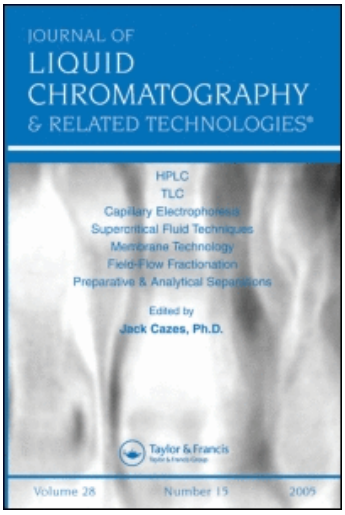

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Foam Countercurrent Chromatography Based on Dual Counter-Current System

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FOAM COUNTERCURRENT CHROMATOGRAPHY BASED ON DUAL COUNTERCURRENT SYSTEM

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ABSTRACT

A novel method of performing foam separation is described. The method utilizes a gas-liquid dual countercurrent flow through a helical column subjected to a particular type of synchronous planetary motion. Samples introduced at the middle portion of the column, in either batch or continuous mode, are separated according to the foam affinity. Any material having an affinity to the foam is quickly carried with the foaming stream and eluted through one end of the column whereas other materials are carried with the liquid stream in the opposite direction and eluted out through the other end of the column. Capability of this foam countercurrent chromatographic method is demonstrated on separations of rhodamine B and Evans blue with an anionic surfactant, SDS, as a collector of rhodamine B. Successful preliminary separation of protein samples, BSA and sheep hemoglobin, indicates that the present method may be effectively applied to separation and purification of various biological samples such as enzymes, membrane receptors, etc.

INTRODUCTION

A variety of countercurrent chromatographic methods (1) developed in the past utilizes a stationary phase that is permanently retained in the

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separation column through which the mobile phase is continuously eluted. This ordinary elution mode, common with many other chromatographic methods, is applied to a batch separation to yield a chromatographic separation of solutes locally charged as a discrete sample volume. Versatility of the method, however, can be further increased, if the system permits continuous separation by continuous sample feeding. A recently developed countercurrent chromatographic method called high-speed countercurrent chromatography (CCC) (2) utilizes a particular combination of the coil orientation and the planetary motion to produce a unique hydrodynamic effect which permits a true countercurrent flow of the two solvent phases through the coiled column. With a proper column design the two phases can be simultaneously eluted through the column in the opposite direction while the sample solution is continuously fed at the middle portion of the column. This dual countercurrent system provides a rich domain of applications such as continuous extraction, enrichment and stripping as well as continuous separation of solutes and particulates.

The present paper describes a preliminary application of this dual countercurrent system to the foam separation method which utilizes foams as one of the phases to collect solutes and/or particulates according to their foam affinity. Although the foam separation method has been widely used during the past fifty years (3), the method has remained rather primitive and inefficient, thus largely limiting its application in the research laboratories. The preliminary results of the foam CCC suggest that this new method would drastically improve both separation times and efficiencies of the conventional methods to become an essential tool for scientific research in the near future.

PRINCIPLE

Dual Countercurrent System

As briefly mentioned earlier, the dual countercurrent system provides the basis for foam CCC. The principle of the dual countercurrent system is illustrated in Figs. 1 and 2. In Fig. 1, a cylindrical coil holder is equipped with a planetary gear which is coupled with an identical stationary sun gear (shaded) placed around the central axis of the centrifuge. This gear arrangement produces a particular type of synchronous planetary motion of the holder: the holder rotates about its own axis and simultaneously revolves around the central axis of the centrifuge at the same angular velocity as indicated by a pair of arrows. This synchronous rotation of the holder unwinds the twist of the flow tubes caused by revolution, thus eliminating the need for the rotary seals. Consequently, the system permits the use of multiple flow channels for performing continuous elution through the rotating column without complications such as leakage and contamination often caused by the rotary seals. This unique capability of the flow-through centrifuge system enables the successful operation of the dual countercurrent elution which necessitates the use of five flow-through channels some under considerably high pressure of 80 psi.

When the tubing is coaxially wound around the holder, the above synchronous planetary motion of the holder distributes the two solvent phases in the coiled tube in such a way that one phase entirely occupies the head side and the other phase, the tail side of the coil. Here, the head-tail relationship of the coil is determined by the Archimedean screw force acting on the coil contents: all objects tend to move from the tail toward the head of the coil. The above unilateral phase distribution renders a unique application to the dual countercurrent system.

