Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Effect of Physicochemical Parameters on the Separation of Proteins from Human Placental Extract by Using a Continuous Foam Fractionating Column

P. Bhattacharya[a]; S. K. Ghosal[a]; K. Sen[a]
[a] Process Engineering Laboratory Chemical Engineering Department, Jadavpur University, Calcutta, India

To cite this Article Bhattacharya, P. , Ghosal, S. K. and Sen, K.(1991) 'Effect of Physicochemical Parameters on the Separation of Proteins from Human Placental Extract by Using a Continuous Foam Fractionating Column', Separation Science and Technology, 26: 10, 1279 — 1293
To link to this Article: DOI: 10.1080/01496399108050532
URL: http://dx.doi.org/10.1080/01496399108050532

SEPARATION SCIENCE AND TECHNOLOGY, 26(10 & 11), pp. 1279–1293, 1991

Effect of Physicochemical Parameters on the Separation of Proteins from Human Placental Extract by Using a Continuous Foam Fractionating Column

P. BHATTACHARYA,* S. K. GHOSAL, and K. SEN
PROCESS ENGINEERING LABORATORY
CHEMICAL ENGINEERING DEPARTMENT
JADAVPUR UNIVERSITY
CALCUTTA 700032, INDIA

Abstract

The recovery of valuable proteins (like proteolytic enzymes) from nonconventional biological sources by using modern separation techniques is becoming increasingly important because of their high commercial potential. In the present investigation, isolation of proteins from human placental extract by using the continuous foam fractionation technique is studied. The effect of different physicochemical parameters on the separation of proteins and purification of proteolytic enzymes are reported. A method for finding a quasi-equilibrium relationship between the solute concentration in the foamate and in the effluent by using the same system is described. The quasi-equilibrium curve is also presented.

INTRODUCTION

The use of proteins and proteinous substances like enzymes is becoming increasingly important in industry, particularly since the advent of immobilization techniques. However, the commercial potential of these substances is not being fully utilized because of economic constraints. One of the main reasons for this is that conventional chemical engineering separation techniques cannot be used here because of the low initial solute concentration which renders the system an unfavorable cost–benefit ratio. Thus, one has to depend on other separation techniques to isolate and purify these compounds. At present the common techniques are salt pre-

*To whom correspondence should be addressed.

cipitation method, chromatographic procedure, gel filtration, etc. These procedures, however, are very slow in operation and cannot be carried out on an industrial scale. This necessitates utilization of a separation technique which is not only relatively fast in operation but can at least be operated on a semicommercial scale.

The foam fractionation technique for the separation of nonenzyme proteins and enzymes from a solution has been used successfully by Ahmad (*1*), Lalchev and Exerowa (*2*), Lalchev et al. (*3*), and Sarkar et al. (*4*). However, except in a few cases, the batch fractionation technique has been utilized, although it has been stated by Ahmad (*1*) that continuous foam fractionation is preferable for separating such substances.

Although it is well known that human placenta contains valuable proteins, only a few attempts have been made to isolate them by using conventional separation techniques. One of the possible reasons for the failure of isolating and purifying such valuable biochemicals is the denaturation of proteins due to changes in the microenvironment. This causes a large loss of enzyme activity. On the other hand, the batch foam fractionation technique has already been successfully applied by Sarkar et al. (*4*) to isolate and purify sensitive proteins like enzymes.

In the present investigation a continuous foam fractionating column is used to isolate valuable proteins like proteolytic enzymes from human placenta. Effects of different physicochemical parameters such as buffer pH, gas flow-rate, liquid flow-rate, foam bed height, solute concentration in the feed, etc. on separation efficiency and enzyme purification have been studied. Since the main intention of the present investigation is to evolve a design procedure for the fractionating column, it is believed that knowledge of the equilibrium relationship between the solute concentration in the foamate and in the effluent will be of great importance.

