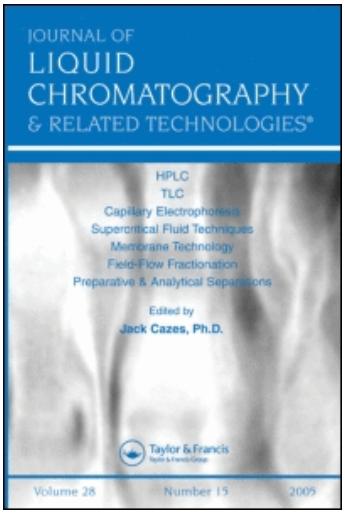

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Versatile Coil Planet Centrifuge for Performing Countercurrent Chromatography: Comparative Studies on Performance on Three Types of Columns

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VERSATILE COIL PLANET CENTRIFUGE FOR PERFORMING COUNTERCURRENT CHROMATOGRAPHY: COMPARATIVE STUDIES ON PERFORMANCE ON THREE TYPES OF COLUMNS

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ABSTRACT

A compact table top model of a coil planet centrifuge has a capability of utilizing various types of separation columns to yield analytical to preparative separations at high partition efficiency of the helical column on the holder. Three types of helical separation columns were examined to evaluate their performance in retention of the stationary phase and partition efficiency using the same set of peptide samples and butanol two-phase solvent systems. The eccentric helical column consisted of eight coil units each prepared by winding two layers of a 1.6 mm i.d. PTFE (polytetrafluoroethylene) tubing onto a 1.25 cm diameter pipe to yield a total volume capacity of about 12 ml. These column units were interconnected in series with narrow-bore PTFE tubing and symmetrically mounted around the holder at a distance approximately 4 cm from the holder axis. The toroidal column was prepared by winding similar tubing onto a 1.25 cm diameter (plastic flexible) core which was again coiled around the holder. The multilayer column was prepared by winding similar tubing directly around the holder making multiple layers with a total capacity of approximately 240 ml. Overall results

indicated that the eccentric helical column yielded higher peak resolution than the toroidal column while the multilayer coil produced the highest peak resolution among all columns. The problem of insufficient retention of viscous sec-butanol/aqueous phase systems in the multilayer column was solved by heating the centrifuge unit at 45-50°C. The toroidal column yielded the lowest efficiency but is most suited for small-scale separations with a narrow-bore coil.

INTRODUCTION

Countercurrent chromatography (CCC) is a unique liquid-liquid separation technique that does not require the use of a solid support matrix. This forgoes the associated problems of peak tailing, sample loss, and sample contamination from the column matrix. In addition, CCC does not require the use of rotating seals [1,2]. This drastically reduces the maintenance requirements of the apparatus. The efficiency and performance of CCC is acutely sensitive to the orientation of the column within the planes of rotation and revolution. Three columns were tested, each differing with respect to this orientation. The performance of each column was assessed by the separation of a pair of standardized peptides (val-typ, trp-leu) in a two-phase solvent mixture of n-butanol, acetic acid, and water (4:1:5) and by the separation of bovine insulin in a solvent system composed of sec-butanol, dichloroacetic acid (DCA) and water (100:1:100) at 50°C.

PRINCIPLE

Countercurrent chromatography is a system based on the hydrodynamic equilibrium established within a column subjected to

a particular acceleration field [3]. This equilibrium results in the retention of one phase (stationary phase) of a two-phase solvent system within the column. This equilibrium is maintained against the flow of the compliment phase (mobile phase) introduced at the inlet of the column. Samples introduced at the inlet of the column along with the mobile phase are partitioned between the two phases and separated chromatographically according to their partition coefficients. The formation of such a hydrodynamic equilibrium is dependent upon variables inherent to both the solvent system used: interfacial tension, tube wall affinity, density differences, viscosity and inherent to the apparatus: revolutions per minute (rpm), internal diameter of tubing used, flow rate, revolutional direction and the ratio of the rotational diameter of the column holder to the revolutional diameter of the apparatus. This ratio is termed the beta value of the apparatus. The formation of this equilibrium is secondary to the acceleration field the column is subjected to. This centripetal field is produced by a synchronous planetary motion whereby the column revolves once with respect the the central horizontal axis of the apparatus and twice with respect to gravity in the same direction and angular velocity [4].

