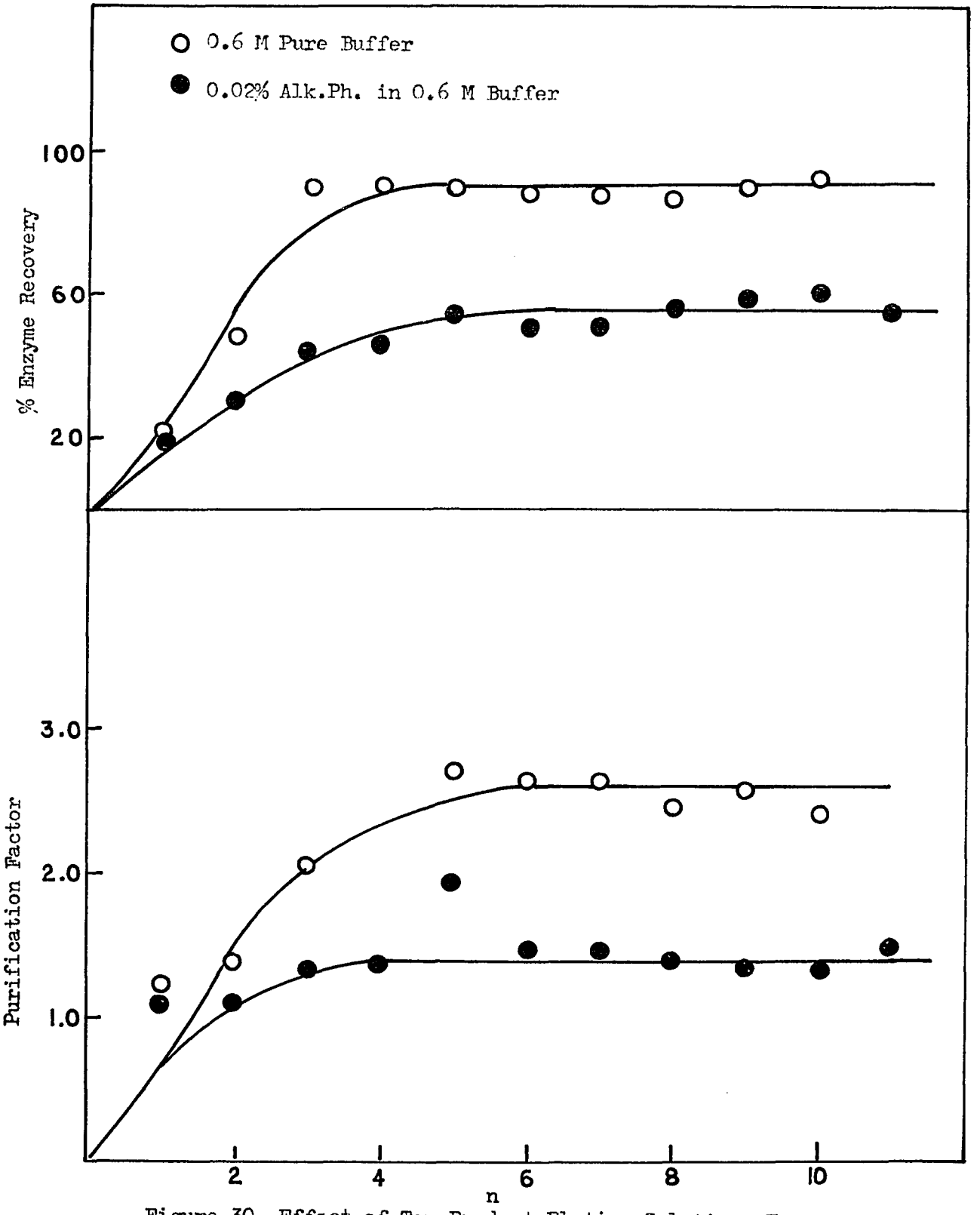


Figure 30. Effect of Top Product Elution Solution, Experimental

TABLE 39

SEMI-CONTINUOUS PARAMETRIC PUMP EXPERIMENTAL RESULTS

EFFECT OF BOTTOM PRODUCT ELUTION SOLUTION

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45.0 cc
Qt	=	15.0 cc

pH 4.0 Elution Buffer	0.02% Alk. Ph. in 0.1M Buffer			0.1M Pure Buffer		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.1486	0.0862	1.724	0.2231	0.1886	1.183
2	0.2843	0.1587	1.791	0.4872	0.3686	1.322
3	0.4455	0.2051	2.172	0.9154	0.4486	2.041
4	0.5602	0.2355	2.378	0.9077	0.3371	2.692
5	0.6917	0.2797	2.473	0.8949	0.3314	2.700
6	0.7511	0.2844	2.641	0.9077	0.3429	2.647
7	0.6960	0.2797	2.488	0.9128	0.3457	2.640
8	0.7701	0.2867	2.686	0.8744	0.3600	2.429
9	0.7638	0.2797	2.731	0.9333	0.3629	2.572
10	0.7129	0.2933	2.431	0.9410	0.3943	2.387
11	0.6938	0.3031	2.289			

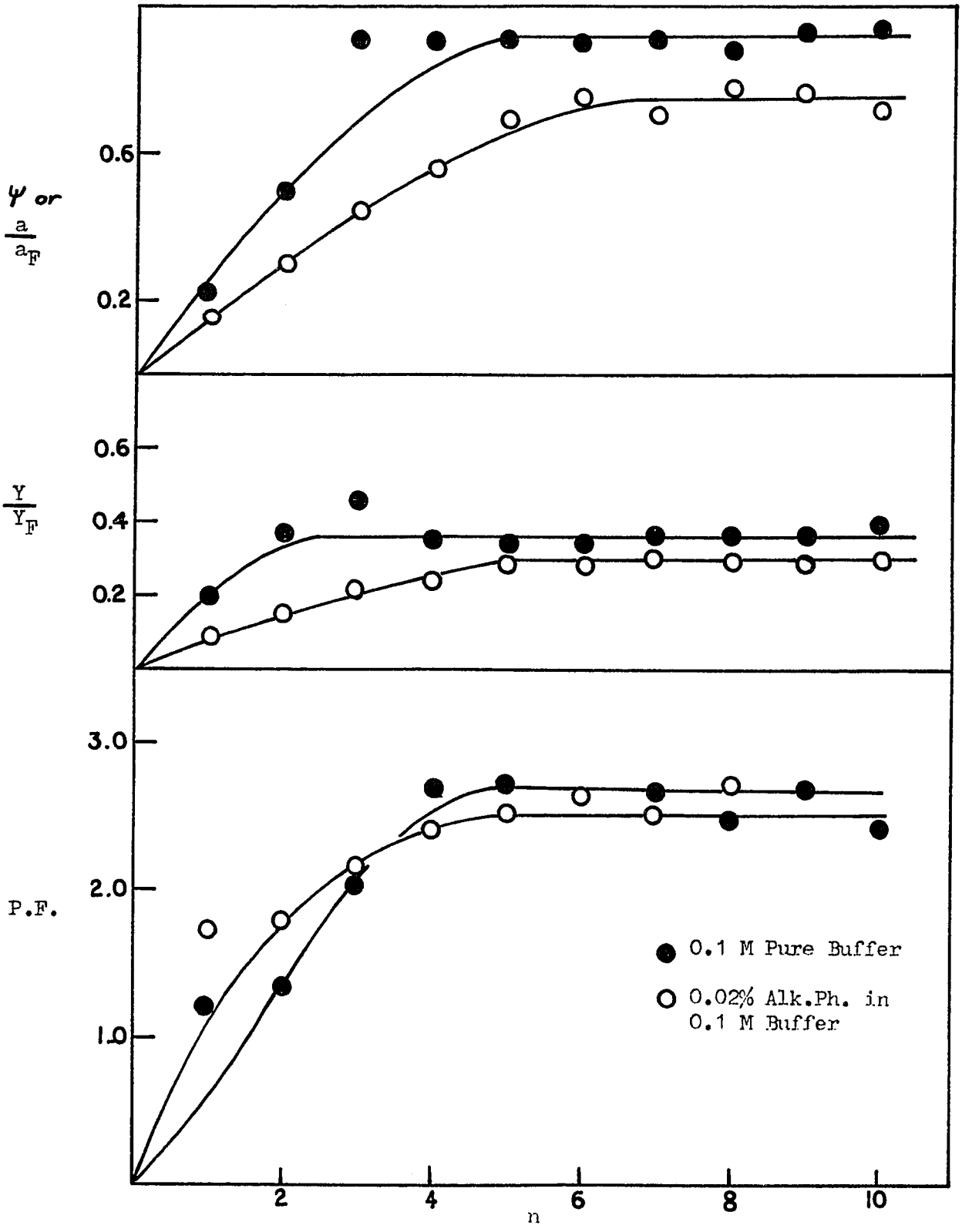
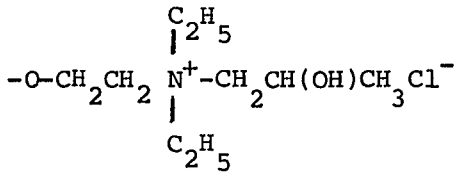


Figure 31. Effect of Bottom Product Elution Solution, Experimental

b- Effect of Adsorbent (Anion)

Two runs designed to examine the effect of adsorbent on purification were carried out using the anion QAE (Quarternary Amino Ethyl),



as an adsorbent. Both runs showed QAE with no significant difference in its selectivity to adsorb and elute the enzyme and the undesired proteins. The purification factor was less than 1.0 for the first five cycles. Then it increased with the increasing in the number of cycles. The steady value obtained is 1.3. This is compared to 2.6 for DEAE under the same operating conditions.

The high capacity of QAE resulted in less enzyme recovery during the elution step. The expansion and contraction properties of QAE which result in changeable flow rate properties make it unsuitable for this system. The typical results of one of the runs are given on Table 40 and Figure 32.

TABLE 40

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, EFFECT OF ADSORBENT

Ion Exchanger	=	Anion
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Qt	=	1.5 cc
Dead Volume	=	45 cc

TYPE OF ANION	DEAE			QAE A 50		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2231	0.1886	1.183	0.2257	0.2646	0.853
2	0.4872	0.3686	1.322	0.4879	0.5169	0.944
3	0.9154	0.4486	2.041	0.5146	0.5323	0.967
4	0.9077	0.3371	2.692	0.3180	0.3846	0.827
5	0.8949	0.3314	2.700	0.4733	0.4985	0.950
6	0.9077	0.3429	2.647	0.5097	0.4892	1.042
7	0.9128	0.3457	2.640	0.6044	0.4585	1.318
8	0.8744	0.3600	2.429	0.6092	0.4923	1.238
9	0.9333	0.3629	2.572	0.5971	0.4554	1.311
10	0.9410	0.3943	2.387	0.5801	0.4492	1.291