A literature review shows that a number of theoretical equations have been proposed by Lemlich (*5*), Goldberg and Rubin (*6*), and Haas and Johnson (*7*) to find an equilibrium correlation; however, no experimental data have been presented so far. In addition, one of the major difficulties in finding the equilibrium relationship in a multicomponent system is to identify the key solute based on which calculation is to be done. It is evident that no true equilibrium relationship can be drawn with such a system. It is possible to obtain only a quasi-equilibrium relationship. Attempts have been made in the present investigation to evolve experimentally a quasi-equilibrium relationship between the solute concentration in the foamate and that in the effluent. It is hoped that such an investigation will help in understanding the system behavior, which can then be utilized for design purposes.

EXPERIMENTAL

Preparation of the Feed Solution

Human placenta was collected from Chittaranjan National Medical College (Calcutta, India). The tissue was kept on ice and was separated from the membrane. It was then cut into small pieces and washed with ice-cold distilled water to remove blood. The tissue was then dried by absorbent paper and homogenized with a suitable amount of Tris-HCl buffer (pH 8.0) until the tissue went into homogeneous dispersion and only fats and cell debris were left behind. The homogenized solution was centrifuged in a cold centrifuge at 10,000 rpm for 30 min, and the supernatant solution (5% placental extract) was stored in a conical flask at 0°C until further use. This stock solution of placental extract was diluted to the desired concentration by adding buffer at the time of the experiment.

Apparatus

A detailed diagram of the continuous foam fractionating column used in the present investigation is shown in Fig. 1. The graduated column, 50 cm high, with an inside diameter of 4 cm, was made of Corning glass. It was fitted with a sparger of 100 μm porosity, a feed inlet, an effluent outlet, and a foam outlet nozzle. In addition, the column was fitted with six intermediate foam outlet nozzles at 5 cm intervals. The foam height was varied with the help of these intermediate nozzles at the time of the experiment.

The flow rate of saturated nitrogen gas, used as a foaming agent, was measured by a rotameter, while the liquid flow-rates were measured by volume collection. Two peristaltic pumps (Gilson) were used to change the liquid flow-rates. The foam outlet nozzles were kept inclined at an angle of 30°. It was observed that foam collapsed while passing through this bend. Hence, no separate arrangement for foam breaking was required.

Protein Concentration/Enzyme Activity Measurement

The surface tension vs concentration plot was established by measuring the surface tension of the placental homogenate by using the Wilhelmy method (*8*). The threshold and CMC limits of protein concentration in placental extract were found to be 0.00435 and 0.425 mg/mL, respectively. The total protease activity was determined by the casein digestion method as suggested by Leonard and coworkers (*9*). The protein measurement was done by the colorimetric method of Lowry (*10*).

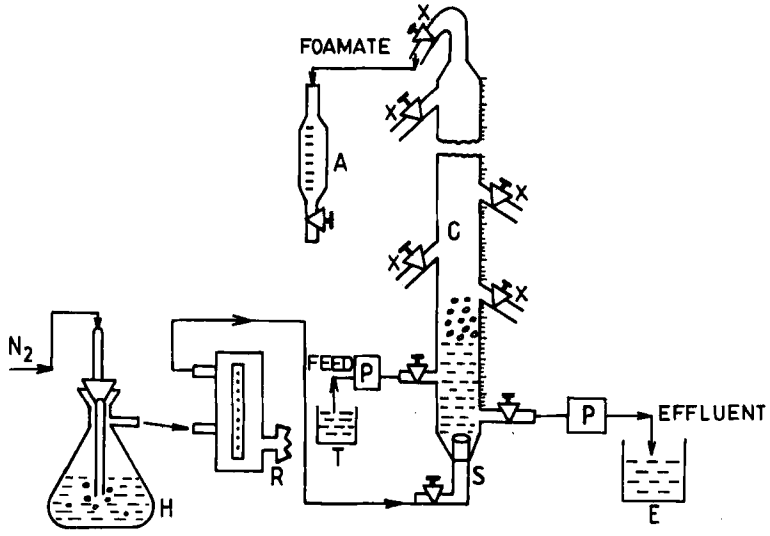

FIG. 1. Schematic diagram of the continuous foam fractionating column.

During the experiment a constant liquid height was maintained inside the column by proper adjustment of the effluent flow-rate. Samples of foamate and effluent were collected for analysis after achieving steady-state during each run. The temperature was generally maintained at 10°C at the time of each run.