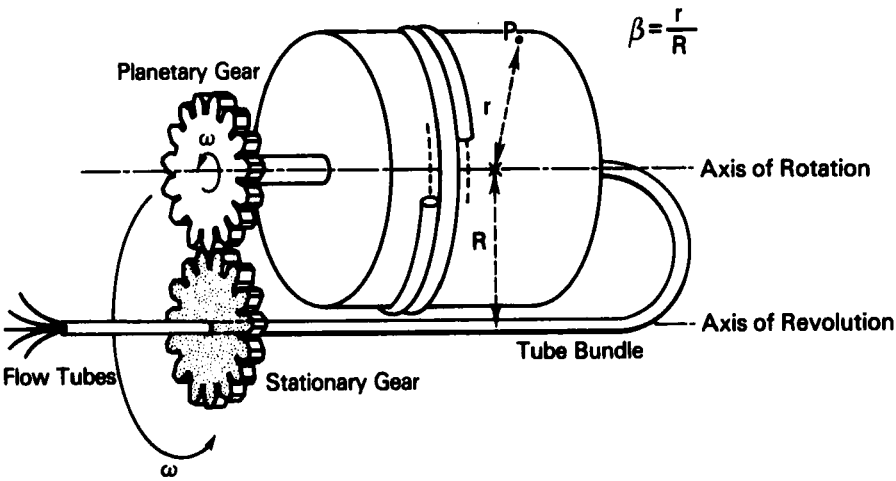

Fig. 1. Synchronous planetary motion of the holder. Parameter β determines the pattern of the centrifugal force field produced by the planetary motion (1, 4).

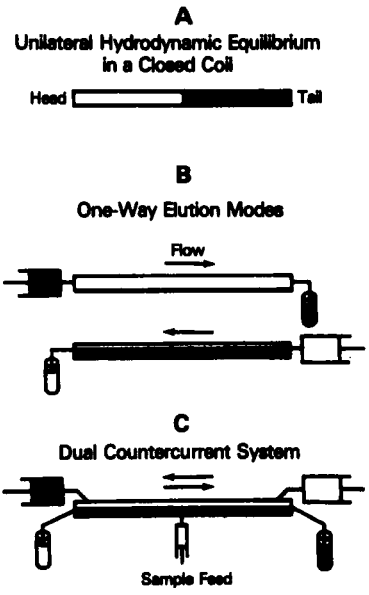

Fig. 2. Mechanism of dual countercurrent system.

The mechanism of the dual countercurrent system is illustrated in Fig. 2 where all coiled tubes are schematically drawn uncoiled to indicate the relative volume distribution of the two solvent phases along the length of the tube. In A the unilateral hydrodynamic equilibrium distributes the white phase in the head half and the black phase in the tail half. This hydrodynamic equilibrium condition clearly indicates that the white phase, if introduced into the black phase, would move toward the head and the black phase, if introduced into the white phase, would move toward the tail. Consequently, the system can be efficiently applied for performing CCC in two different ways. The coil is first entirely filled with the white phase and the black phase is pumped through the head of the coil (B, top). Alternatively, the coil is first entirely filled with the black phase and the white phase is pumped through the tail of the coil (B, bottom). The system further permits simultaneous introduction of the two phases through the respective end of the coil to produce the true countercurrent flow. This requires an additional flow channel at each end of the coil to collect the effluent and also a sample feed line at the middle portion of the coil (C). This dual countercurrent system can be applied to the foam separation as described below.

Foam CCC

Foam CCC uses a particular form of the dual countercurrent system in which one of the solvent phases is replaced with a gas phase to produce foams to collect the foam active materials. In this foam CCC, the foaming stream moves from the head toward to tail, opposing to the liquid stream moving from the tail toward the head of the coil.

Fig. 2 illustrates a schematic column design for foam CCC. The coiled column is equipped with five flow channels. The liquid phase is

introduced through the liquid feed line located near the tail and drained through the liquid collection line at the head of the coil. The gas phase is similarly introduced through the gas feed line located near the head and the generated foams are harvested through the foam collection line at the tail of the coil. The sample solution may be introduced through the sample feed line opened at the middle portion of the coil.

In a typical operation mode the liquid phase containing surfactant and N_2 gas are simultaneously introduced through the respective lines into the rotating column while the sample solution is continuously fed through the sample feed line. Consequently, the samples are separated according to their foam affinity. Solutes or particulates having an affinity to the foam are quickly carried with the foaming stream toward the tail and harvested through the foam collection line while other materials in the sample solution are carried with the liquid stream in the opposite direction toward the head and eluted out through the liquid collection line.

This foam CCC method can be applied to a broad spectrum of samples having a foam affinity which may be classified into the following two categories: 1) Direct affinity to the gas-liquid interface. Detergents and many other foam-producing materials can be harvested through the foam collection line without any special treatment. 2) Affinity to the foam-producing carrier or collector molecule. Samples which lack a direct affinity to the gas-liquid interface can be indirectly adsorbed to the foam if they have an affinity to the foam-producing agents lining the gas-liquid interface in the foams. This type of affinity effectively used for foam separation may vary in a wide range from a nonspecific form such as surface electric charges to a highly specific form such as enzyme-substrate or -inhibitor binding provided that one of the pair has either direct or indirect foam affinity.