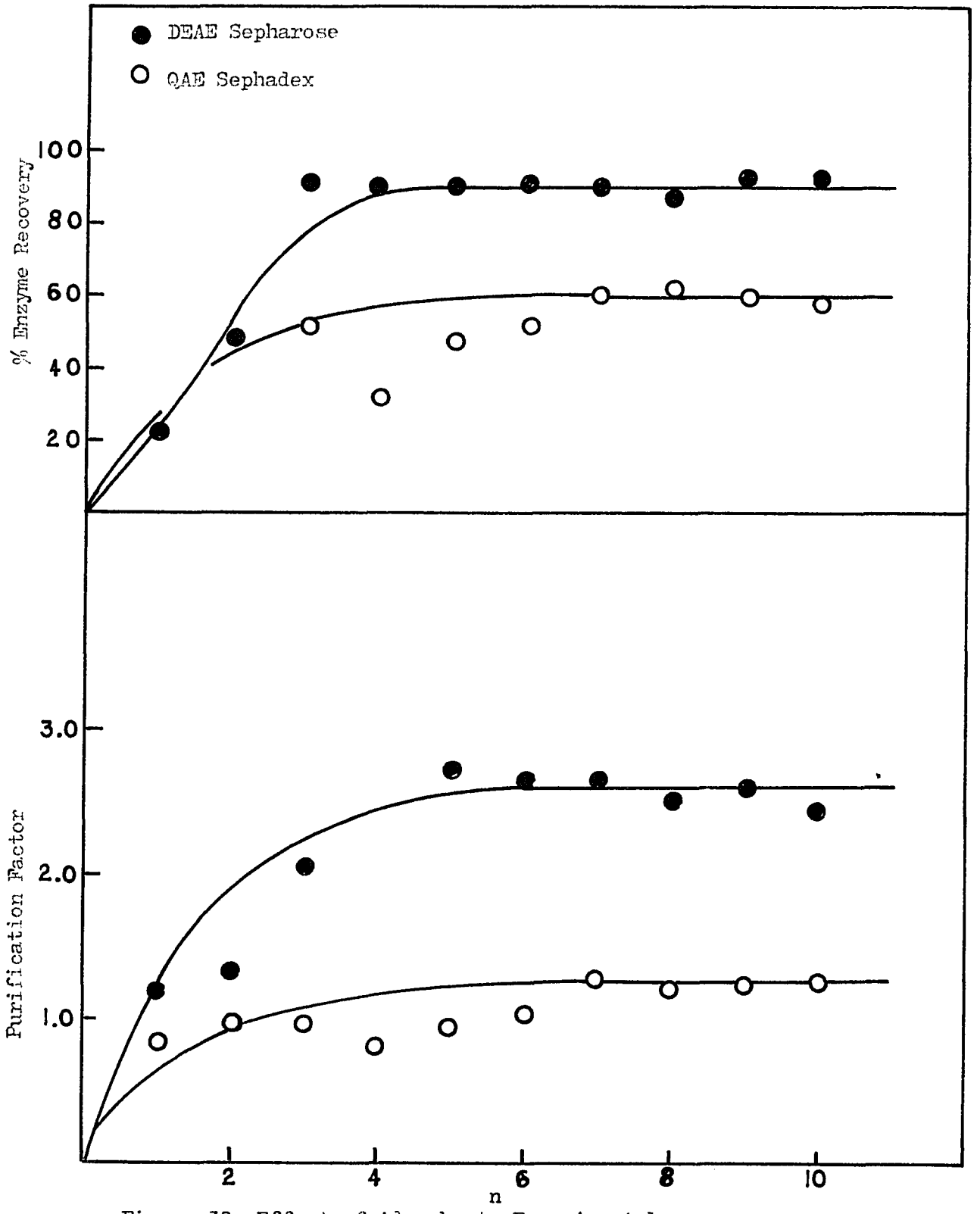


Figure 32. Effect of Adsorbent, Experimental

c- Effect of Circulation

Circulation of the fluid between the reservoirs and the column has been thought and was proved to be of great importance in assuring the shifting of buffer pH and ionic strength in the column to exact required values. Circulation takes place between the fluid in the top reservoir and the column during Step 3. Preliminary work to determine the optimum time for circulation indicated that circulation for 45 to 60 minutes would be adequate. Hence, a 60 minute circulation period was adopted. The pH of the fluid in the reservoirs was maintained constant by two automatic titrators. Two hollow fiber dialyzers were used to keep the ionic strengths constant.

Two cases were picked to study the effect of circulation. The flow rate for the first case was 1.0 cc/min everywhere. For the second case, it was 1.0 cc/min for each stream except the top product stream for which the flow rate was 0.5 cc/min. The results obtained are shown on Tables 41 and 42 and Figure 34. It is found that circulation has a definite effect on the enzyme recovery and the purification factor. In the first case, the steady state value of the purification factor rose from 2.1 for the no circulation run to 2.8 and values as high as 3.0 were obtained in some cycles. For the second case, the purification factor rose from 2.6 for the no circulation case to 3.1 for the circulation one.

It should be noticed that the circulation effect is taken into consideration in developing the mathematical model equations.

TABLE 41

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, EFFECT OF CIRCULATION

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
Dead Volume	=	45 cc
Qt	=	15 cc

	Circulation (60 min) Top Product			No Circulation Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1.	0.2000	0.1455	1.37	0.2138	0.2886	0.741
2	0.3838	0.1848	2.06	0.6449	0.4362	1.478
3	0.5351	0.2212	2.40	0.8080	0.4262	1.896
4	0.6270	0.2242	2.78	0.6377	0.3624	1.760
5	0.6757	0.2455	2.74	0.6413	0.2987	2.147
6	0.7595	0.2484	3.04	0.6558	0.3322	1.974
7	0.8054	0.2576	3.07	0.6630	0.3087	2.148
8	0.7424	0.2818	2.58	0.6014	0.3121	1.927
9	0.7432	0.2242	3.30	0.6051	0.2785	2.172
10	0.7514	0.2667	2.80	0.6450	0.3221	2.002
11	0.7730	0.2727	2.81			
12	0.7676	0.2697	2.83			

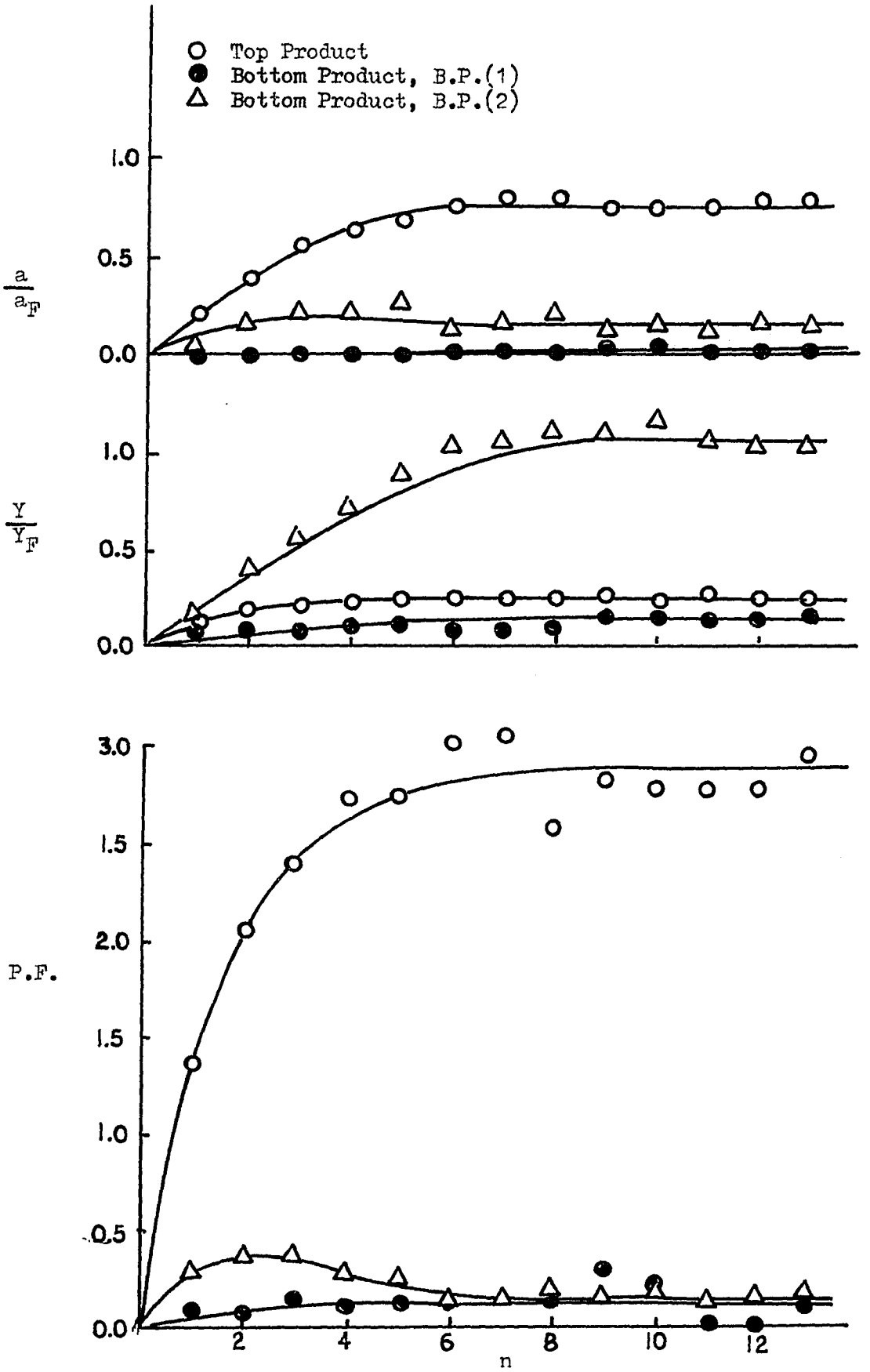


Figure33. Semi-Continuous Parametric Pump, Effect of Circulation
Experimental

TABLE 42

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, EFFECT OF CIRCULATION

Ion Exchanger	=	Anion (DEAE)
Feed	=	0.02% Alk. Phosphatase
h	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Qt	=	15 cc
Dead Volume	=	45 cc

	Circulation (60 min) Top Product			No Circularion Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2513	0.1601	1.570	0.2231	0.1886	1.183
2	0.4412	0.2255	1.957	0.4872	0.3686	1.322
3	0.5471	0.3072	2.106	0.9154	0.4486	2.041
4	0.6316	0.2778	2.994	0.9077	0.3371	2.692
5	0.6850	0.2908	3.043	0.8949	0.3314	2.700
6	0.7837	0.3039	2.974	0.9077	0.3429	2.647
7	0.8118	0.3072	2.968	0.9128	0.3457	2.640
8	0.9251	0.3105	2.980	0.8744	0.3600	2.429
9	0.9118	0.3007	3.032	0.9333	0.3629	2.572
10	0.9085	0.3072	3.085	0.9410	0.3943	2.387
11	0.8984	0.3007	2.988			

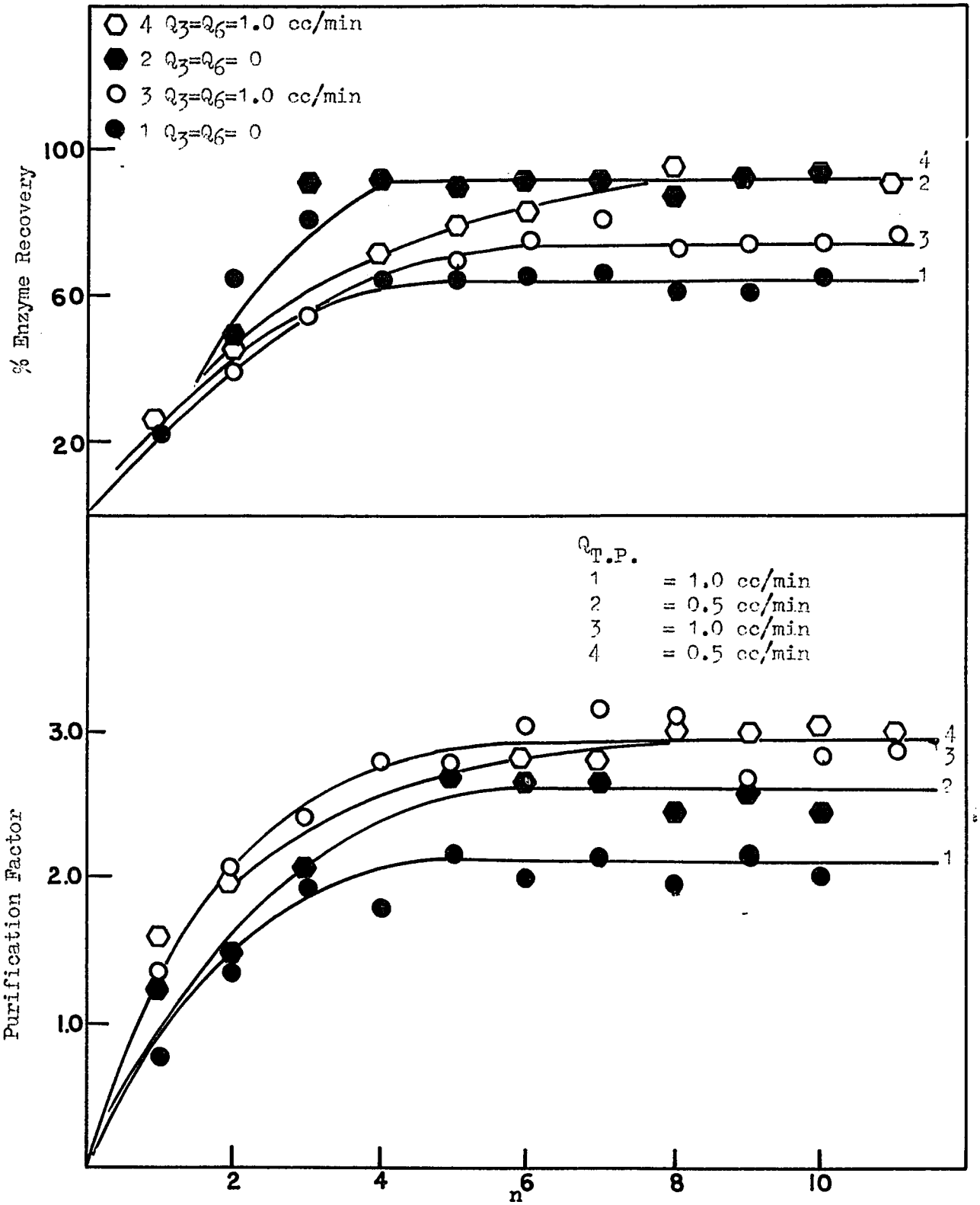


Figure34.Effect of Circulation, Experimental

8-Two Column Systems

A set of four runs designed to study the effect of using two column system with different configuration on the recovery and the purification of the enzyme HPAP (alkaline phosphatase) was carried out. Circulation was used in each of these runs except the one in which the two column (anions) were connected in series. The results are shown on Tables 43, 44, 45 and 46 and Figure 38 and Figure 40.