Equilibrium Data Collection

The fractionator was used in the batch mode for evaluating the equilibrium data. The placental homogenate stock solution was diluted with buffer up to a certain protein concentration. A definite quantity of this solution was used as the feed. Saturated nitrogen gas was allowed to pass at a certain moderate rate until no more foam rose in the column. Samples of foamate and effluent were collected for analysis.

By keeping the quantity and concentration of the feed the same as in the first run, the subsequent experiments were carried out by gradually decreasing the nitrogen flow-rate until a further decrease ensured no rise of foam bubbles in the column. The above method was repeated for different feed concentrations.

Bubble Diameter Determination

Bubble size was measured for each operating condition by photographing (Pentax) a section of the graduated column above the liquid height and then by reading the enlarged photographic prints. The diameters of several bubbles were measured on each print, and the mean of a normal distribution has been reported following the method given by Lemlich (*5*).

Data Processing

Experimental data have been processed using standard linear and nonlinear regression methods in order to get a linear plot or a smooth curve wherever necessary.

RESULTS AND DISCUSSION

Effect of pH

In any enzyme system it is essential to establish the optimum pH at which other physicochemical parameters should be studied. This is important because it is well known that the partition of protein is altered by changing the pH of the solution. It is expected that at the isoelectric condition, the surface adsorption of the desired protein will be enhanced as a result of both decreased repulsive force and solubility at this isoelectric pH.

The effect of pH on enzyme purification and protein enrichment for the present system in batch operation has been reported by Sarkar et al. (*4*). In order to compare this value with that obtained in continuous operation, a plot of enrichment of protein as well as the purification of enzyme (Fig. 2) for a pH range of 7.0–9.0 (covering isoelectric pH) has been made. It is evident from the figure that at pH 8.0, while enzyme purification is a maximum, protein enrichment shows a minimum value. The reason for enhanced purification at pH 8.0 is mainly due to less protein concentration in the foamate, as explained by Sarkar et al. (*4*). It is also apparent that by changing the contacting pattern of mass transfer, no shift in the optimum pH is observed, although the values of both fold purification and protein enrichment increase significantly.

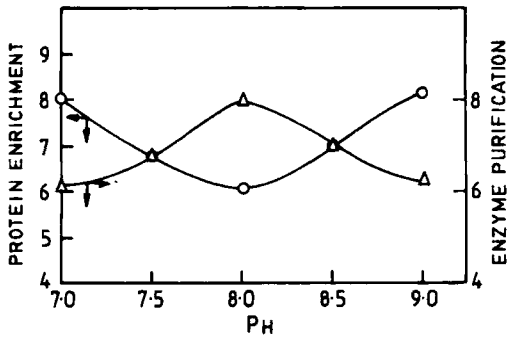

FIG. 2. Effect of pH on protein enrichment and enzyme purification.

Protein Enrichment/Separation Efficiency

Effect of Nitrogen Flow-Rate

It is expected that the flow rate of the carrier gas will influence both the enrichment of the desired solute and the degree of stripping. Figure 3 shows the effect of nitrogen flow-rate on the enrichment ratio for different feed flow-rates, and the effect of nitrogen flow-rate on the degree of stripping. It is evident from the figure that at a particular feed flow-rate, the enrichment ratio decreases with an increase in the nitrogen flow-rate, but at the same time the degree of stripping increases. By realizing that the rate of bubble surface production and the rate of liquid entrainment in the foam both increase with an increase in the gas flow-rate, this behavior is to be expected. From the mechanistic point of view, the extent of agitation will be more at a higher gas flow-rate, and this will lead to increased foamate formation. Thus, the solute transferred in the foamate will find more fluid volume. As a result, its concentration will drop. Again, more foam bubbles will collapse at a higher gas flow-rate, leading to increased liquid entrainment and simultaneously causing an increase in the degree of stripping.