APPARATUS

The preliminary experiments for foam CCC have been performed with a table top model of the combined horizontal flow-through coil planet centrifuge at a 20 cm revolutional radius (4). The apparatus carries a pair of column holders each subjected to a different type of synchronous planetary motion. Among these the gear-driven holder, which produces the suitable planetary motion illustrated in Fig. 1, was exclusively employed for the present study.

Fig. 3 shows a cross-sectional view through the central axis of the apparatus. The motor drives the rotary frame around the central stationary pipe (shaded). The rotary frame, consisting of a pair of aluminum plates rigidly bridged with links, holds a column holder and the associated countershaft on each side in the symmetrical locations. The central stationary pipe is equipped with a stationary gear and a stationary toothed pulley for introduction of the particular planetary motion to each holder.

On the gear-driven side of the holder (upper), the stationary gear is coupled to an identical gear on the countershaft to produce rotation of the countershaft on the rotary frame. This motion is further conveyed to the holder with a pair of toothed pulleys and a toothed belt. This arrangement produces a desired planetary motion of the holder, i.e., rotation about its own axis and revolution around the central axis of the centrifuge at the same angular velocity. On the pulley-driven side of the holder (lower), the central stationary pulley is coupled with a toothed belt to an identical pulley on the countershaft to produce counterrotation of the countershaft on the rotary frame. This motion is similarly conveyed to the holder with a pair of toothed pulleys and a

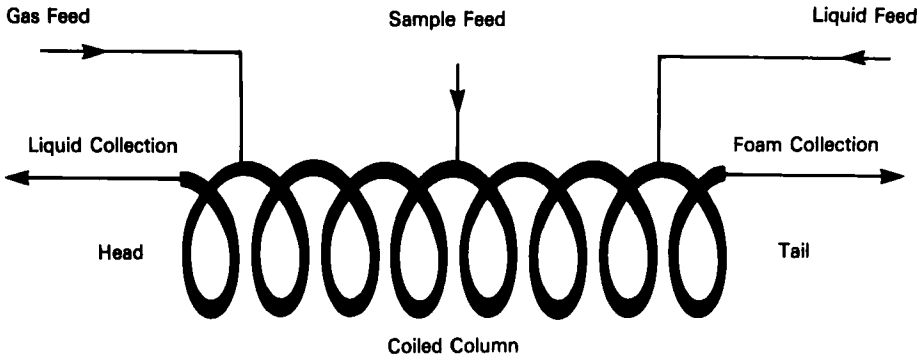

Fig. 3. Schematic column design for foam CCC.

toothed belt. Consequently, the pulley-driven holder counterrotates about its own axis as it revolves around the central axis of the centrifuge. This type of planetary motion, though applicable to the foam separation method (5), tends to produce high column pressure and constant discharge of the gas phase through the liquid collection line. In the present study this holder was used to mount a counterweight for balancing the centrifuge. Each holder is made removable from the rotary frame by loosening a pair of screws on each bearing block. The position of the holder on the rotary frame is adjustable to 15 cm or 20 cm from the central axis of the centrifuge by choosing the respective bearing holes.

The separation column was prepared from a 10 m long, 2.6 mm i.d. PTFE tube (Zeus Industrial Products, Raritan, N.J.) by winding it coaxially onto the holder (12.5 cm diameter) making two coiled layers with a total capacity of about 50 ml. In order to facilitate the countercurrent process, the head terminal was located at the outer layer and the tail terminal at the inner layer of the coiled column. Overall layout of the flow lines on the coiled column is illustrated in Fig. 5. Each terminal is equipped with a 3-way Kel-F

(polytrifluoromonochloroethylene) adaptor which connects inlet and outlet flow tubes to the coiled column whereas at the middle portion of the column a sample feed tube opens through another 3-way Kel-F adaptor. At each terminal the feed line is passed through the adaptor to extend into the separation column for about 50 cm or one complete helical turn. This prevents the introduced phases from flowing back toward the immediate outlet opening at each terminal. The foam and liquid collection lines were made of 0.85 mm i.d. PTFE tubes and other three feed lines, 0.55 mm i.d. tubes.

The five flow tubes from the separation column were bundled, lubricated with silicone grease and protected with a piece of flexible tubing (Tygon) to prevent direct contract with metal parts. The passage of these bundled flow tubes is illustrated in Fig. 4. The tube bundle is first led through the hole of the holder shaft and then passed through a side hole of the coupling pipe mounted on the central axis to reach the opening of the central stationary pipe. As mentioned earlier, these flow tubes are free from twisting and can last several months to one year under the normal operational condition.