In the first run, two anion exchangers were connected such that the enzyme enriched product stream of the first column is feed to the second (Figure 35). The ionic strength (0.6) of this product stream was changed to 0.1 before fed to the second column. The change which is necessary in order that the adsorption step can take place on the second exchanger was conducted through the use of a dialyzer. The results obtained show an increase in the steady state value of the purification factor from an average value of 3.15 for the first column to 3.75 for the second column. The enzyme recovered from the second column was less than that from the first, but so was the concentration of the undesired proteins in the final enriched enzyme product stream. This is obvious since the feed to the second column, which is a product of the first column, contained a lesser amount of enzyme and undesired proteins if compared to the feed to the first column.

For the two column in series run, the feed was introduced to both columns (both anions) and no circulation was used (Figure 36). The percentage of the enzyme recovered and the purification factor obtained were almost the same as in the one column system with no circulation. This is clear, since the product withdrawn was the product of the two

column at the same time. This case could be considered close to a one column system with a height equal to two times the usual height (8.0 cm).

In the third run, the enriched enzyme product stream of the first anion column was directed to the 0.6M reservoir of the second anion column as shown in Figure 37 . The aim was to enrich the concentration of the enzyme in the top reservoir (0.6 M) of the second column, hence enhancing the enzyme concentration in the final enriched enzyme product stream obtained from the second column. Feed was also introduced to the second column. The enzyme recovered in the final enriched enzyme product stream was only 94% of that presented in the original feed that was fed to the first column. This was less than what we anticipated since fresh feed was also introduced to the second column. The purification factor did not increase if compared with the one column with circulation system. It is possible that the second column was to some extent saturated with the enzyme and part of the enzyme that appeared in its top product was not enzyme retained by the column. This conclusion is supported by the unusual, noticable enzyme concentration in the bottom product. Values as high as 25% of the original enzyme in the feed stream to the first column were observed. This compared to almost zero enzyme concentration in bottom products obtained in the case of a one column system.

The last run of this set was conducted using anion-cation system. The same operating conditions were used except for the feed stream where the feed buffer pH and ionic strength were 8.4 and 0.6M respectively. Breakthrough experiments indicated strong adsorption of the enzyme on

the anion exchanger for this condition. Our purpose was to guarantee that enzyme desorped by the cation exchanger would be readsorped on the anion exchanger during the first circulation step, (see Figure 39). A recovery as high as 0.75 was obtained and the purification factor steady value was about 2.1.

In conclusion, the two anion column system with the enriched enzyme product stream of the first used as feed to the second is the most promising configuration if a two column system is used to enhance the purification of the enzyme.

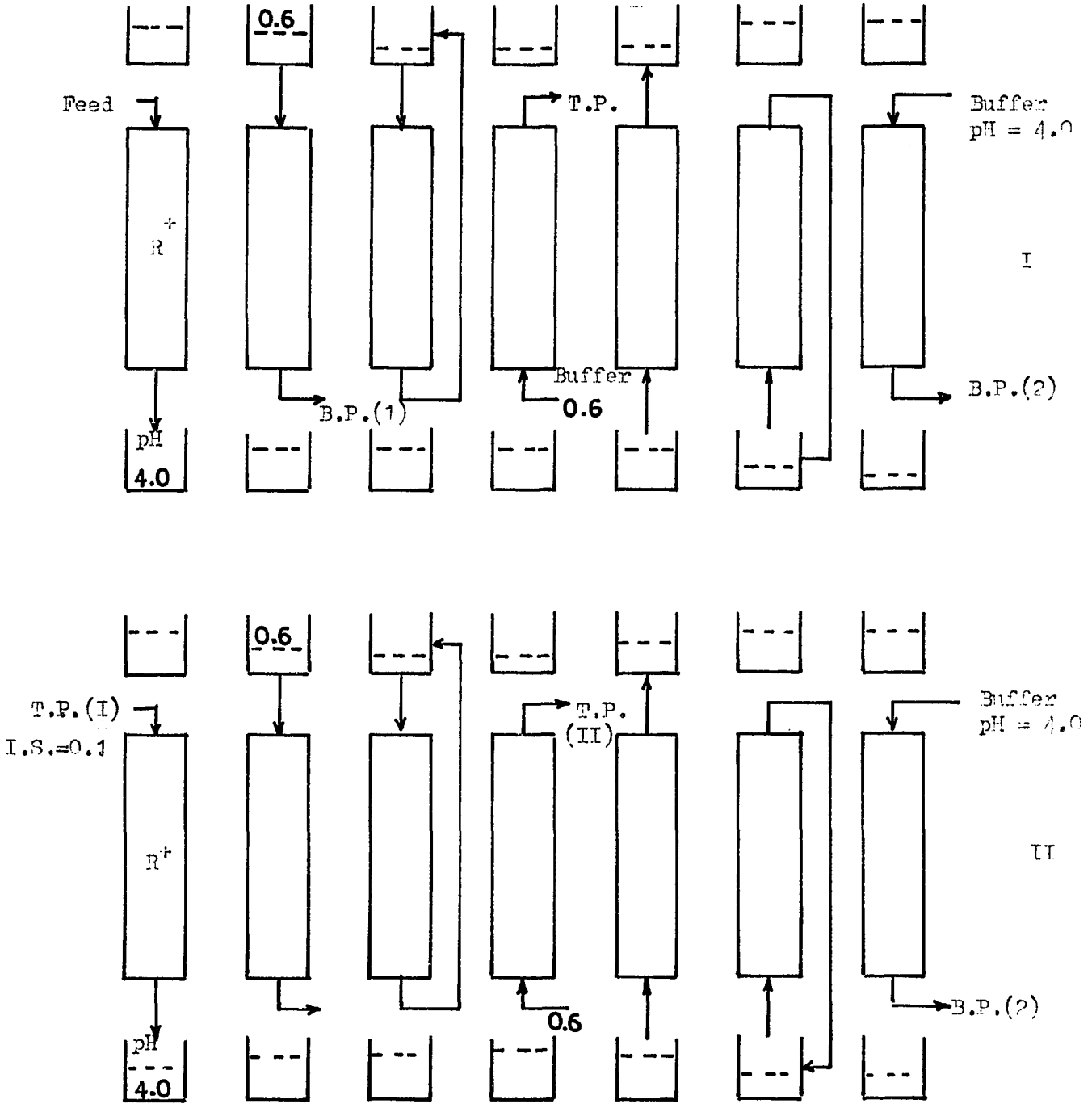


Figure 35. Two Column With Top Product of the First Used as Feed to the Second

TABLE 43

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, TWO COLUMN SYSTEM

WITH THE TOP PRODUCT OF THE FIRST AS FEED.TO THE SECOND

Ion Exchanger	=	Anion (DEAE)
$h_1 = h_2$	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Dead Volume	=	45 cc
Qt	=	15 cc

	COLUMN I			COLUMN II		
	Top Product			Top Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.1086	0.1438	0.76	0.0263	0.0813	0.32
2	0.3914	0.3125	1.25	0.0789	0.0688	1.15
3	0.3980	0.3438	1.16	0.1480	0.1156	1.28
4	0.6546	0.2844	2.30	0.2664	0.1125	2.37
5	0.6053	0.2625	2.30	0.3059	0.1063	2.87
6	0.7270	0.3063	2.37	0.3980	0.1250	3.18
7	0.7105	0.2844	2.50	0.4079	0.1344	3.04
8	0.9079	0.2594	3.50	0.4770	0.1125	4.30
9	0.9243	0.2969	3.11	0.5329	0.1387	3.87
10	0.9145	0.3063	2.99	0.5987	0.1594	3.70
11	0.9211	0.2781	3.30	0.6118	0.1750	3.50
12	0.8914	0.2844	3.13	0.5986	0.1594	3.76

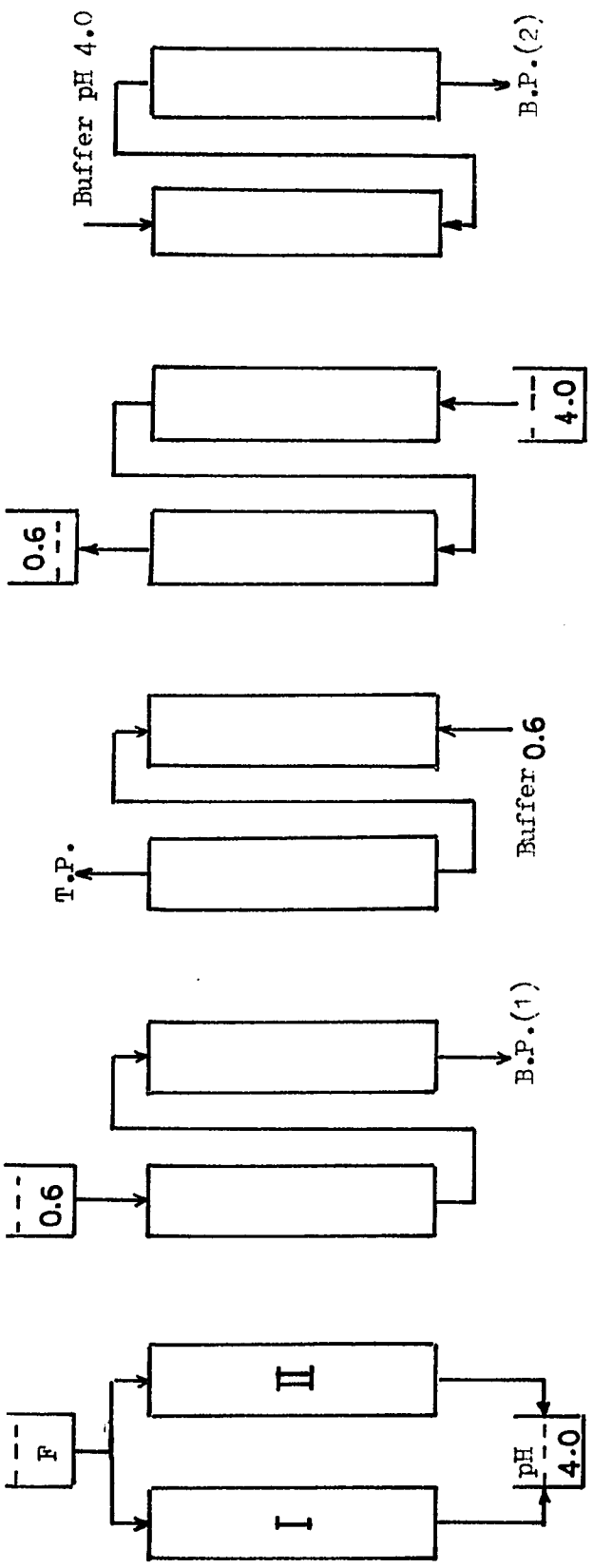


Figure 36. Two Column in Series (Anion-Anion)