Effect of Gas/Liquid

Figure 4 shows the effect of the gas flow-rate to liquid flow-rate ratio on separation efficiency at a given feed concentration. It is apparent from the figure that the efficiency increases as the ratio increases up to a certain critical value. Once this value is reached, no further increase in efficiency is observed, even after increasing the flow-rate ratio. It is well known that an increase in gas flow-rate at a constant liquid flow-rate will increase foaming, resulting in more solute transfer from the liquid phase to the foam phase and increasing the separation efficiency. However, when the

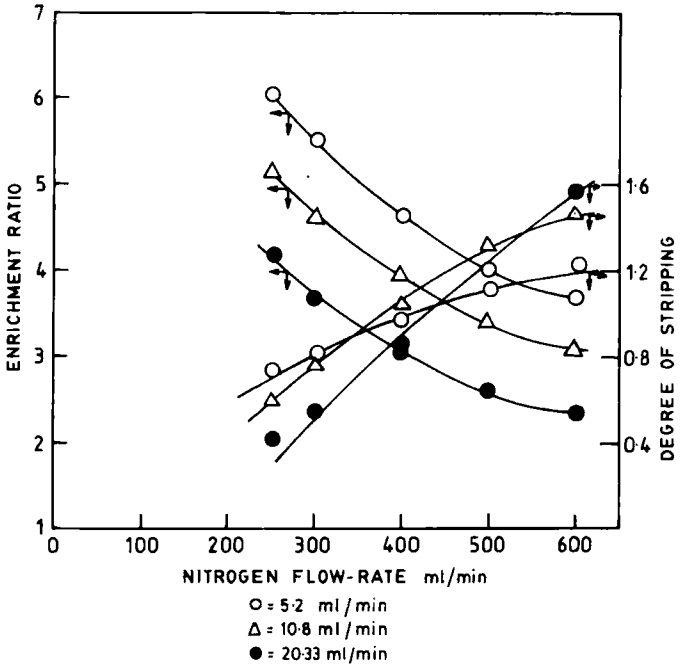

FIG. 3. Effect of nitrogen flow-rate on protein enrichment ratio and on degree of stripping. Feed concentration = 0.204 mg/mL. Foam bed height = 15 cm. pH 8.0.

critical value is reached, the tendency of foam breaking inside the column will increase. The column will shift from its stable operation to instability, very much like the flooding operation encountered in two-phase mass-transfer operations.

Effect of Feed Concentration

In order to test the effect of feed concentration on the enrichment ratio, experiments were conducted to identify its effects at a constant liquid and gas flow-rate. The results are shown in Fig. 5. It is observed that the enrichment ratio decreases with an increase in feed concentration. This behavior can be explained by recognizing that a bubble surface can adsorb only a specific quantity of solute at specified operating conditions, and the enrichment ratio has to fall above that. Mechanistically, the gas flow-rate is not sufficient to cause transfer of solute in the foam phase compared to that in the lower feed concentration. This was further evidenced when the column effluent analysis showed an increase in protein concentration at higher feed concentrations.

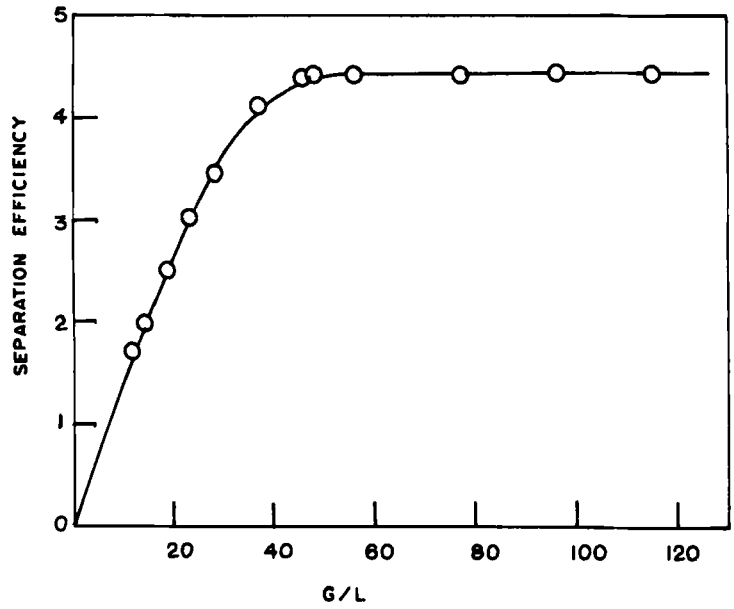

FIG. 4. Effect of gas–liquid (G/L) ratio on protein separation efficiency. Feed concentration = 0.204 mg/mL. Foam bed height = 15 cm. pH 8.0.