The rotational speed of the apparatus is continuously adjustable up to 1000 rpm while the rate was limited to 500 rpm (about 56 x g on the holder axis) throughout the present study. Two metering pumps (Milton Roy Minipump) were used to pump the liquid phase, one through the liquid feed line and the other through the sample feed line, whereas N_2 gas was introduced through the gas feed line at constant pressure of 80 psi which produced a gas flow rate of 600 ml/min measured at the outlet of the foam collection line. The flow rate through the liquid collection line was regulated by the use of a needle valve (Model SS-1SG, Potomac Valve & Fitting, Inc., Rockville, MD) equipped with a 50 cm length of 0.3 mm i.d.

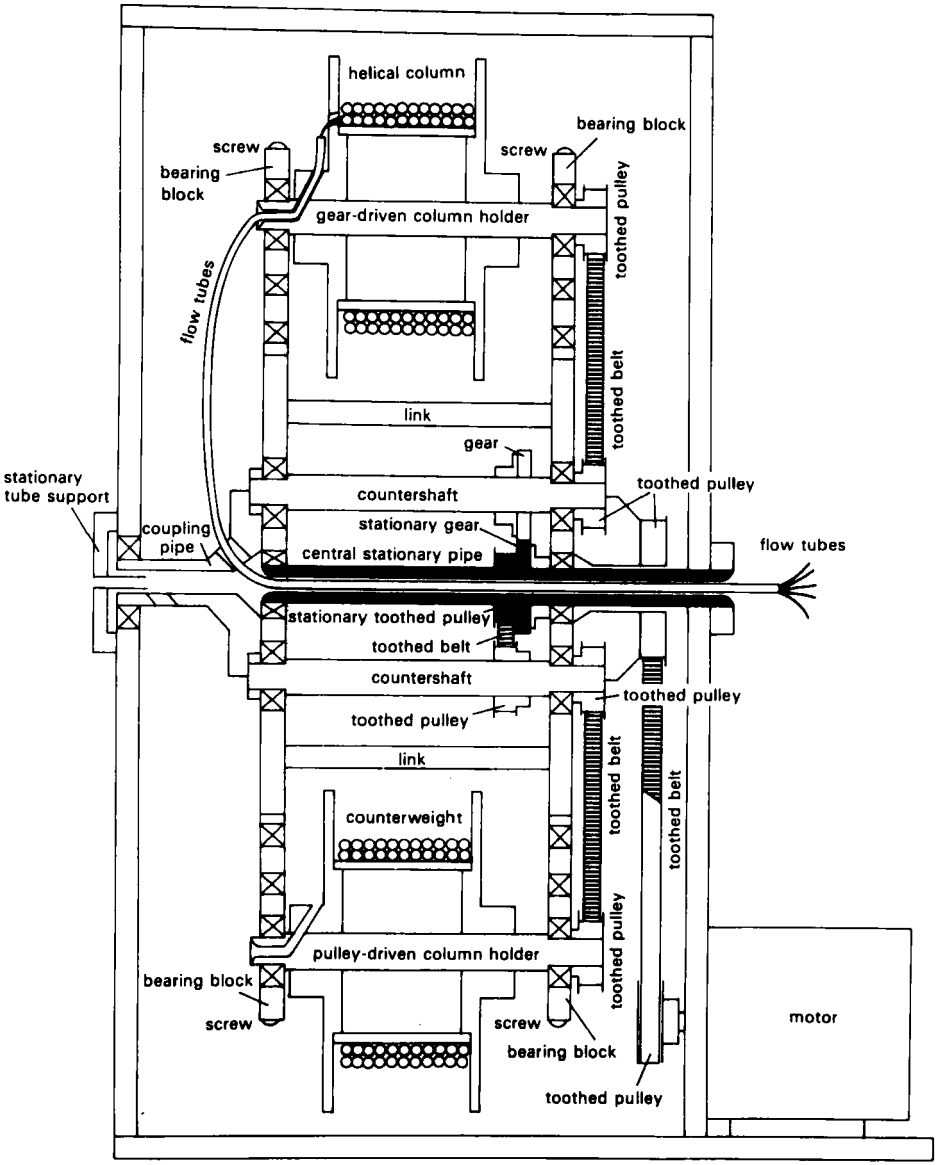

Fig. 4. Cross-sectional view of the apparatus.