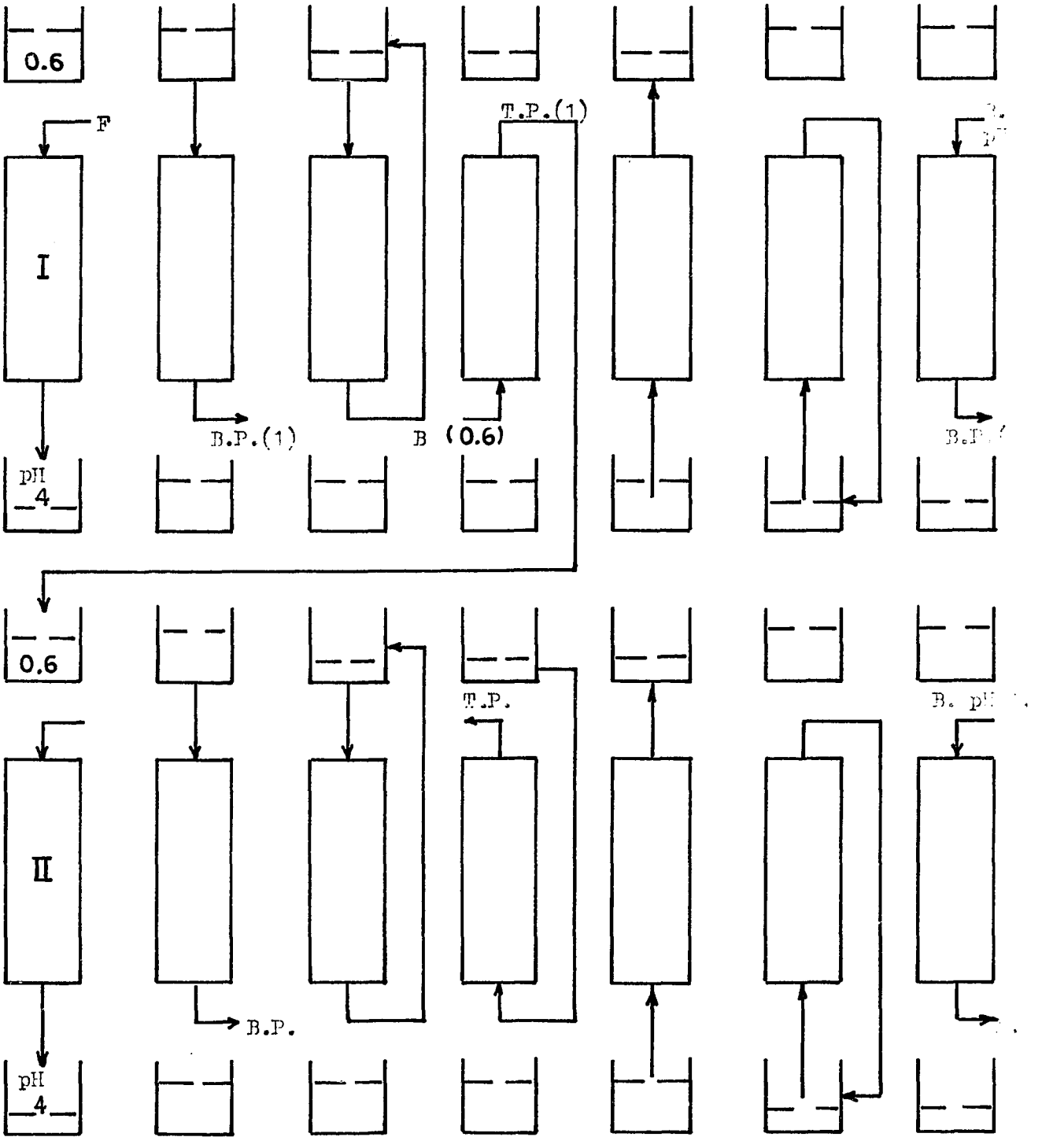
TABLE 44

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, TWO COLUMN IN SERIES

Ion Exchanger	=	Anion (DEAE)
$h_1 - h_2$	=	8 cm
Q	=	1.0 cc/min
Qt	=	30 cc (15 cc for each column)
Dead Volume	=	45 cc
Feed	=	0.02% Alk. Phosphatase
Product Volume	=	30 cc

	Major Product		
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.5424	0.3018	1.79
2	0.8362	0.7059	1.18
3	0.6525	0.5371	1.21
4	0.7373	0.4092	1.80
5	0.7626	0.4373	1.76
6	0.7175	0.4066	1.76
7	0.7712	0.2864	2.69
8	0.7429	0.3453	2.15
9	0.7768	0.2966	2.61
10	0.7514	0.2834	2.64



F = Feed
B = Buffer

Figure 37. Anion-Anion With T.P. of the First Column Directed to 0.6 Reservoir of the Second

TABLE 45

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, TWO COLUMN SYSTEM WITH TOP PRODUCT OF THE FIRST DIRECTED TO THE 0.6 RESERVOIR OF THE SECOND COLUMN

Ion Exchanger	=	Anion (DEAE)
$h_1 = h_2$	=	8.0 cm
Q	=	1.0 cc/min
$Q_{T.P.}$	+	0.5 cc/min
Feed	=	0.02% Alk. Phosphatase
Dead Volume	=	45 cc
Qt	=	15 cc

Cycle No.:	Top Product $\frac{a}{a_F}$	Top Product $\frac{Y}{Y_F}$	P.F.
1.	0.3373	0.1804	1.870
2	0.4627	0.3165	1.462
3	0.5522	0.4177	1.322
4	0.5831	0.2468	2.370
5	0.6627	0.2437	2.720
6	0.7612	0.2848	2.672
7	0.7373	0.2943	2.505
8	0.7493	0.2975	2.519
9	0.9284	0.3418	2.716
10	0.9313	0.3354	2.776
11	0.9433	0.3196	2.951

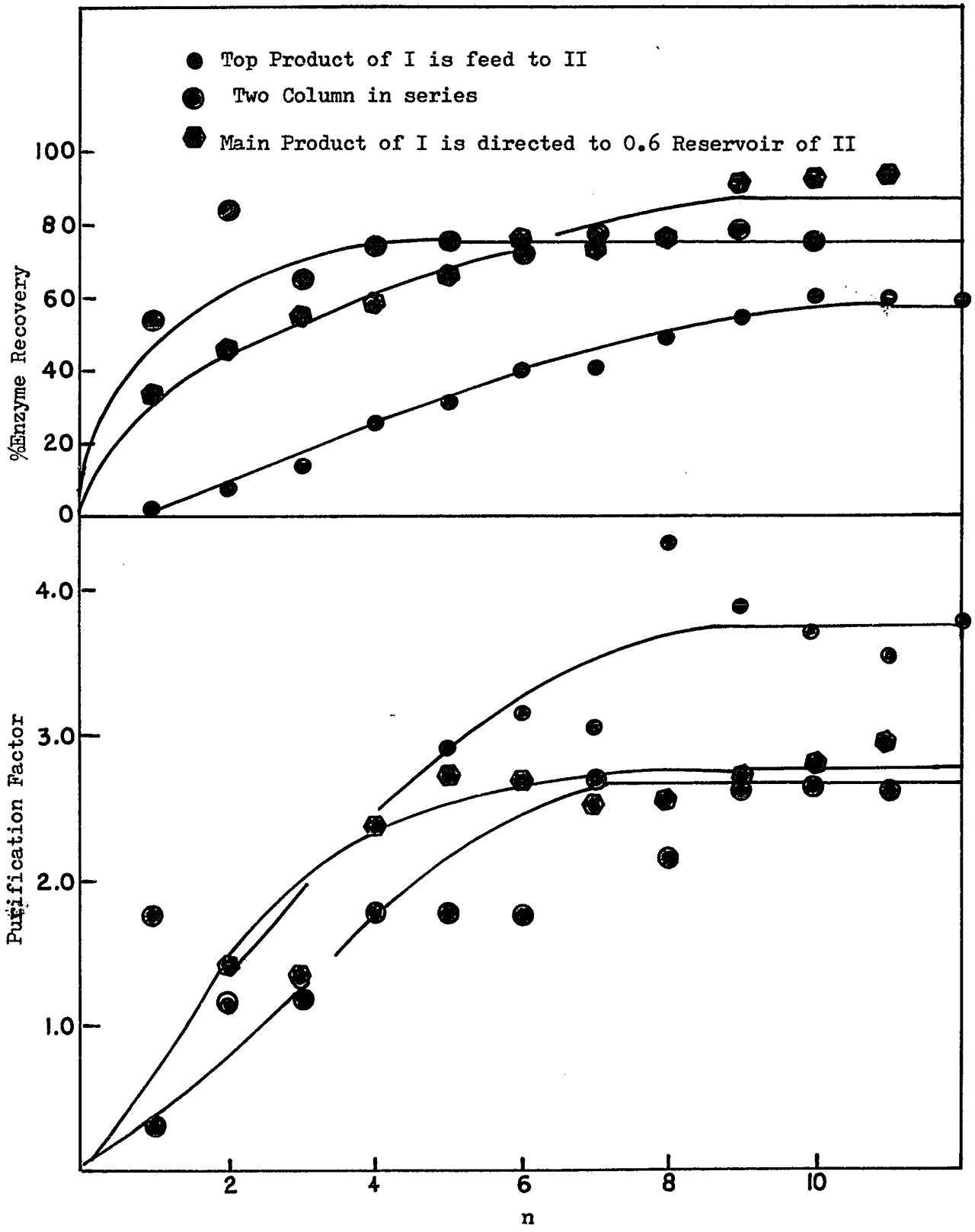


Figure 38. Anion - Anion Parametric Pump ,Experimental

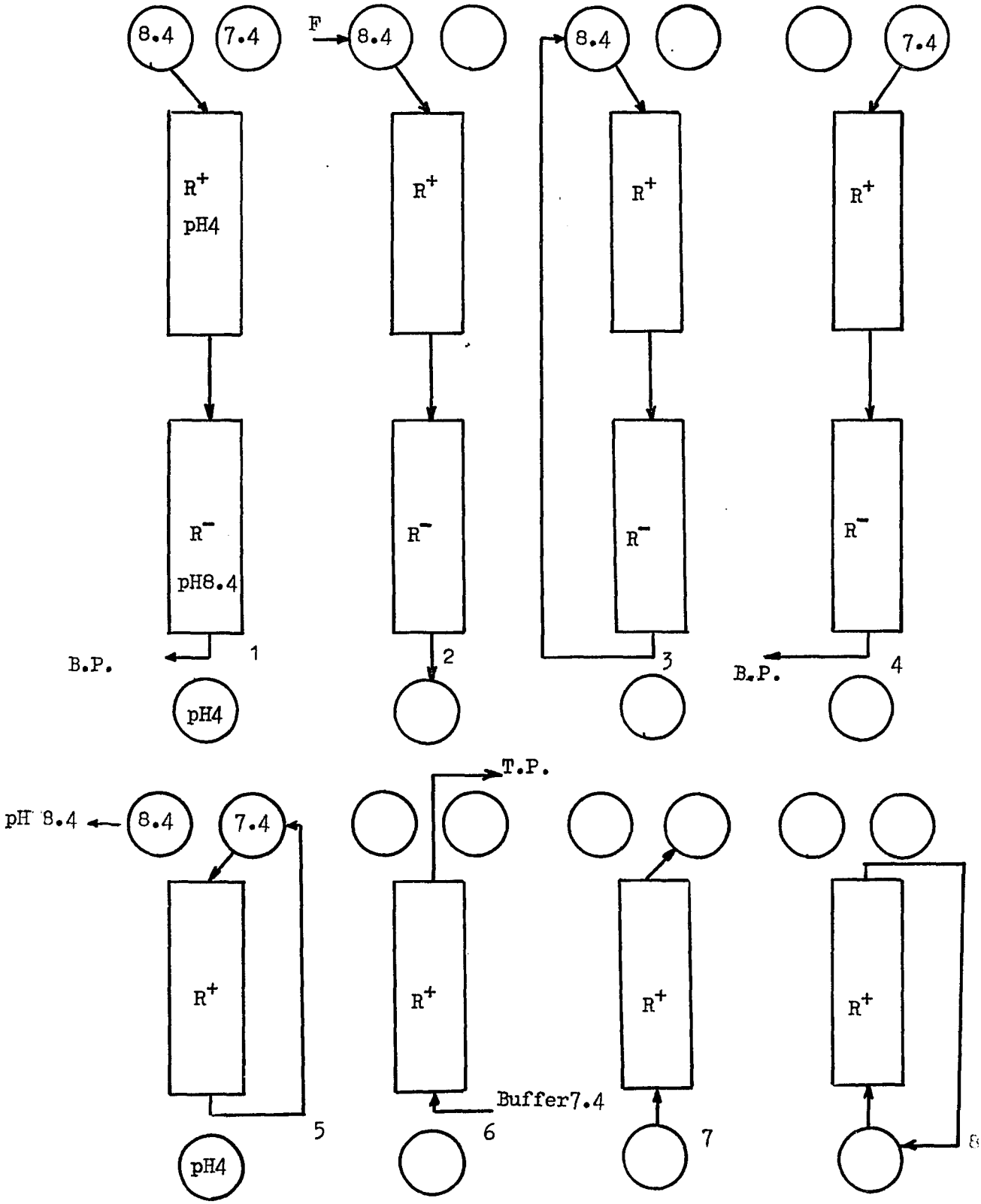


Figure 39. Anion - Cation Parametric Pump

TABLE 46

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, ANION-CATION

Anion	=	DEAE Sepharose
Cation	=	CM Sepharose
$h_1 = h_2$	=	8.0 cm
Q	=	1.0 cc/min
Dead Volume	=	45.0 cc
Feed	=	0.02% Alk.Ph. pH 8.4,I.S.=0.4
Elution Buffers	=	pH 7.4, I.S.=0.6 and pH 4.0, I.S.=0.1
Qt	=	15 cc