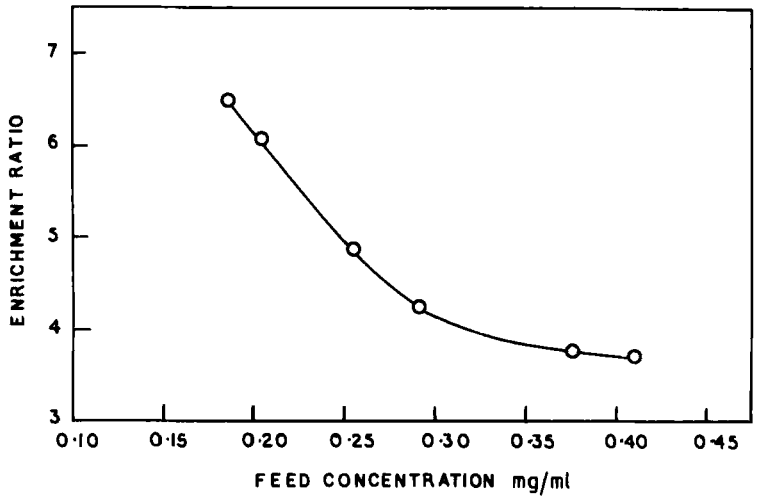

FIG. 5. Effect of feed concentration on protein enrichment ratio. G = 250 mL/min. L = 5.2 mL/min. pH 8.0. Foam bed height = 15 cm.

Effect of Bubble Diameter

In the present investigation, mainly the effects of primary variables on enrichment ratio/separation efficiency have been studied. It is believed that the bubble diameter is a secondary variable and should not be used as a basis for estimating separation efficiency. On the other hand, the importance of bubble size on the design aspects and hydrodynamic behavior should be realized. Since a change of gas velocity at a fixed feed flow-rate has a direct bearing on bubble size, the effect of bubble diameter on the enrichment ratio will be of the same order as that of a change in gas flow-rate. On the other hand, the effect of bubble diameter on the enrichment ratio at fixed gas and liquid flow-rates will be the reverse of the effect of a change in feed concentration.

Effect of Foam Bed Height

In order to find out how separation efficiency changes along the length of the column, a plot (Fig. 6) has been made showing the separation efficiency as a function of foam bed height. It is evident that the efficiency increases at first and then decreases with an increase in foam bed height. It may therefore be concluded that for a fixed gas–liquid flow-rate ratio, there will be a definite foam height up to which foam is stable and separation of solute increases gradually. A further increase in foam height will cause a spontaneous rupture of foam bubbles, giving a sudden drop in the separation efficiency. Physical observation through a transparent glass column confirms this conclusion.

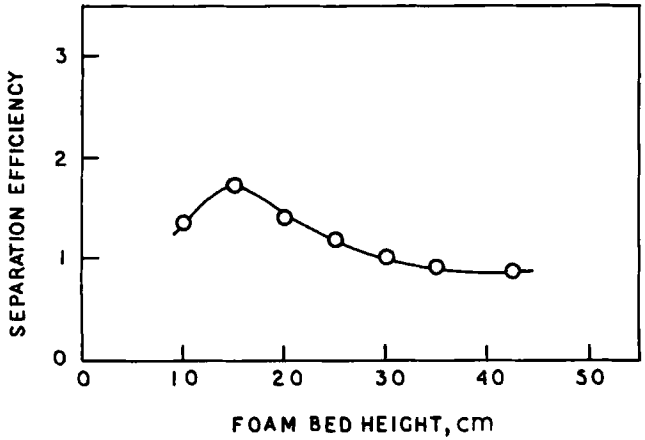

FIG. 6. Effect of foam bed height on protein separation efficiency. G = 250 mL/min. L = 20.83 mL/min. Feed concentration = 0.204 mg/mL. pH 8.0.