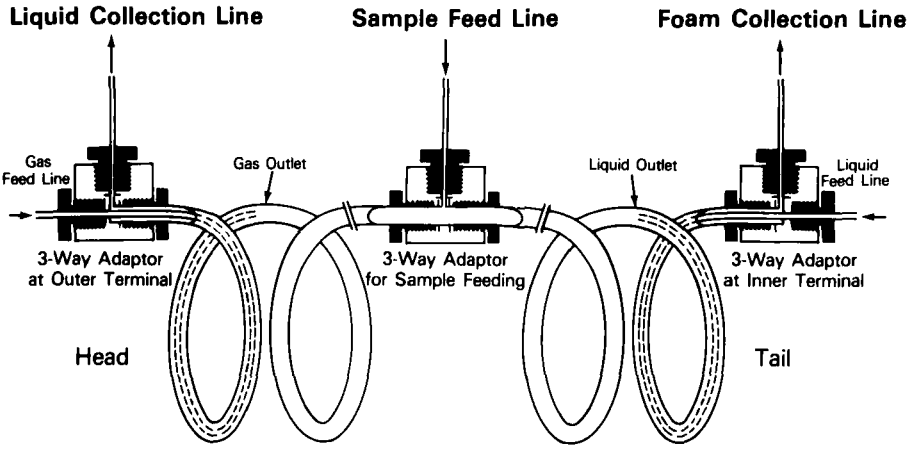

Fig. 5. Layout of five flow channels on the foam CCC column. Note: In the actual configuration of the coiled column consisting of the double layers, the first and the second layers have different handedness of the coil.

PTFE tubing at the outlet of the valve to restrict the flow rate. The foam collection line was left open without restriction, the foam collection rate being determined by the difference between the sum of the liquid feed rates (through the liquid feed line and sample feed line) and the liquid collection rate through the liquid collection line.

EXPERIMENTAL

Reagents

Rhodamine B and Evans blue were obtained from Fisher Scientific Company, Pittsburgh, PA; sodium dodecyl sulfate (SDS) and 35% bovine serum albumin (BSA) from Sigma Chemical Company, St. Louis, MO; and sodium phosphates from J. T. Baker Chemical Company, Phillipsburg, NJ.

Methanol used for absorbance measurement was obtained from Burdick and Jackson Laboratories, Inc., Muskegon, MI.

Preparation of Hemoglobin Solution

About 3 ml of EDTA-treated sheep blood was delivered into a 12 ml graduated plastic centrifuge tube and spun at 2000 rpm for 10 min. Then, the supernatant plasma was removed and the red cell sediment was washed with 10 ml of 0.9% saline solution three times by repeating gentle mixing, centrifugation and decanting the supernatant. Finally, 1 ml of loosely packed red cells was mixed with 9 ml of distilled water for hemolysis. The protein sample mixture was prepared by combining equal volumes of the above hemoglobin solution and 0.2% BSA.

Experimental Procedures

Preliminary studies were performed in the following three elution modes.

1) Continuous enrichment and stripping without using the sample feed line.

A large volume of the sample solution containing a small amounts of material is pumped through the liquid feed line while the N_2 gas is introduced through the gas feed line at constant pressure of 80 psi. The enriched foam is continuously collected through the foam collection line and the stripped liquid, through the liquid collection line.

2) Batch separation with sample injection through the sample feed line.

Continuous countercurrent streams of gas and liquid phases are introduced along the length of the column by pumping the liquid phase through the liquid feed line under N_2 gas pressure of 80 psi applied through the gas feed line. After the steady state hydrodynamic equilibrium is established, a small volume of the sample solution is

locally injected through the sample feed line. Foam and liquid eluted through the respective collection lines are separately fractionated into a series of test tubes at suitable intervals.

3) Continuous separation by continuous sample feeding through the sample feed line.

The steady state equilibrium of the gas-liquid countercurrent flow is first established in the column as in the batch separation experiment

TABLE 1

Experimental Conditions Applied to the Present Study

FACTORS	VARIABLE ITEMS	APPLIED CONDITIONS
CENTRIFUGE	Planetary motion	Synchronous planetary motion on gear-driven holder
	Revolutional radius (R)	20 cm
	Rotational radius (r)	6.4-6.7 cm
	β value (r/R)	0.32 - 0.34
	Revolutional speed	500 rpm
COLUMN	Diameter	2.6 mm i.d.
	Length	10 m
	Configuration	Coaxial to holder axis two layers of coil
FLOW	Liquid feed rate	214 ml/h
	Gas feed rate	600 ml/min (80 psi)
	Sample feed rate	Varied
	Foam collection rate	Varied
	Liquid collection rate	Varied

described above. Then, the sample solution is continuously introduced through the sample feed line. Foam from the foam collection line and the effluent from the liquid collection line are each separately collected into a graduated cylinder.