	Top Product			Bottom Product (2)
Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{Y}{Y_F}$
1	0.1718	0.1856	0.926	0.0526
2	0.3128	0.2355	1.328	0.2687
3	0.4308	0.3268	1.318	0.3629
4	0.6179	0.3241	1.907	0.4986
5	0.6718	0.3241	2.073	0.5346
6	0.6026	0.3186	1.891	0.4875
7	0.6436	0.3102	2.075	0.5374
8	0.6179	0.3241	1.907	0.5291
9	0.6667	0.3213	2.075	0.4460
10	0.7026	0.3352	2.096	0.3041
11	0.7462	0.3463	2.155	0.3313
12	0.7538	0.3573	2.110	0.3324

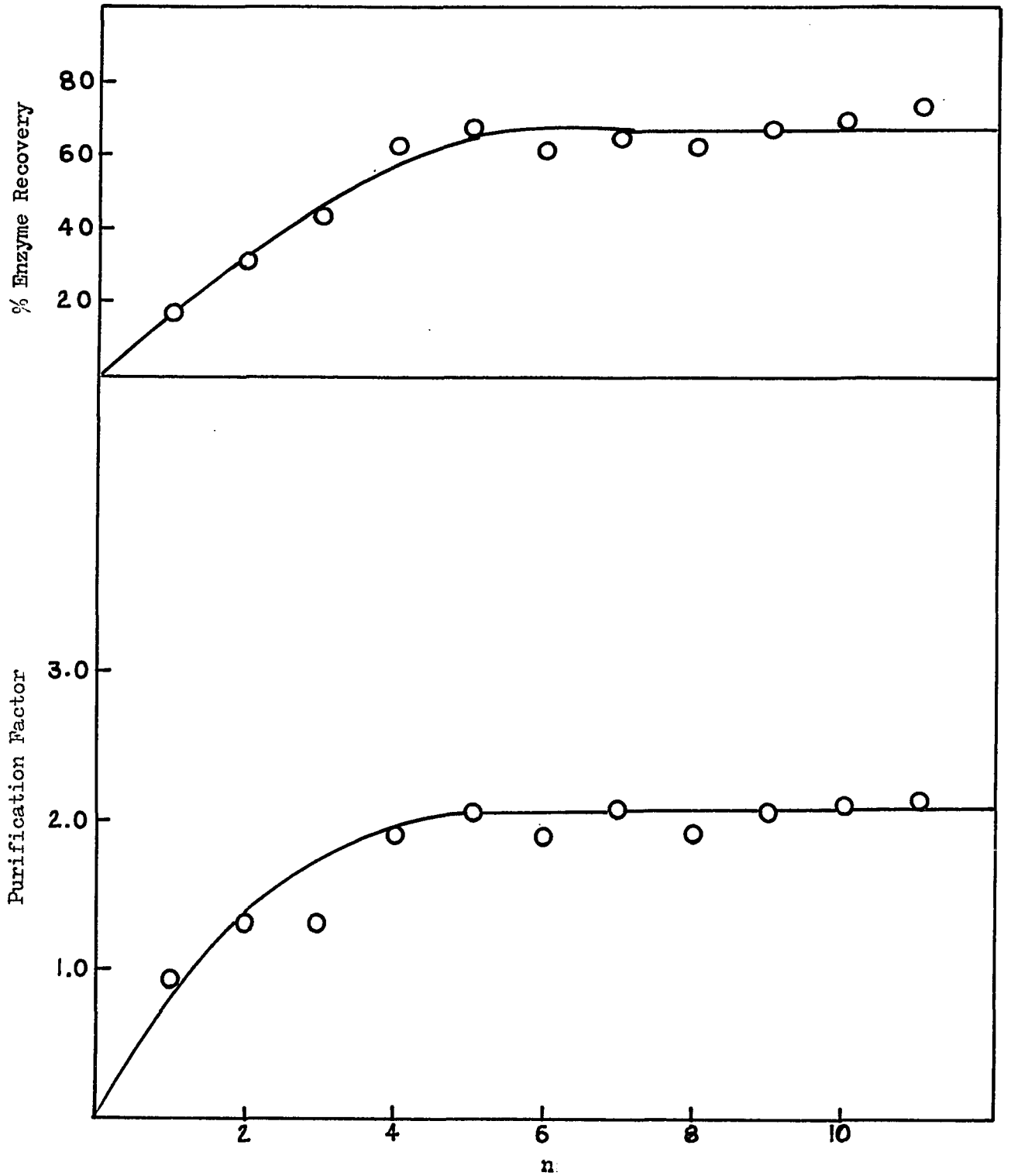


Figure 40. Anion – Cation Parametric Pump

9- Comparison of Parametric Pumping With Cycling Zone Adsorption

The difference between parametric pumping and cycling zone adsorption is only in the method of operation. The cycling zone adsorption flows in a single direction.

The cycling zone is an open system which after several cycles reaches a repeating state where each cycle is exactly a repeat of the preceding cycle. This is analogous to steady state in parametric pumping. The reversal of the flow in parametric pumping imposes limitations on production while longitudinal mixing in cycling zone adsorption imposes limitations on separation.

More mass transfer between the solid and fluid phases at any given position in the column takes place in parametric pumping than in cycling zone adsorption because of longer contact time between the phases in the case of parametric pumping.

The enzyme recovery and purification using the two techniques were compared. This comparison requires a fixed flow rate in a column of fixed size and fixed amount of feed. Two runs, one for each under the same operating conditions with flow rate equal to 1.0 cc/min, were picked.

In order to make such a comparison, we set $\tau_1 = t_2 = t_3 = t_1$ (cycling zone) (Figure 11). The average values of a_p and Y_p over each time interval, t_1, are used for the comparison. For each cycle, there are three average values ($\alpha_1, \alpha_2, \alpha_3$ and $\beta_1, \beta_2, \beta_3$ shown in Figure 11) corresponding to three product concentrations. The parametric pumping run results are given on Table 41. The comparison for the purified product stream for the two processes is given on Table 47.

One can see that parametric pumping has a higher purification factor and greater % enzyme activity recovered than cycling zone adsorption system, while the latter has a larger rate of production of product.

TABLE 47

COMPARISON OF PARAMETRIC PUMPING WITH

CYCLING ZONE ADSORPTION

At Steady State $n \longrightarrow \infty$	Parametric Pumping	Cycling Zone Adsorption
Purification Factor, P.F.	2.8	1.6*
% Enzyme Activity Recovered,	75%	59%**
Rate of Production of Product (based on column of height = 8.0 cm and column diameter = 1.6 cm), International Units/min	0.065	0.121

* based on α_2/β_2

** based on α_2

CONCLUSIONS

Many enzyme purification processes are batch-wise. The parametric pumping device described here offers the possibility of semicontinuous processing, thereby tending to minimize both processing time and degradation. Further more, it can yield high purification factors using small equipment.

In enzyme purification, small molecules can be easily removed by dialysis. However, the separation of the enzyme from the undesired proteins presented in the mixture is a serious problem. Enzyme concentration can be determined by measuring its activity. The activity is expressed as international units/cc. This activity in relation to the total amount of proteins present in the sample determines the purity of the enzyme. Therefore maximizing the quantity a/Y, i.e., the purification factor is very important in the purification process.

In the purification of alkaline phosphatase by pH-ionic strength parametric pumping using DEAE-Sepharose as an adsorbent several factors were found to affect the purification process. The most pronounced are: the flow rate, the displacement, feed concentration and the amount of product withdrawn. Other factors that have also an effect on the process include type of adsorbent, hence its capacity, type of elution buffer and the technique of operation used.

Slower flow rate increases the mass transfer between the solid and the fluid phases resulting in more separation. However, an optimum flow

rate should be used because too long a time might cause the undesired proteins to desorb with the enzyme. Intermixing might also take place. A displacement slightly higher than the void volume of the column will assure the shifting of the pH and ionic strength inside the column. This will guarantee that the entire column will experience the maximum change in the thermodynamic variables of the process.

Lower feed concentration increases the capacity of the adsorbent and results in too strong adsorption of enzyme molecules on the adsorbent. Thus the desorption step might not be easy. In the meantime higher feed concentration saturates the adsorbent and enzyme eluted might not be the enzyme retained by the exchanger, hence less separation is obtained. Higher concentration might also cause interaction between the large molecules.

An elution buffer that does not saturate the column is required to facilitate the removal of the enzyme molecules into the fluid phase during the elution step.

Circulation between the fluid in the column and the reservoirs (where the pH and ionic strength are maintained constants) to ensure the shifting of pH and ionic strength to the required values proved to be of great importance. The calculated values agreed reasonably well with the experimental values obtained from runs with circulation introduced as a step in the process.

One pH and several ionic strengths i.e. graduate elution would be an advantage if the variable ionic strengths are further apart and no mixing between them that will change their original values occured.

Further applications involving two or multi-column systems are more promising especially if the columns are of the same type and if the product from the previous column is used as a feed to the next column. However, less enzyme is recovered but high purification is attained.

Some proteins could carry negative charges at pH higher than their isoelectric points and still bind to cation exchanger especially in cases of low ionic strengths. The reverse holds true for anion exchangers (in cases of low ionic strengths). Hence optimum ionic strengths should be used for both the adsorption and desorption steps.

The recuperative mode in cases of pH and ionic strength parametric pumping is more feasible and practical since it is very difficult to introduce changes in the pH or the ionic strength in a direct more without disordering the concentration profiles inside the column. The recuperative mode is also considered as adiabatic while the entering fluid changes its thermodynamic variable.

No unique set of equations, hence no single solutions could be obtained for all cases of this non-symmetric open flow system. This is due to the fact that for each combination of reflux ratio (volume displaced from the column into the reservoir at one end of the column to the total volume of products withdrawn at that end) there are different patterns of concentration regions inside the column. These patterns make up the product stream. Therefore, several solutions are required. Each one of them is valid only under the conditions for which it was devised. As an example, if we displace the void volume of the column, then the set of equations given in Theory (pages 21, 23, 24) may be used. However, if the displacement is more or less than the void volume of the column, then the set of equations given in Appendix A should be used.

The experimental results agree reasonably with those predicted from the mathematical analyses (see Tables A-1, A-2 and Figure A-2). Those analyses were based on the assumption of linear equilibrium between the solid and fluid phases. The operating conditions and the results for a typical optimized run for the purification of alkaline phosphatase by the technique we developed are given on Table 42 (circulation included).

NOMENCLATURE

a	enzyme activity, defined in Appendix D	
A	area	m^2
B.R.	bottom reservoir	
E	enzyme concentration in fluid phase	gm/cc
F	feed	cc
h	column height	m
I.S.	buffer concentration	gm mole/cc
L	height	m
m	slope of the equilibrium distribution isotherm	
n	number of cycles	
P.F.	purification factor = $(\langle a \rangle_n / \langle Y \rangle_n)/(a_F/Y_F)$	
Q	volumetric flow rate	cc/min
R^+	anion	
R^-	cation	
t	time duration	min
T.R.	top reservoir	
u	solute velocity	cc/min
v	fluid velocity	cc/min
V	volume of fluid phase on stage	cc
$\bar{V}$	volume of solid phase on stage	cc
V_D	dead volume in reservoir	cc
x	concentration of enzyme in solid phase	gm/cc
Y	total protein concentration	gm/cc
$\langle\ \rangle$	average	

NOMENCLATURE (CONTINUED)

Subscripts

a	step number in the process
b	step number in the process
c	step number in the process
d	step number in the process
e	step number in the process
F	feed
J	stage number
n	n^{th} cycle
P	product
T.P.	top product
∞	steady state