This synchronous planetary motion yields a high percentage of one phase remaining in the column. This high stationary phase retention is of critical importance to sample peak resolution, i.e., peak resolution is increased with higher stationary phase retention. The partitioning process of the sample between the

solvent pairs at the inlet of the column represents the basic principle behind CCC; however, different hydrodynamic equilibrium conditions arise with the use of different column orientations with respect to the central axis of the apparatus. This partitioning process is dependent upon the stationary phase retention which is in turn dependent upon the hydrodynamic equilibria established.

Three columns with different orientations were tested in order to elucidate their respective stationary phase retention and peak resolution capabilities (Fig. 1). In the multilayer orientation of the column (Fig. 1, right), the centrifugal force field induced by rotation establishes an equilibrium whereby a unilateral distribution of the two phases occurs. Depending on which type of solvent system is used and at what temperature, one phase will totally occupy the head end of the column while the other phase occupies the tail end. (The head-tail relationship is conventionally defined on the basis of an Archimedean screw effect where all objects of different density move from the tail toward the head of the rotating coil.) This equilibrium is dependent upon the beta value of the apparatus. When the beta value exceeds 0.5, the centrifugal force field pattern is such that the centrifugal force vectors are always pointing outwardly from the column and fluctuates in both magnitude and direction during each revolutional cycle [3]. During rotation the formation of a mixing zone (area of intense agitation) occurs in a portion of tubing in each helical turn closest to the central

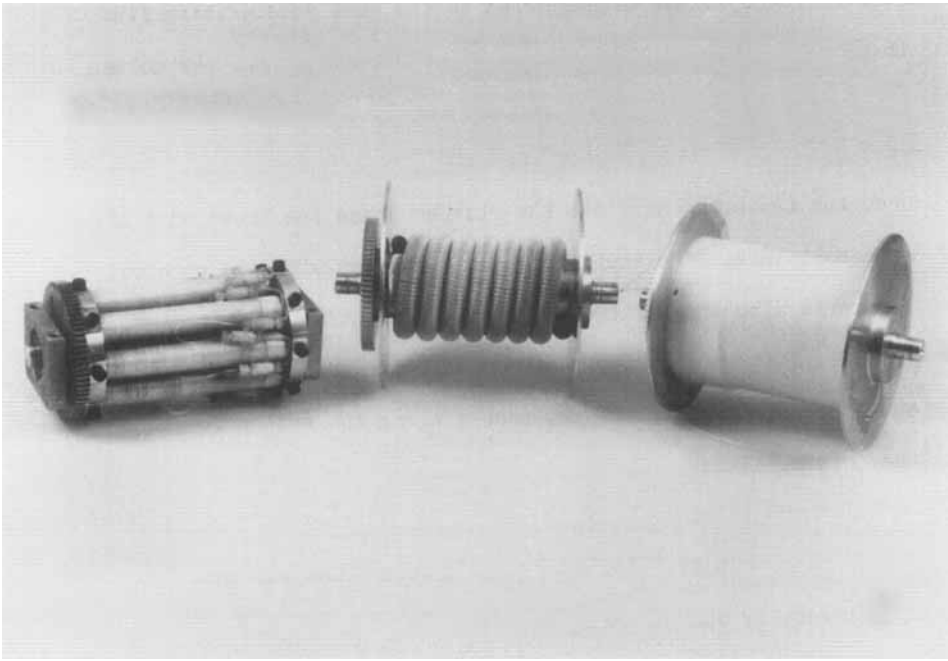

Fig. 1: Column configuration: eccentric (left), toroidal (center), multilayer (right).