Sample Analysis

Analysis of dye fractions was made by diluting an aliquot of each fraction with methanol and measuring the absorbance with a Zeiss spectrophotometer. Rhodamine B and Evans blue were analyzed at 556 nm and 620 nm, respectively. The absorbance values were multiplied with the dilution factor and the total fraction volume to determine the relative sample dose in each fraction. A minute amount of rhodamine B present in the stripped sample solution was fluorometrically analyzed with an AmincoBowman Fluorometer using the excitation and emission wavelengths at 560 nm and 600 nm, respectively. Analysis of protein fractions was performed by diluting each fraction with distilled water and measuring the absorbance at 280 nm and 540 nm for BSA and sheep hemoglobin, respectively.

RESULTS AND DISCUSSION

A series of preliminary experiments has been performed to demonstrate the capability of the foam CCC method under a set of experimental conditions listed in Table 1. Because of a large number of factors involved, no attempt has been systematically made to optimize all those conditions. Instead, efforts were directed to select one workable set of experimental conditions for exploration of the potential capability of the method.

Continuous Enrichment and Stripping

This experiment was performed to demonstrate the capability of the method to concentrate and/or eliminate a minute amount of material present in a large volume of sample solution. The sample solution containing rhodamine B at a 10^{-6} M concentration and SDS at 10^{-3} M as a collector was introduced through the liquid feed line at 214 ml/h against N_2 flow through the gas feed line at 80 psi, while the apparatus was run at 500 rpm. The sample feed line was not used in this experiment. The liquid collection rate was adjusted at a level slightly below the liquid feed rate so that the foam collection rate became as small as several hundred microliters per hour which yielded the foam highly enriched with rhodamine B. After 1 liter of the sample solution was eluted, the liquid collection line was closed to elute rhodamine B remaining in the column through the foam collection line. The stripped liquid collected through the liquid collection line was fluorometrically analyzed to determine the concentration of rhodamine B. The results showed that the dye concentration in the stripped solution was 1.3×10^{-9} M while over 99% of rhodamine B was recovered through the foam collection line within a 2ml volume resulting in over 500-fold enrichment.

Batch Separation with Sample Injection through Sample Feed Line

This experiment was initiated by establishing a liquid-gas countercurrent flow equilibrium through the coiled column. At the revolutional speed of 500 rpm a surfactant solution containing SDS at 10^{-3} M was pumped through the liquid feed line while the N_2 gas flow was introduced through the gas feed line at 80 psi. After the hydrodynamic equilibrium was reached, 0.5 ml of sample solution containing rhodamine B and Evans blue each at 5×10^{-4} M was injected through the sample feed line. The needle valve on the liquid collection line was adjusted to

make a 1:3 volume ratio between the foam and liquid fractions. Effluents from both collection lines were separately fractionated into a series of test tubes at 30 second intervals. The concentration of each dye in the fractions was spectrophotometrically determined using 556 nm for rhodamine B and 620 nm for Evans blue. Fig. 6 shows the typical experimental result obtained with the present method. The upper chromatogram obtained through the foam collection line shows a sharp single peak entirely consisting of rhodamine B with the peak maximum at one minute after sample injection. The lower chromatogram obtained through the liquid collection line shows a broad symmetrical peak of Evans blue with the peak maximum at 2.75 minutes after sample injection. These results are quite reproducible and injection of the single component produced the similar peak through the respected collection line. The volume of the liquid phase present in the column under a steady state hydrodynamic equilibrium in these experiments ranged between 4 and 5 ml which amounted to approximately 10% of the total column capacity.

Continuous Separation by Continuous Sample Feeding through the Sample Feed Line.

Rapid and clean separation of the two dyes in the batch separation method described above indicated the feasibility of continuous separation by steadily feeding the sample mixture at a proper rate. Under otherwise identical experimental conditions used in the batch separation, the sample solution was continuously introduced through the sample feed line at various flow rates. The satisfactory separations were obtained at sample feed rates of 0.36 ml/min or less, which separated each sample at the maximum rate of 1.8×10^{-7} mol/min. The application of higher flow rates resulted in initial accumulation of rhodamine B in the column which was later followed by elution of rhodamine B through the liquid collection line.