Enzyme Purification

Human placental extract has been identified by many workers as a potential source of proteolytic enzymes. However, little attempt has been made so far to isolate and purify proteolytic enzymes from hospital waste. One of the main objectives of this investigation was to see how enzyme purification was affected by changing the operating parameters of a continuous foam fractionator. Fold purification has been determined by measuring the proteolytic activity of the enzyme separated in the foamate. On the other hand, fold purification has been defined as the ratio of specific activity of protease in the foamate to that in the original feed, where "specific activity" means the increase in optical density per milligram of protein. Results on fold purification of enzyme from placental extract by using batch foam fractionation has been reported by Sarkar et al. (*4*). The present work compares these values with the data obtained in a continuous foam fractionator.

Effect of Nitrogen Flow-Rate

Figure 7 shows the effect of nitrogen flow-rate on fold purification of protease. It is observed that with an increase in nitrogen flow-rate, fold purification decreases. This is possibly due to the fact that with an increase in gas flow-rate, entrainment increases, which in turn decreases the specific

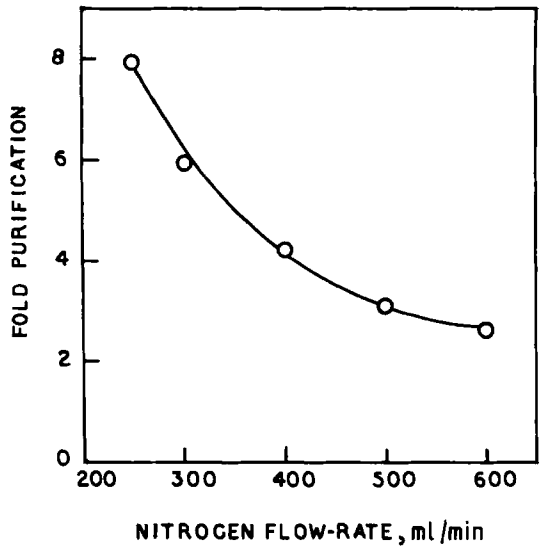

FIG. 7. Effect of nitrogen flow-rate on fold purification of enzymes. L = 5.2 mL/min. Feed concentration = 0.204 mg/mL. Foam bed height = 15 cm. pH 8.0.

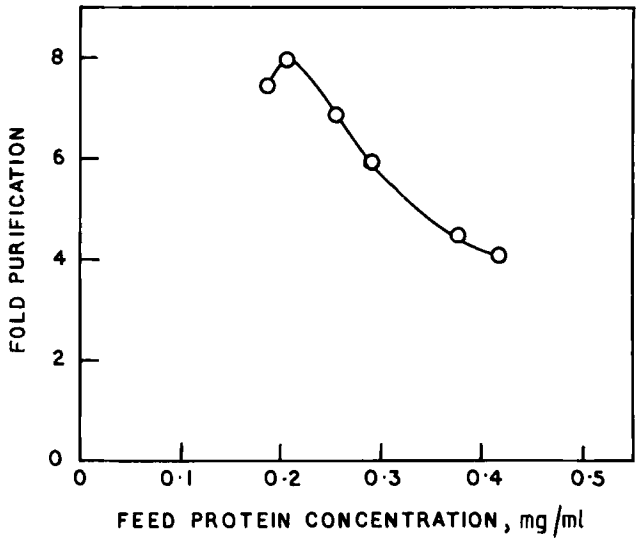

FIG. 8. Effect of feed protein concentration on fold purification of enzymes. G/L = 12. pH 8.0. Foam bed height = 15 cm.

activity of protease. A similar observation was made in the case of a batch fractionation column by Sarkar et al. (*4*).

It is interesting to compare the results obtained for the protein enrichment ratio with fold purification of enzymes. A similar behavior is observed in both cases. It is therefore advisable to conduct experiments at lower gas flow-rate in order to obtain either a higher fold purification of enzymes or a higher protein enrichment ratio.

Effect of Feed Concentration

In order to study the effect of feed protein concentration on enzyme purification, a plot (Fig. 8) of fold purification against feed protein concentration was made. It was observed that with an increase in protein concentration, enzyme purification increases up to a certain point. After this critical value, purification drops rapidly. This is possibly because excess protein in the feed solution makes the foam too stable to allow for effective drainage. At the same time, extraneous proteins rather than protease concentrate more in the foamate, giving rise to a poor purification factor.