axis of revolution. The remainder of each helical turn represents a settling zone (formation of two layers) in which the lower phase occupies the peripheral half of each helical turn while the upper phase occupies the innermost half. The mixing zone propagates along the coil and ensures good mixing and mass transfer between the two phases. The revolutional rate determines how quickly per unit time the mixing zone is formed. In the toroidal column (Fig. 1, center) the hydrodynamic equilibrium differs from that of the multilayer column. Upon

rotation, the column is subjected to the same acceleration field in that the acceleration vectors point outwardly from the column and undulates in both magnitude and direction. This results in the separation of the two phases whereby the heavier phase occupies the outer half and the lighter phase the inner half of each coil unit. The equilibrium pattern seen with the toroidal column is also true of the eccentric orientation of the column (Fig. 1, left). This separation of the two phases represents an equal distribution of the two phases along the entire length of the column. This implies that the maximal stationary phase retention values in both the toroidal and eccentrical columns would not exceed 50% whereas values greater than 50% and experimentally observed values of up to 90% have been demonstrated with the multilayer configuration. Although retention values of over 30% are usually adequate for satisfactory separation, higher retention values will substantially improve the peak resolution, especially in early eluting peaks.

APPARATUS

A horizontal-flow through coil planet centrifuge equipped with a temperature control system was utilized in the present study (Fig. 2). The rotary frame of the apparatus was coupled to the central shaft by a pair of toothed pulleys and toothed belt and revolves around the stationary central shaft of the apparatus. The rotary frame consists of two symmetrically rotating holders each with their central shafts parallel to the central axis of

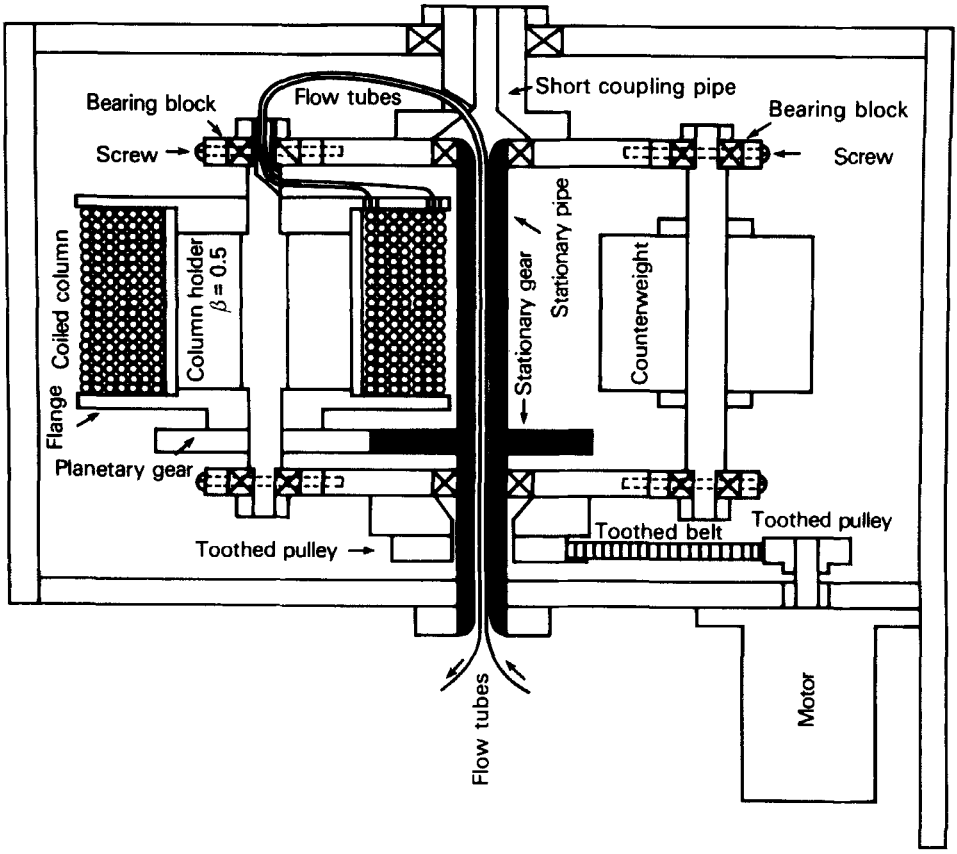

Fig. 2: Cross-sectional view through the central axis of the apparatus.