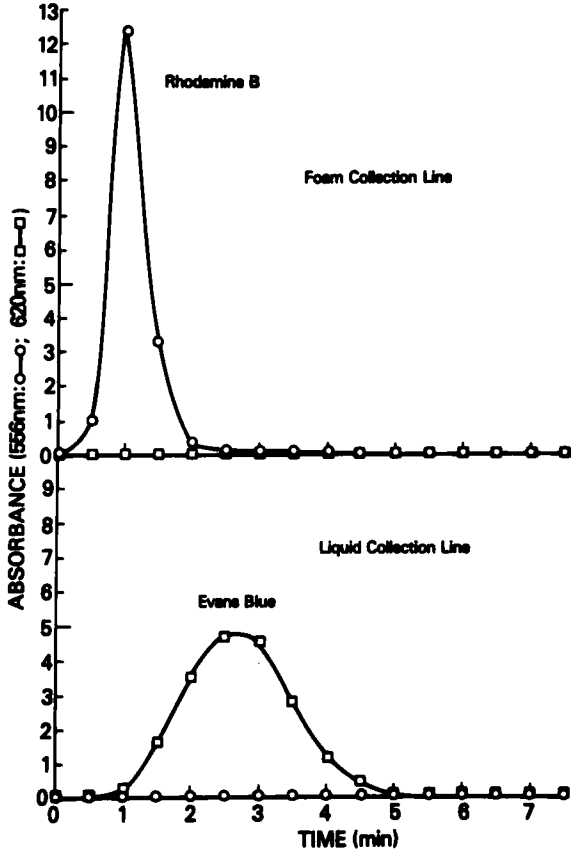

Fig. 6. Batch separation of rhodamine B and Evans blue with foam CCC. Rhodamine B having foam affinity was quickly eluted through the foam collection line (upper chromatogram) while Evans blue was carried with the liquid stream in the opposite direction and eluted through the liquid collection line (lower chromatogram). Note: In analysis, Evans blue absorbed the light at both wavelengths at a ratio of 0.6 (556nm/620nm). Therefore, the 556nm curve in the lower chromatogram, which represents the elution profile of rhodamine B, was given by the total absorbance at 556nm less the absorbance at 620nm multiplied by 0.6.

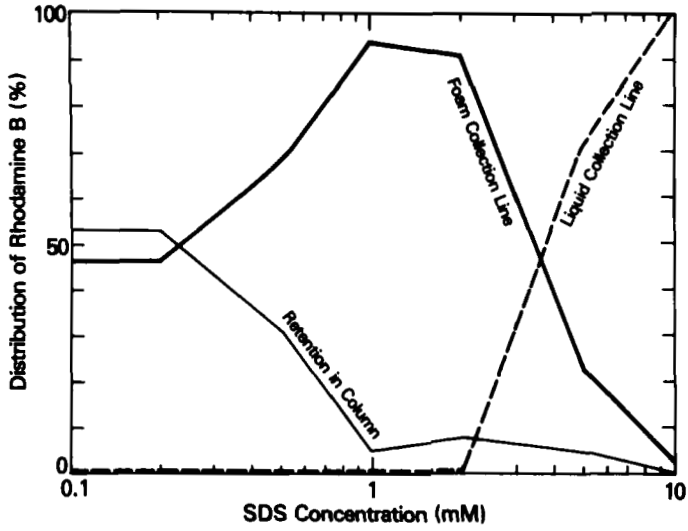

Fig. 7. Distribution of rhodamine B at various SDS concentrations in the liquid phase.

The experiments were continued to study the effects of SDS concentration on the hydrodynamic behavior of rhodamine B in the foam separation column under a high sample feed rate of 3.8×10^{-7} mol/min. The results are summarized in Fig. 7 where the percentage distribution of the rhodamine sample is plotted for the applied SDS concentration expressed in a log scale in abscissa. Among three curves drawn in the diagram, the thick solid curve indicates the amounts of dye eluted through the foam collection line; the thin solid curve, the amounts of dye retained within the column; and the broken curve, the amounts of dye eluted through the liquid collection line. At a high SDS concentration of 10^{-2} M, the dye exhibited little affinity to the foam and mostly eluted through the liquid collection line. As the SDS concentration was decreased, the dye rapidly developed the foam affinity and, at 10^{-3} to 2

x 10^{-3} M SDS concentrations, over 90% of the dye was collected through the foam collection line while the liquid collection line eluted clear liquid almost free of rhodamine B. Further decrease of the SDS concentration resulted in a decreased foam recovery rate of rhodamine B causing the retention within the separation column. If the sample feeding is stopped at this stage, elution of the dye through the foam collection line continues at the same rate until the retained dye is completely recovered. If the sample feeding is continued, the dye continuously accumulates in the column and finally appears through the liquid collection line. The results clearly indicate that the foam recovery rate of rhodamine B is largely governed by the SDS concentration which yields the highest recovery rate at 10^{-3} M to 2 x 10^{-3} M under the present experimental condition.

Preliminary Experiments for Protein Separation

The present method has been applied to the separation of proteins without the use of a surfactant collector. As is well known, exposure of proteins such as BSA to a gas-liquid interface may cause denaturation to alter the physiological function of the molecule. The preliminary studies were conducted to test vulnerability and foam-producing capacity of proteins with the present system by injecting the sample solution into the running column through the sample feed line. Several kinds of proteins including BSA, human and sheep hemoglobin, and ovalbumin were examined. Among these only BSA showed an active foam-producing ability and was collected through the foam collection line whereas other proteins were mostly eluted through the liquid collection line without any visible evidence of denaturation. BSA fractions eluted through the foam collection line showed various degree of turbidity apparently due to denaturation of the molecule. Further experiments revealed that the

intensity of turbidity highly depended upon the composition of the applied liquid phase. The use of salt-free distilled water or dilute acid solution caused most intensive turbidity. Addition of a surfactant to the liquid phase decreased the degree of turbidity but at the same time lowered the foam recovery rate of BSA. Sodium phosphate solution of slightly alkaline pH (7.2 - 8.9) at a relatively high ionic strength (0.2 - 0.5 M) produced minimum turbidity with a high BSA recovery of over 90% through the foam collection line.