Greek letters

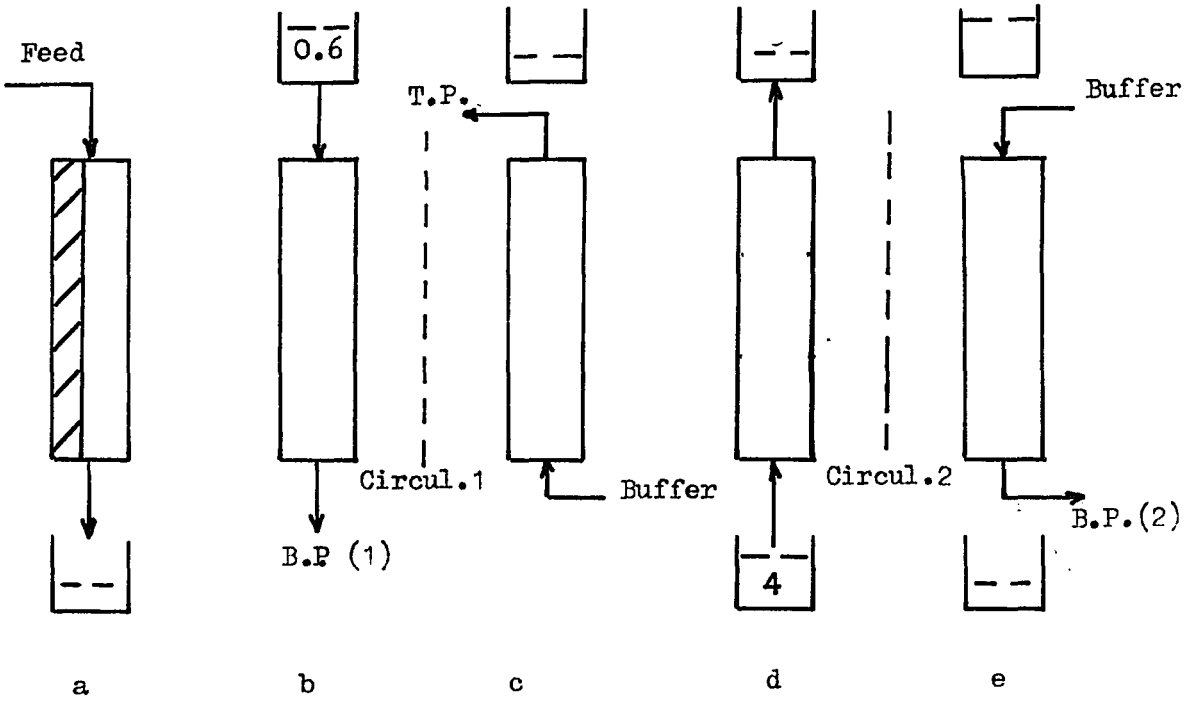
α	average value of a_p (cycling zone)
β	average value of Y_p (cycling zone)
ϵ	void fraction in column
ρ	ration of $V/\overline{V}$
ρ_D	ration of $V_D/\overline{V}$
η	intensive system variable
ψ	% of enzyme activity recovered

APPENDIX A

CALCULATED RESULTS

For the model presented in the theoretical part of this work, the equations were derived for displacement which is equal to the column void volume. Here we extend the derivation to displacements less or greater than the column void volume. The assumptions mentioned in the theory section still hold. The same nomenclature is used.

The experimental results for the enzyme concentration in the product streams agree reasonably with theoretical predictions. Some deviation in the case of the undesired proteins is observed. This is due to the nonhomogenity of the undesired proteins since they are a mixture of many proteins, (some of them are unknown). Therefore they might undergo different passes through the adsorbent. The calculated results for two cases are given at the end of this appendix.



Circul.1 ; Circulation between top reservoir and column.

Circul.2 ; Circulation between bottom reservoir and column.

Figure A-1 pH - Ionic Strength Parametric Pump, Scheme of Operation

For displacement less than or equal to the column void volume

$$X_n^a\bar{V} + VE_n^a = X_{n-1}^e\bar{V} + VrE_F + (V-Vr)E_{n-1}^e \quad \text{A-1}$$

$$X_n^b\bar{V} + (V + V_D)E_n^b = X_n^a\bar{V} + (V-Vr)E_n^a + (V_D + Vr)E_{n-1}^{T.R.} \quad \text{A-2}$$

$$X_n^c\bar{V} + VE_n^c = X_n^b\bar{V} + (V-Vr)E_n^b \quad \text{A-3}$$

$$X_n^d\bar{V} + (V + V_D)E_n^d = X_n^c\bar{V} + (V-Vr)E^c + (V_D + Vr)E_{n-1}^{B.R.} \quad \text{A-4}$$

$$X_n^e\bar{V} + VE_n^e = X_n^d\bar{V} + (V-Vr)E_n^d \quad \text{A-5}$$

let

$$\frac{V}{\bar{V}} = \rho \quad \text{A-6}$$

$$x^i = mE^i \quad \text{A-7}$$

$$\frac{Vr}{\bar{V}} = \rho_r \quad \text{A-8}$$

$$\frac{V}{\bar{V}} = \rho_D \quad \text{A-9}$$

$\therefore$

$$E_n^a(\rho + m^a) = m^e E_{n-1}^e + (\rho - \rho_r)E_{n-1}^e + \rho_r E_F \quad \text{A-10}$$

$$E_n^a = \frac{1}{\rho + m^a}\left\{E_{n-1}^e(m^e + \rho - \rho_r) + \rho_r E_F\right\} \quad \text{A-11}$$

$$m^b E_n^b + (\rho + \rho_D)E_n^b = m^a E_n^a + (\rho - \rho_r)E_n^a + E_{n-1}^{T.R.}(\rho_D + \rho_r) \quad \text{A-12}$$

$$E^{T.R.}_{n-1} = (E^{b}_{n-1} V_D + VrE^{c}_{n-1}) / (V_D + Vr) \qquad A\text{-}13$$

$$E^{c}_{n} = \frac{1}{\rho + m^{c}} \left\{ E^{b}_{n} m^{b} + (\rho - \rho_r) E^{b}_{n} \right\} \qquad A\text{-}14$$

$$E^{c}_{n} = \frac{1}{\rho + m^{c}} E^{b}_{n} (m^{b} + (\rho - \rho_r)) \qquad A\text{-}15$$

$\therefore$

$$E^{T.R.}_{n-1} = \left[E^{b}_{n-1} V_D + VrE^{b}_{n-1} (m^{b} + \rho - \rho_r)/(\rho + m^{c}) \right] / (V_D + Vr) \qquad A\text{-}16$$

$$= E^{b}_{n-1} \left\{ V_D + Vr \frac{(m^{b} + \rho - \rho_r)}{+ m^{c}} \right\} / (V_D + Vr) \qquad A\text{-}17$$

$$= E^{b}_{n-1} \left\{ \frac{V_D}{V_D + Vr} + \frac{Vr}{V_D + Vr} \frac{m^{b} + \rho - \rho_r}{\rho + m^{c}} \right\} \qquad A\text{-}18$$

$$= E^{b}_{n-1} \left\{ \frac{\rho_D}{\rho_D + \rho_r} + \frac{(\rho_r)}{\rho_D + \rho_r} \frac{m^{b} + \rho - \rho_r}{\rho + m^{c}} \right\} \qquad A\text{-}19$$

$$= E^{b}_{n-1} \left\{ \frac{\rho_D}{\rho_D + \rho_r} + \frac{\rho_r}{\rho_D + \rho_r} \frac{m^{b} + \rho - \rho_r}{\rho + m^{c}} \right\} \qquad A\text{-}20$$

$\therefore$

$$E^{b}_{n} = \frac{1}{m^{b} + \rho + \rho_D} \left\{ E^{a}_{n} (m^{a} + \rho - \rho_r) + E^{b}_{n-1} \left(\frac{\rho_D + \rho_r}{\rho_D + \rho_r}\right) * \right.$$

$$\left. (\rho_D + \rho_r \frac{m^{b} + \rho - \rho_r)}{\rho + m^{c}} \right\} \qquad A\text{-}21$$

$$E_n^b = \frac{1}{m^b + \rho + \rho_D} \left\{ E_n^a(m^a + \rho - \rho_r) + E_{n-1}^b \left(\rho_D + \rho_r \frac{m^b + \rho - \rho_r}{\rho + m^c} \right) \right\} \quad \text{A-22}$$

$$E_n^d(m^d + \rho_D + \rho) = m^c E_n^c + (\rho - \rho_r)E_n^c + (\rho_r + \rho_D)E_{n-1}^{B.R.} \quad \text{A-23}$$

$$E_{n-1}^{B.R.}(V_D + Vr) = V_D E_{n-1}^d + VrE_{n-1}^e \quad \text{A-24}$$

$\therefore$

$$E_n^d = \frac{1}{m^d + \rho + \rho_D} \left\{ E_n^c (m^c + \rho - \rho_r) + E_{n-1}^d \left(\rho_D + \rho_r \frac{m^d + \rho - \rho_r}{\rho + m^e} \right) \right\} \quad \text{A-25}$$

$$E_n^e = \frac{1}{\rho + m^e} E_n^d (m^d + \rho - \rho_r) \quad \text{A-26}$$

For the case where displacement is greater than the void volume of the column with the assumption of complete adsorption during step a, the equations are:

$$E_n^a = \frac{1}{\rho + m^a} \left\{ m^e E_{n-1}^e + \rho_r E_F \right\} \quad \text{A-27}$$

$$E_n^b = \frac{1}{\rho_D + \rho + m^b} \left\{ m^a E_n^a + (\rho_D + \rho)(E_{n-1}^{T.R.}) \right\} \quad \text{A-28}$$

but

$$E^{T.R.}_{n-1} = (E^{b}_{n-1} V_D + VE^{c}_{n-1} + (Vr - V)E^{e}_{n-1}) / (V_D + Vr) \qquad A\text{-}29$$

$$E^{c}_{n-1} = \frac{1}{\rho + m^{c}} m^{b} E^{b}_{n-1} \qquad A\text{-}30$$

i.e.,

$$E^{c}_{n} = \frac{1}{\rho + m^{c}} m^{b} E^{b}_{n} \qquad A\text{-}31$$

for bottom reservoir we have

$$E^{B.R.}_{n-1} = (V_D E^{d}_{n-1} + VE^{e}_{n-1} + (Vr-V)(0)) / V_D + Vr \qquad A\text{-}32$$

$$= \frac{1}{\rho_D + \rho_r} (\rho E^{d}_{n-1} + \rho E^{e}_{n-1}) \qquad A\text{-}33$$

and

$$E^{d}_{n} = \frac{1}{m^{d} + \rho + \rho_D} \left\{ m^{c} E^{c}_{n} + (\rho + \rho_D) E^{B.R.}_{n=1} \right\} \qquad A\text{-}34$$

$$= \frac{1}{m^{d} + \rho + \rho_D} \left\{ m^{c} E^{c}_{n} + \frac{\rho + \rho_D}{\rho_D + \rho_r} E^{d}_{n-1} (\rho_D + \frac{m^{d} \rho}{\rho + m^{e}}) \right\} \qquad A\text{-}35$$

Finally

$$E^{e}_{n} = \frac{1}{\rho + m^{e}} m^{d} E^{d}_{n} \qquad A\text{-}36$$

TABLE A-1

SEMI-CONTINUOUS PARAMETRIC PUMP

CALCULATED RESULTS, VARIABLE FLOW RATE AND DISPLACEMENT

Q = 1.0 cc/min

Q_t = 15 cc

Top Product

Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2039	0.1724	1.1827
2	0.3582	0.2795	1.282
3	0.4706	0.3460	1.360
4	0.5538	0.3879	1.428
5	0.6152	0.4140	1.486
6	0.6607	0.4305	1.535
7	0.6944	0.4408	1.575
8	0.7193	0.4475	1.607
9	0.7377	0.4517	1.633
10	0.7514	0.4574	1.654
11	0.7615	0.4563	1.669
12	0.7691	0.4575	1.682
13	0.7746	0.4582	1.691
14	0.7787	0.4588	1.697
15	0.7818	0.4592	1.703
16	0.7841	0.4594	1.707

TABLE A-2

SEMI-CONTINUOUS PARAMETRIC PUMP

CALCULATED RESULTS, VARIABLE FLOW RATE AND DISPLACEMENT

Q	=	1.0 cc/min
$Q_{T.P.}$	=	0.5 cc/min
Qt	=	15.0 cc

Top Product

Cycle No.:	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.2082	0.1745	1.193
2	0.3637	0.2842	1.280
3	0.4800	0.3535	1.358
4	0.5667	0.3973	1.4264
5	0.6315	0.4250	1.486
6	0.6800	0.4427	1.536
7	0.7162	0.4540	1.578
8	0.7433	0.4612	1.612
9	0.7636	0.4659	1.639
10	0.7789	0.4690	1.661
11	0.7902	0.4710	1.678
12	0.7987	0.4723	1.691
13	0.8051	0.4731	1.702
14	0.8098	0.4738	1.709
15	0.8134	0.4742	1.715