the apparatus. One holder serves as the experimental column and is held to the rotary frame by a pair of removable sealed ball bearing assemblies at a fixed distance of four inches from the central axis of the apparatus. The other holder serves as a counterbalance. The shaft of each column holder is equipped with a metal planetary gear which is coupled to an identical

stationary metal sun gear affixed to the central shaft of the apparatus. This gear arrangement produces a synchronous planetary motion that allows for a system that does not require rotating seals.

The apparatus was modified in order to maintain desirable operating temperatures (Fig.3). The outside of the apparatus was insulated with sheets of polyurethane foam glued to the outer surface of the aluminum walls of the apparatus. Four electric heating pads along with a heat sensor were glued to the three inside walls of the apparatus, the fourth wall is of transparent plexiglass. A thermometer was taped to the side of the plexiglass shield in order to observe the operating temperature of the apparatus. Temperature was regulated by a temperature controller obtained from FHL Industries, Inc., Boonton, New Jersey.

Revolutional speed was regulated by an Electro-Craft E-652-M control unit. Solvent was pumped through the column with either a Beckman Accu-Flow pump or a Chromatronix Cheminert metering pump. The effluent was monitored by an LKB Uvicord S at 280 nm and collected into fractions with an LKB Ultrorac fraction collector.

The columns were prepared by winding continuous lengths of PTFE tubing around the column holder. A pair of flow tubes from the column was first passed through a central hole in the holder shaft and then through a side opening of a hollow central stationary shaft of the apparatus. To prevent direct metal

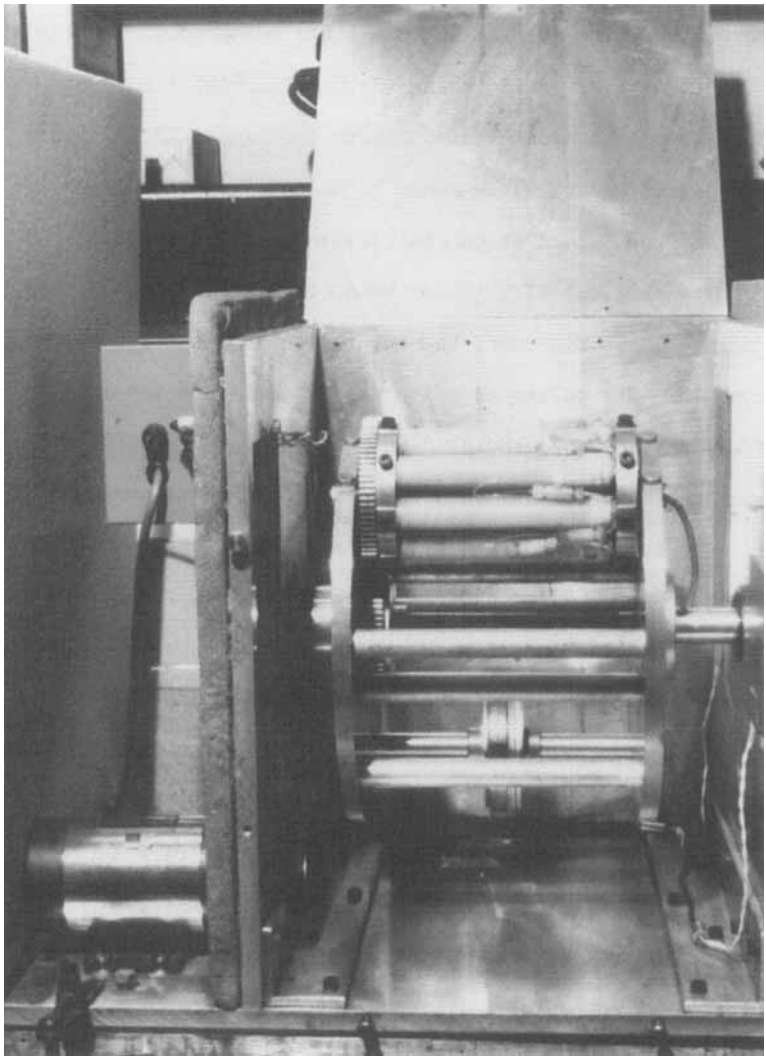

Fig. 3: Horizontal flow-through coil planet centrifuge modified with a temperature control system.