Fig. 8 illustrates a preliminary result of the batch separation of BSA and sheep hemoglobin obtained with a liquid phase composed of 0.2 M dibasic sodium phosphate solution (pH 8.9) under the standard experimental condition previously applied to the dye separation. BSA with a foam-producing capacity was quickly eluted through the foam collection line within 6 minutes while sheep hemoglobin was entirely recovered through the liquid collection line in about 10 minutes.

The preliminary studies on foam separation of proteins described above furnish a useful guidance for further development of the present method. Although simple adjustment of pH and ionic concentration of the liquid phase worked out well for separation of BSA, many other proteins lack an active foam-producing capability and therefore require the selective collectors to acquire the foam affinity. Hopefully, the use of such collectors would also prevent the protein molecules from direct contact with the gas-liquid interface, thus reducing the possibility of denaturation. One useful future application of the present method may be the separation of macromolecules or particulates with foam affinity CCC using highly specific collector molecules. For example, a long hydrocarbon chain is attached to a substrate or inhibitor molecule of the aimed enzyme to form a hydrophobic terminal so that the derived molecule

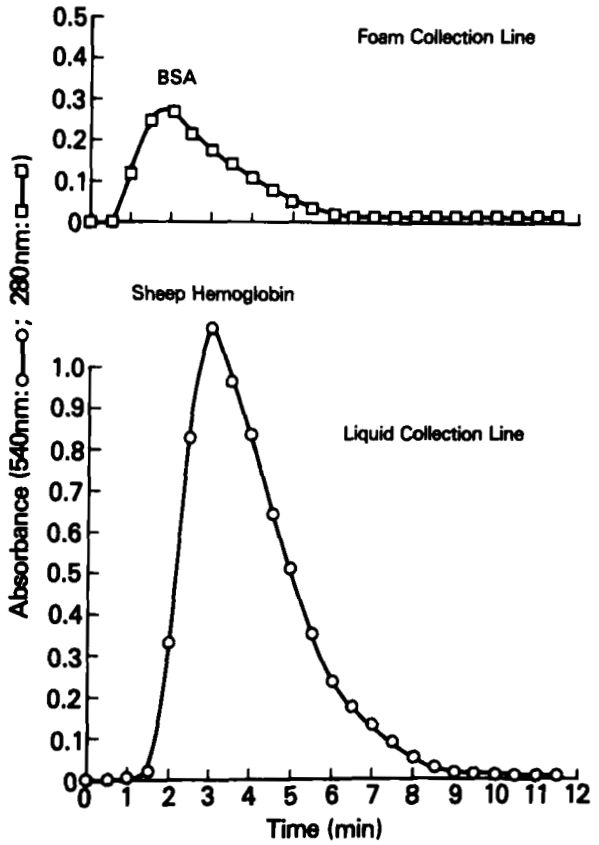

Fig. 8. Preliminary separation of proteins with the foam CCC method. BSA having a foam-producing capacity was collected through the foam collection line while sheep hemoglobin was entirely recovered through the liquid collection line.

acquires a foam affinity to carry the enzyme. The enzyme prebound to such collector molecules may be efficiently concentrated and isolated through the foam collection line in a short period of time. Achievement of the above goal would make a great contribution to a broad field of biological sciences including protein chemistry, cell physiology, and genetic engineering.

REFERENCES

1. Ito, Y., Development of countercurrent chromatography, Anal. Chem., 56, 534A, 1984.

2. Ito, Y., Sandlin, J., and Bowers, W. G., High-speed preparative counter-current chromatography with a coil planet centrifuge, J. Chromatogr., 244, 247, 1982.

3. Somasundaran, P., Foam separation methods, Sep. Purif. Methods, 1, 117, 1972.

4. Ito, Y., Experimental observations of the hydrodynamic behavior of solvent systems in high-speed counter-current chromatography: I. Hydrodynamic distribution of two solvent phases in a helical column subjected to two types of synchronous planetary motion, J. Chromatogr., 301, 377, 1984.

5. Ito, Y. and Bowman, R. L., Foam countercurrent chromatography: New foam separation technique with flow-through coil planet centrifuge, Separation Science, 11 (3), 201, 1976.