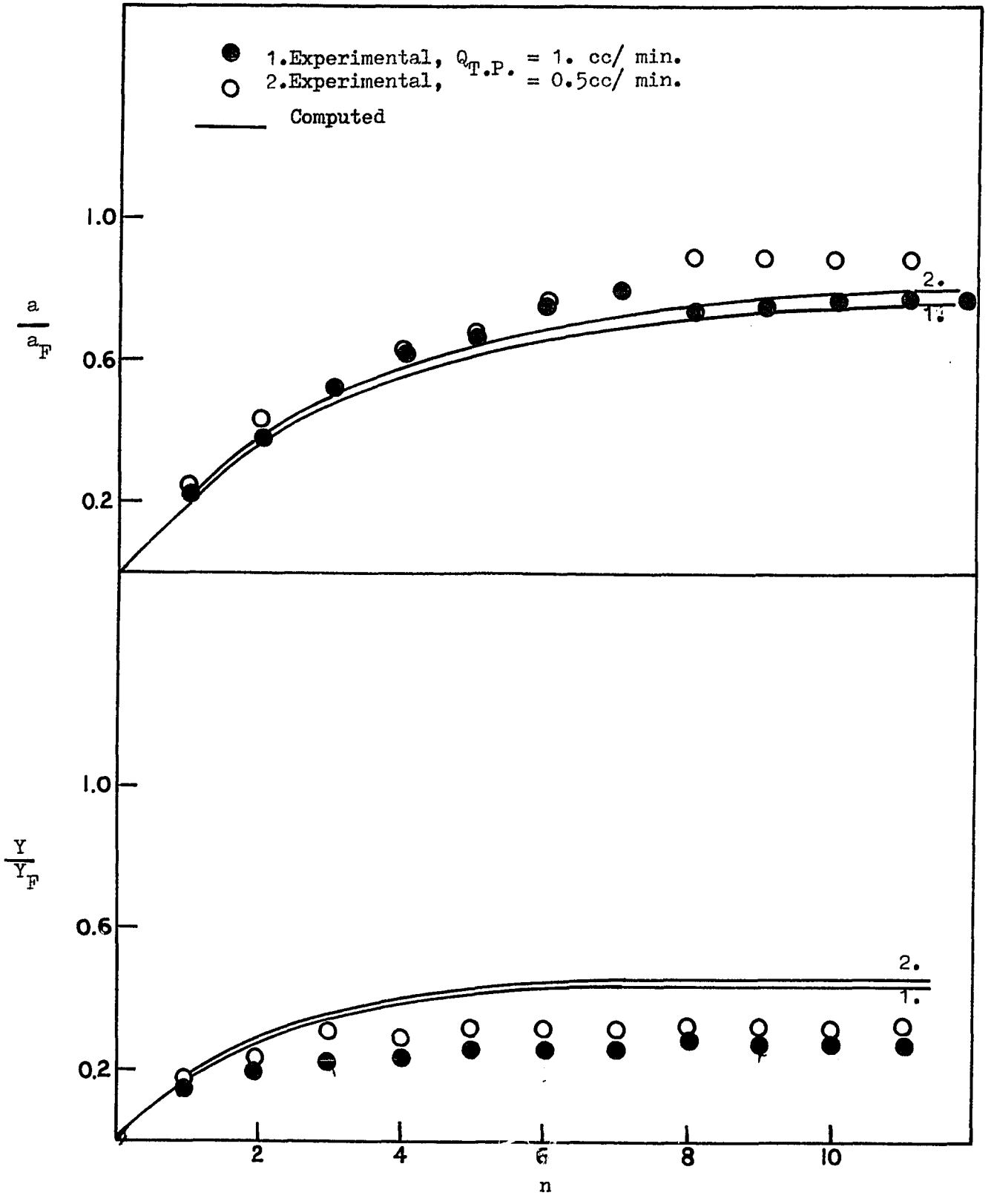


Figure A-2. Calculated Results

APPENDIX B

ONE pH, SEVERAL IONIC STRENGTHS PARAMETRIC PUMP

The using of one thermodynamic variable as a deriving force for both the purification process and the regeneration of the column would make the process much easier to operate and control.

To our knowledge few studies have been devoted to investigate the use of the concentration changes inside the column as a deriving force for separation in cyclic separation processes.

The ionic strength parametric pump would have the advantage of achieving the required range of the separation power without series changes in the composition of the eluting buffer. The feasibility of such a technique was examined through a series of experimental runs. The typical results of two of these runs are shown on Table B-1 and B-2. The feed stream in each of these runs was 0.02% alkaline phosphatase in 0.1M, Tris + HCl (pH 7.4) buffer. The step-wise desorption of the enzyme and the undesired proteins was carried out using the same buffer but with ionic strengths of 0.6 and 0.8M respectively.

For the one product scheme parametric pump, the concentration of the enzyme in the top reservoir increased with the number of cycles. The same holds true in the case of the undesired proteins in the bottom reservoir.

In the case of the regular three product parametric pump scheme, the enzyme concentration in the top product increased with the number of cycles and purification factors above 2.0 were obtained.

Increasing the ionic strength decreased the interaction between the adsorbent and the adsorbate by increasing the force of dissociation. Therefore breaking the electric linkage between the protein molecules and the exchanger.

The results shown here together with the ionic strength cycling zone results indicate that, the ionic strength parametric pump has the capability of purifying the enzyme to a satisfactory level.

While the pH-ionic strength parametric pump showed a slightly higher purification factor, it is believed that the ionic strength parametric pump is easy to operate since only one thermodynamic variable needs to be controlled. Sketches of the operating scheme and experimental results are given on Figures B-1 and B-2.

TABLE B-1

SEMI-CONTINUOUS - PARAMETRIC PUMP

EXPERIMENTAL RESULTS, ONE pH, VARIABLE IONIC STRENGTHS

ONE PRODUCT SCHEME

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
Feed	=	0.02% Alk.Phosphatase, I.S.=0.1
h	=	8.0 cm
Q	=	1.0 cc/min
Qt	=	15.0 cc
Dead Volume	=	45.0 cc
pH	=	7.4

Elution Buffer	I.S. = 0.6			I.S. = 0.8		
Cycle No.:	Top Reservoir			Bottom Reservoir		
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.
1	0.1379	0.1098	1.27	0.0586	0.0289	2.04
2	0.3379	0.3295	1.03	0.1276	0.0925	1.39
3	0.5448	0.5144	1.07	0.1966	0.1214	1.63
4	0.6000	0.5462	1.11	0.2207	0.2207	1.26
5.	0.7103	0.7168	1.00	0.4517	0.7428	0.61
6.	0.8759	0.5751	1.54	0.9310	0.8613	1.09
7	0.8345	0.5462	1.54	0.9000	0.8382	1.08

TABLE B-2

SEMI-CONTINUOUS PARAMETRIC PUMP

EXPERIMENTAL RESULTS, ONE pH, VARIABLE IONIC STRENGTHS

Ion Exchanger	=	Anion (DEAE)
Buffer	=	Tris + HCl
Feed	=	0.02% Alk.Phosphatase,I.S.=0.1
h	=	8.0 cm
Q	=	1.0 cc/min
Qt_i	=	15.0 cc
Dead Volume	=	45.0 cc
pH	=	7.4
Elution Buffers	=	I.S.=0.6, I.S.=0.8

Cycle No.:	Top Product			Bottom Product B.P.(2)	
	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$	P.F.	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
1	0.161	0.091	1.71	0.062	0.101
2	0.384	0.210	1.82	0.181	0.221
3	0.472	0.242	1.96	0.110	0.161
4	0.581	0.263	2.20	0.143	0.253
5.	0.615	0.311	1.93	0.170	0.362
6.	0.634	0.260	2.41	0.191	0.341
7	0.703	0.264	2.69	0.171	0.336
8	0.664	0.340	1.96	0.182	0.340
9	0.715	0.341	2.07	0.219	0.361
10	0.830	0.462	1.80	0.169	0.349
11	0.825	0.342	2.41	0.260	0.347
12	0.821	0.340	2.40	0.179	0.341