Effect of Foam Bed Height

The effect of foam bed height on fold purification of protease is shown in Fig. 9. It is observed that with an increase in foam height, fold purifi-

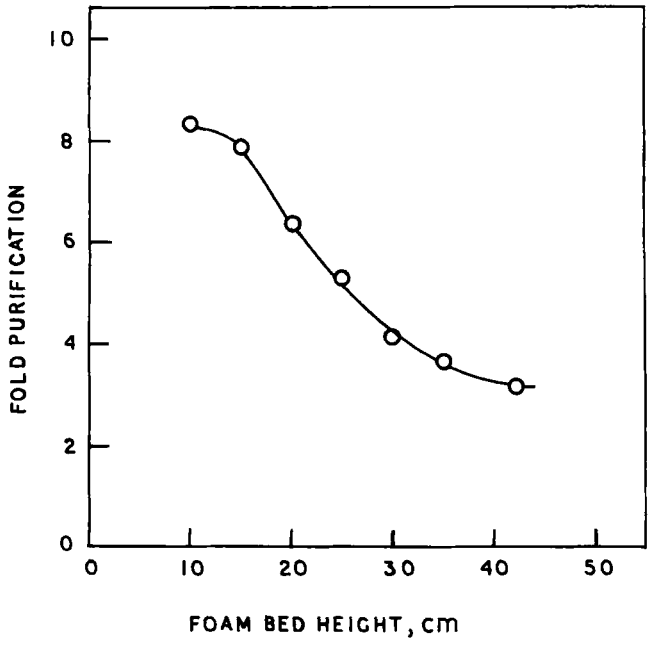

FIG. 9. Effect of foam bed height on fold purification of enzymes. G/L = 12. pH 8.0. Feed concentration = 0.204 mg/mL.

cation decreases. This observation can possibly be explained by the fact that as foam height increases, enzymes remain in a stretched condition for a longer time, resulting in denaturation. Hence, the activity decreases.

Equilibrium Curve

As mentioned previously, it is not possible to construct a true equilibrium diagram for the present system because the solute protein is not an unique component here. From our knowledge of human placental extract, it can be said that besides other nonprotein substances, the main constituents are a mixture of nonenzymic proteins and enzymes. Fortunately, nonprotein substances are not surface active, and thus they will not be separated during foam fractionation. However, all protein components, being surface active, will compete for adsorption on the foam bubble surface. This creates a large problem in constructing a true equilibrium curve. On the other hand, remembering the great importance of such an equilibrium curve in designing a column, an attempt has been made in the present investigation to find a quasi-equilibrium curve showing the equilibrium relationship between the protein concentration in the foamate and that in the effluent

liquid. The total protein analyzed during the experiment was considered to be the solute. It is believed that such a quasi-equilibrium curve will be of great use in our design approach in the absence of available data for the present system. While true equilibrium is a function of intensive thermodynamic properties only, the need for such a quasi-equilibrium curve

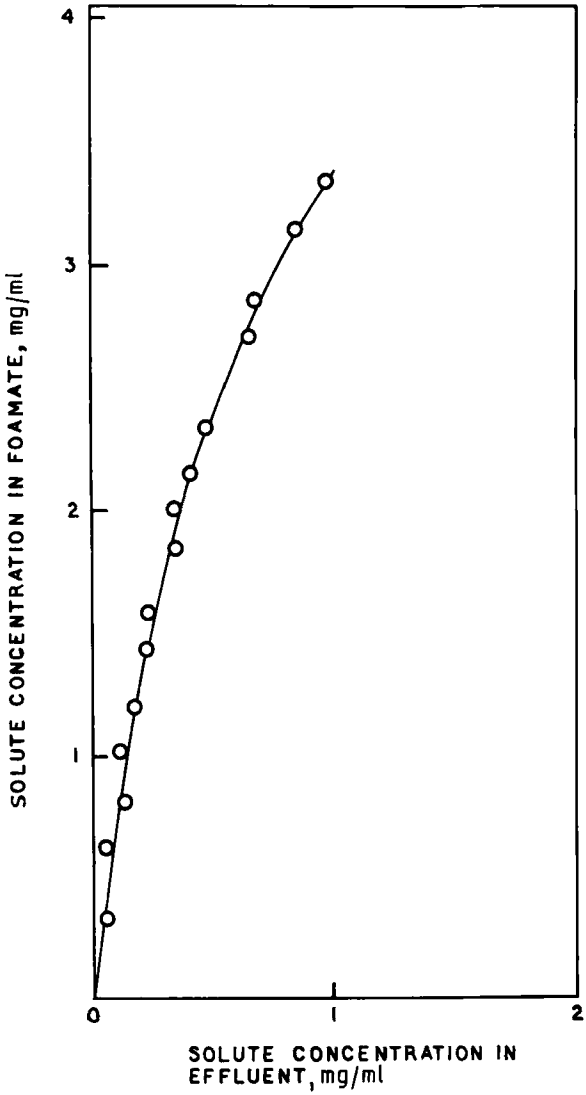

FIG. 10. Equilibrium curve for placental homogenate at 10°C.

is apparent because kinetics, flow behavior, hydrodynamics, and other rate effects are expected to alter the equilibrium condition, as in the present case.

The quasi-equilibrium relationship between the protein or solute concentration in the foamate and in the effluent is shown in Fig. 10. The procedure for obtaining the relevant data has already been described. The temperature was kept constant at 10°C during the experimental runs. The idea behind this procedure is that the solute concentration at the lowest gas flow-rate evolves the equilibrium relationship. Under ideal condition, this will lead to the highest residence time of a single foam bubble in the column, thereby giving the necessary time to reach equilibrium. However, in the present system some bubbles collapsed and some ascending foam was found entrained in the liquid. These unfavorable behaviors could not be arrested during the experimental runs. If it is permitted to speak of the residence time of foam bubbles in the present system, it is concluded that the actual residence time of a single bubble (if it did not collapse) is less than in the ideal case.

APPENDIX

Definitions for some of the terms used in the text are given below:

$$\text{Enrichment ratio} = \frac{\text{concentration of protein in the foamate}}{\text{concentration of protein in the effluent}}$$

$$= X_F/X_B$$

$$\text{Degree of stripping} = F/B$$

Separation efficiency

$$= \frac{(\text{concentration of protein in the foamate}) \times (\text{flow rate of foamate})}{(\text{concentration of protein in the effluent}) \times (\text{flow rate of effluent})}$$

$$= X_F F/X_B B$$

Foam bed height = distance from the upper level of the constant liquid height maintained in the column to the point of foam exit

Residence time = time required by a foam bubble to rise through the foam bed height

NOMENCLATURE

L flow rate of the feed solution (mL/min)
B flow rate of the effluent (mL/min)
F flow rate of the foamate (mL/min)
G gas flow-rate (mL/min)
X_t concentration of protein in the overflowing foam on a gas-free basis (mg/mL)
X_B concentration of protein in the effluent (mg/mL)

Acknowledgment

One of the authors (K.S.) gratefully acknowledges the financial support given by Council of Scientific and Industrial Research, New Delhi, India.

REFERENCES

1. S. I. Ahmad, *Sep. Sci.*, *10*(6), 673 (1975).
2. Z. Lalchev and D. Exerowa, *Biotechnol. Bioeng.*, *23*, 669 (1981).
3. Z. Lalchev, L. Dimitrova, P. Tzvetkova, and D. Exerowa, *Ibid.*, *24*, 2253 (1982).
4. P. Sarkar, P. Bhattacharya, R. N. Mukherjea, and M. Mukherjea, *Ibid.*, *29*, 934 (1987).
5. R. Lemlich, *Ind. Eng. Chem.*, *60*(10) (1968).
6. M. Goldberg and E. Rubin, *Sep. Sci.*, 7(1), 51 (1972).
7. P. A. Haas and H. F. Johnson, *AIChE J.*, *11*, 319 (1965).
8. H. B. Bull, *Physical Biochemistry*, Wiley, New York, 1945.
9. L. Keay and B. S. Wildi, *Biotechnol. Bioeng.*, *12*, 179 (1970).
10. P. H. Lowry, N. J. Rusebrough, A. L. Fall, and R. J. Randan, *J. Biol. Chem.*, *193*, 265 (1951).

Received by editor September 10, 1990