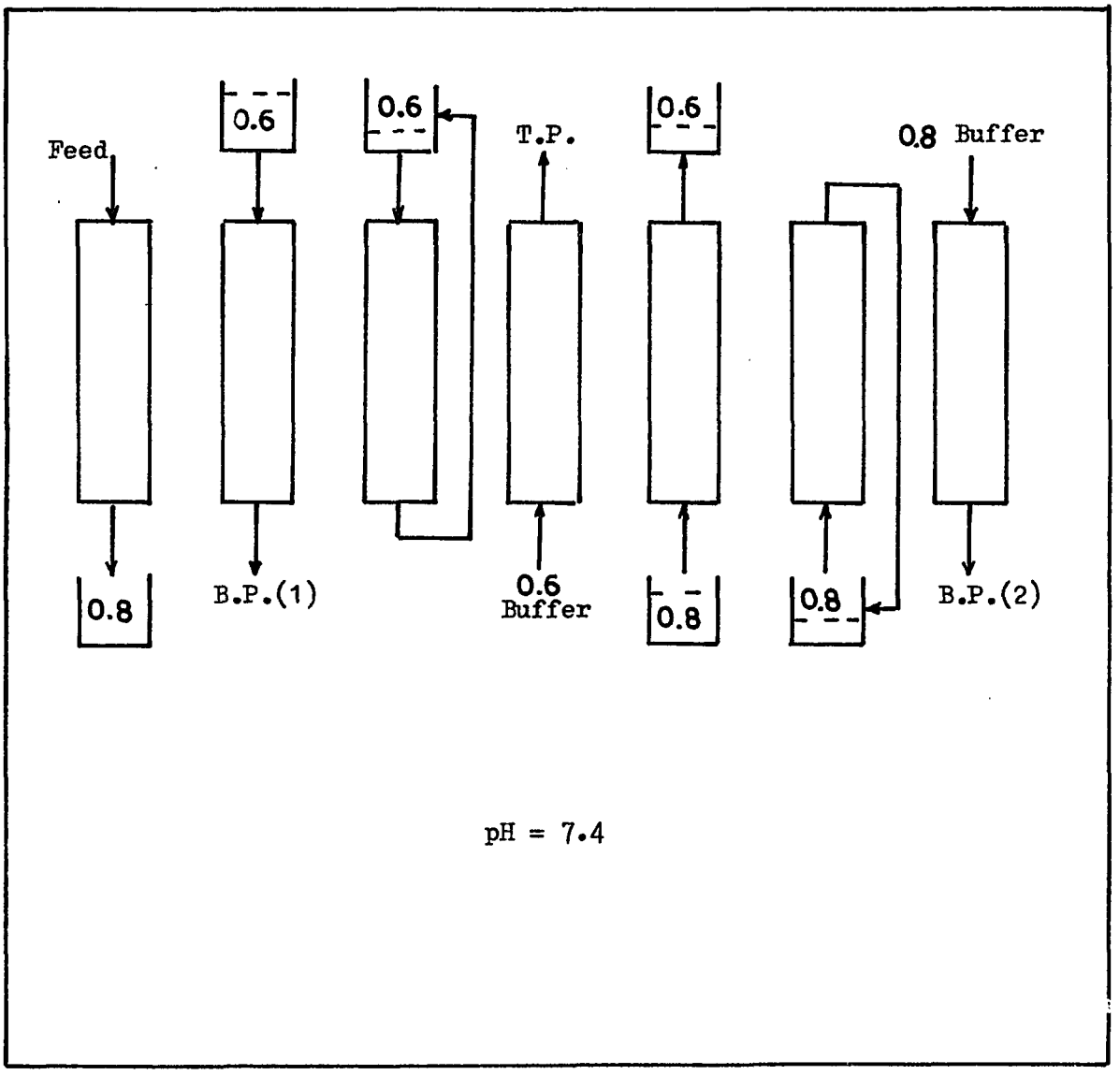


Figure B-1 Scheme of One pH, Different Ionic Strength Parametric Pump

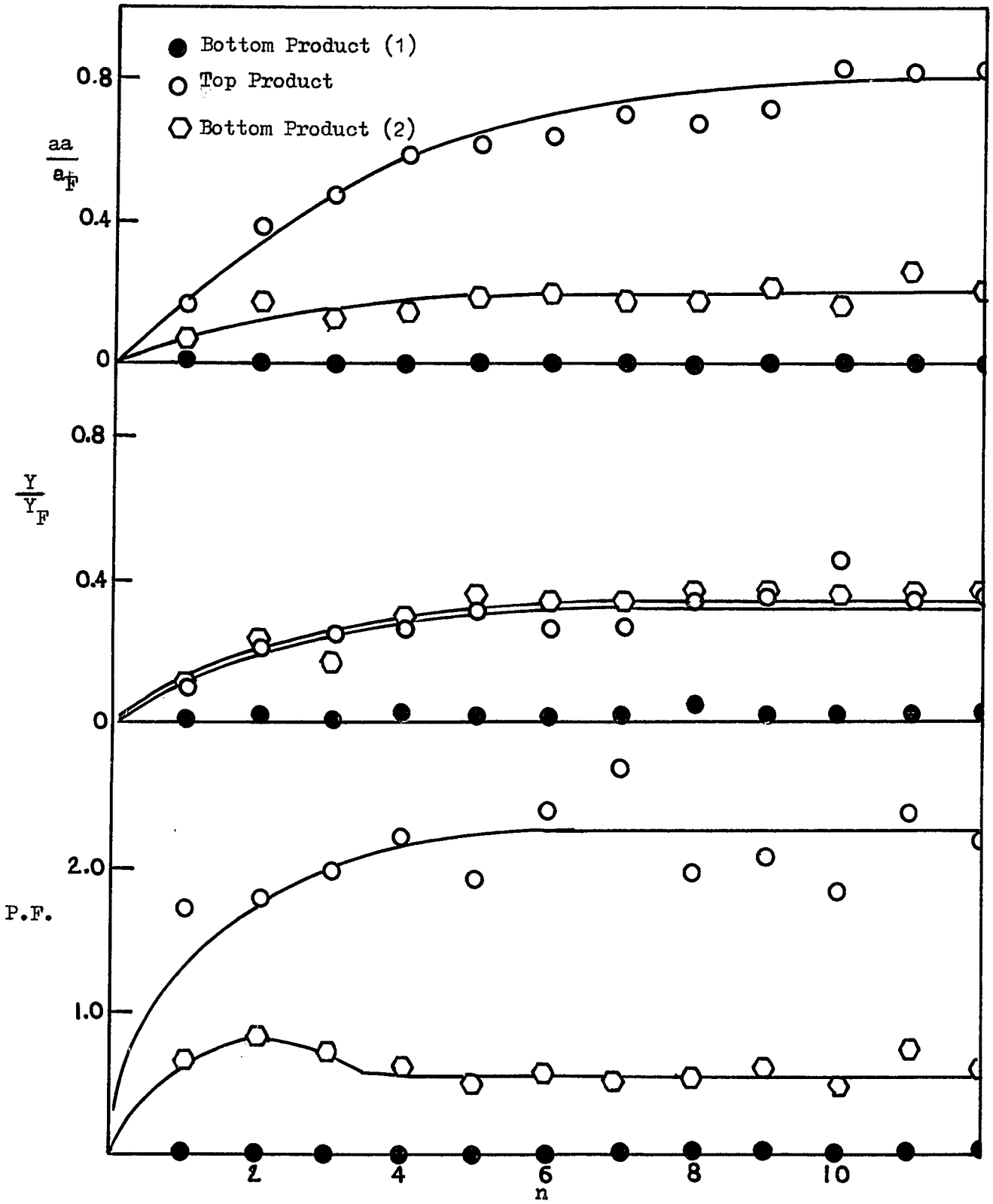


Figure B-2. Ionic Strength Parametric Pump

APPENDIX C

CATION EXCHANGER

The feasibility of using a cation exchanger to purify the alkaline phosphatase in a one column parametric pumping system was explored through a series of adsorption/desorption experiments. The cation exchanger CM-Sepharose was used. In each one of these experiments the feed stream (0.02% alk.ph) was of pH 4.0. For the desorption step two pH, 7.4 and 8.4 were examined. The results of those expeiments are shown on Tables C-1, 2, 3, 4, 5, 6, 7, 8.

Since at pH 4.0 the enzyme mixture carries positive charges, we would expect a strong binding on the exchanger at this pH. Indeed this was the case. Nevertheless, when a higher pH (above the isoelectric point) was used during the desorption step, the enzyme desorption was insignificant.

At pH 7.4, the undesired proteins desorped at a relatively higher ionic strength (0.4M) and it took almost 10 column void volumes to desorb. Unexpectedly the enzyme did not desorb.

When the pH for the desorption buffer was raised to 8.4, the same results were observed. However, the undesired proteins desorped much faster. Increasing the ionic strength of the pH 8.4 buffer to 0.6M caused some desorption (10%) of the enzyme.

An increase of the ionic strength of the feed (pH 4.0) to 0.4M resulted in a relatively higher enzyme desorption (20%) when the pH 8.4 buffer was used to desorb the mixture. A large quantity was also

observed in the desorbed stream. This is due to the relatively weak binding of the enzyme mixture at this higher ionic strength as a result of less interaction between the adsorbate and the adsorbent because of higher competition among the protein molecules.

The above results indicate the poor performance of the cation CM-Sepharose if compared with the anion DEAE. Therefore it is not recommended as an adsorbent when purifying the enzyme alkaline phosphatase in a one-column parametric pumping system.

TABLE C-1

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = Cation (CM-Sepharose)

h = 8.0 cm

Q = 1.0 cc/min

Feed	pH	I.S.	Volume Displaced (cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk. Ph. in Buffer Acetate	4.0	0.1	3	0.0000	0.0000
			6	0.0000	0.0000
			9	0.0000	0.0000
			15	0.0053	0.0084
			21	0.0000	0.0042
			27	0.0000	0.0000
			33	0.0000	0.0084
			39	0.0000	0.0000
Pure Buffer Tris + HCl	7.4	0.02	45	0.0000	0.0000
			51	0.0053	0.0168
			57	0.0000	0.0252
			63	0.0053	0.0378
			69	0.0106	0.0378
			75	0.0053	0.0294
			81	0.0053	0.0252
			87	0.0000	0.0084

TABLE C-2

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = Cation (CM-Sepharose)

h = 8.0 cm

Feed	pH	I.S.	Volume Displaced (cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk. Ph. in Buffer Acetate	4.0	0.1	6	0.0000	0.0000
			12	0.0000	0.0000
			18	0.0000	0.0000
			24	0.0000	0.0000
			30	0.0000	0.0000
			36	0.0000	0.0000
Pure Buffer Tris + HCl	7.4	0.4	42	0.0000	0.0000
			48	0.0000	0.0000
			54	0.0000	0.0000
			60	0.0000	0.0000
			66	0.0000	0.0000
			186	0.0000	0.0038
			198	0.0253	2.9080
			210	0.0000	0.2069
			212	0.0000	0.0000

TABLE C-3

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = Cation (CM - Sepharose)

h = 8.0 cm

Q = 1.0 cc/min

Feed	pH	I.S.	Volume Displaced(cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk. Ph. in Buffer Acetate	4.0	0.2	9	0.0000	0.0000
			15	0.0000	0.0000
			21	0.0000	0.0201
			27	0.0000	0.0000
			33	0.0000	0.0161
			39	0.0000	0.0000
Pure Buffer Tris + HCl	7.4	0.2	45	0.0000	0.0000
			51	0.0000	0.0402
			57	0.0000	0.0361
			63	0.0000	0.0562
			69	0.0000	0.0482
			75	0.0000	0.0442
			81	0.0000	0.0000
			87	0.0000	0.0000

TABLE C-4

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = Cation (CM-Sepharose)

h = 8.0 cm

Q = 1.0 cc/min

Feed	pH	I.S.	Volume Displaced(cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk. Ph. in Buffer Acetate	4.0	0.2	9	0.0000	0.0000
			15	0.0000	0.0000
			21	0.0000	0.0038
			27	0.0000	0.0000
			33	0.0000	0.0077
			39	0.0000	0.0000
Pure Buffer Tris + HCl	7.4	0.4	45	0.0000	0.0000
			51	0.0000	0.0077
			57	0.0000	0.0000
			63	0.0000	0.0000
			69	0.0000	0.0000
			75	0.0000	0.0000
			81	0.0000	0.0000
			89	0.0000	0.0000

TABLE C-5

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = Cation (CM-Sepharose)

h = 8.0 cm

Q = 1.0 cc/min

Feed	pH	I.S.	Volume Displaced (cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk.Ph. in Buffer Acetate	4.0	0.1	9	0.0000	0.0000
			15	0.0000	0.0000
			21	0.0100	0.0157
			33	0.0000	0.0157
Pure Buffer Tris + HCl	8.4	0.4	39	0.0000	0.0196
			45	0.0000	0.0196
			57	0.0050	0.0235
			63	0.0000	0.0196
			69	0.0000	0.0275
			75	0.0250	0.6078
			81	0.1600	3.3647
			84	0.1660	3.4627
			87	0.0450	1.0784
			90	0.0250	0.0471
			96	0.0000	0.0039

TABLE C-6

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = CM-Sepharose)

h = 8.0 cm

Q = 1.0 cc/min

Feed	pH	I.S.	Volume Displaced(cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk.Ph. in Buffer Acetate	4.0	0.1	6	0.0000	0.0000
			12	0.0000	0.0000
			18	0.0000	0.0000
			24	0.0000	0.0000
			30	0.0000	0.0000
			33	0.0000	0.0000
Pure Buffer Tris + HCl	8.4	0.6	39	0.0000	0.0000
			45	0.0000	0.0000
			57	0.0000	0.0175
			63	0.0191	0.3706
			66	0.1720	3.3042
			69	0.1274	2.4091
			72	0.0446	0.8147
			75	0.0255	0.2238
			81	0.0064	0.1084
			87	0.0000	0.0000

TABLE C-7

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = Cation (CM-Sepharose)

h = 8.0 cm

Q = 1.0 cc/min

Feed	pH	I.S.	Volume Displaced (cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk.Ph. in Buffer Acetate	4.0	0.2	9	0.0035	0.0050
			15	0.0000	0.0149
			21	0.0070	0.0000
			27	0.0000	0.0000
			33	0.0035	0.0000
Pure Buffer Tris + HCl	8.4	0.2	39	0.0035	0.0000
			45	0.0000	0.0000
			54	0.0000	0.0000
			60	0.0035	0.0000
			66	0.0070	0.0000
			72	0.0000	0.0000
			78	0.0000	0.0000
			84	0.0035	0.0000
			90	0.0000	0.0000

TABLE AC-8

ADSORPTION-DESORPTION BREAKTHROUGH DATA

Ion Exchanger = Cation (CM-Sepharose)

h = 8.0 cm

Q = 1.0 cc/min

Feed	pH	I.S.	Volume Displaced(cc)	$\frac{a}{a_F}$	$\frac{Y}{Y_F}$
0.02% Alk. Ph. in Buffer Acetate	4.0	0.4	9	0.0000	0.1261
			15	0.0000	0.0721
			21	0.0000	0.0721
			27	0.0000	0.0360
Pure Buffer	8.4	0.4	33	0.0000	0.0000
			39	0.4358	6.4505
			45	0.3462	5.4775
			51	0.0385	0.3784

APPENDIX (D)

Methods of Enzyme Analysis

Enzyme:

The reagent used to determine the concentration of the enzyme by detecting its activity at 405 μm and 30°C was that suggested by Worthington, and it consists of two parts. Their methods of preparation are as follows:

Part A: 8.512 grams of 2-amino-2 methyl-1 propanol (Eastman Kodak, U.S.A.) were stirred with 70 ml of distilled water and the solution was adjusted to pH = 10.0 using concentrated HCL (2-5 M.), then, completed to 90 ml using distilled water. 0.372 grams of NaCL were then dissolved into the new solution and the pH was adjusted to 10.2. The mixture was then completed to 100 ml by adding distilled water and filtered.

Part B: 0.1841 grams of para nitrophenyl phosphate disodium salt (Baker Chemical Co. U.S.A.) were stirred in 18 ml distilled water for 30 minutes. 0.0023 grams of Mg acetate were then dissolved into the solution and the pH was adjusted to 7.0 using dilute HCL. The mixture was completed to 20 ml using distilled water.

The reagent was prepared by mixing A and B in the ratio 13 to 3, respectively. For analysis, 0.1 ml of the sample was added to 3.0 ml of the reagent. The mixture was allowed to react for two to three minutes at 30°C; then a reading was taken on the spectrophotometer at

405 µn. The activity is calculated as international units/litre.

$$U/l = \frac{(\Delta A/min)(1000)(3.1)(1000)T}{18,750\ (LP)\ (ml\ of\ sample\ used)}$$

where;

$\Delta A/min$ = Change in absorbence/min

LP = 1.0 cm

3.1 = total reaction volume in ml.

T = 30^0C

$$U/ml = \frac{(\Delta A/min)(1000)(3.1)(1000)T}{(1000)(18,750(1)(0.1)}$$

$$= (\Delta A/min)(1.6533)$$

Total Protein:

The dye reagent used for determing the total protein (Enzyme + Undesired protein) in the mixture was that recommended by Bio-Rad Laboratories (U.S.A.). One volume of the dye was diluted with four volumes of distilled water, and the diluted volume was filtered on Whatman No. 1 paper. 0.2 ml of the product was added to 4.0 ml of the dye reagent, and the mixture was vortexed and allowed to react for five to ten minutes. A reading at 595 µn was then taken at 30^0C versus reagent blank and compared with that of the feed.

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