contact of the flow tubes to the apparatus, the flow tubes were lubricated with silicone grease and surrounded by a piece of plastic tubing. The multilayer column was prepared by winding a piece of 1.6 mm i.d., PTFE tubing (Zeus Industrial Products, Raritan, NJ) onto a spool-shaped holder 1.5" in diameter. This corresponded to a beta value range of 0.19-0.42. The column capacity was 260 ml. A second multilayer coil consisted of a piece of 1.6 mm i.d., PTFE tubing wound in multiple layers upon a spool holder 5" in diameter, thus corresponding to a beta value of 0.56-0.75. The column capacity was 240 ml. The toroidal column was prepared by winding PTFE tubing onto a flexible core of rubber tubing which was then wound onto the spool-shaped holder. The tubing was 1.6 mm i.d. and 91 ml in capacity. The column holder for the eccentrical coils consisted of eight evenly spaced aluminum rods radially arranged around the central shaft of the holder. At the ends of each rod is an aluminum fitting which fits snugly onto the holder. Each rod was attached to the holder via a single Allen screw in each aluminum fitting. On each rod was wound a single layer of 1.6 mm i.d., PTFE tubing, 5 meters long and 12 ml in capacity. Each coiled unit was connected in series by a 10cm long piece of tubing, 0.46 mm i.d. to make a total column capacity of 96 ml.

EXPERIMENTAL

Solvent System and Sample Solution

In the present study, two phase systems were used. The first consisted of n-butanol (Burdick & Jackson Laboratories,

Inc., Muskegon, MI), glacial acetic acid (J. T. Baker Chemical Co., Phillipsburg, NJ), and distilled water at a volume ratio of 4:1:5. The second phase system was composed of sec-butanol (Burdick & Jackson Laboratories, Inc., Muskegon, MI), dichloroacetic acid (Aldrich Chemical Co., Milwaukee, WI) and distilled water at varying volume ratios. The n-butanol solvent system was equilibrated in a separatory funnel at room temperature and separated before use. The sec-butanol solvent system was equilibrated in a water bath at 50°C and separated before use.

A sample solution of L-tryptophyl-L-leucine (trp-leu) and L-valyl-L-tyrosine (val-tyr), obtained from Sigma Chemical Co., Saint Louis, MO, was used in conjunction with the n-butanol, acetic acid, and water system (BAW), while a sample solution of bovine insulin, also from Sigma Chemical Co., was used with the sec-butanol system. Twenty-five milligrams of each peptide were dissolved in equal volumes of upper and lower phases at room temperature for a final sample volume of 2 ml. One hundred milligrams of insulin were dissolved in equal volumes of upper and lower phases at 50°C for a total sample volume of 3 ml.

Separation Procedure

In the separation of the dipeptides, the column was first filled entirely with the stationary phase at room temperature and then followed by the injection of the sample solution through the sample port. Following sample injection the apparatus was rotated at the designated speed (600 or 800 rpm) while the mobile

phase was pumped into the column at the desired flow rate of 50 ml/hr. For the separation of insulin, the apparatus was first equilibrated to 50°C. This was followed by introduction of the heat equilibrated mobile phase. The sample solution was introduced into the rotating apparatus following the appearance of the solvent front.

The effluent was continuously monitored at 280 nm with an LKB 2138 Uvicord S detector and collected with an LKB Ultrorac fraction collector. An aliquot of each fraction (200 ul) was diluted with 3 ml methanol and analyzed with a Beckman model 35 spectrophotometer at 280 nm. Following the separation, the column contents were voided into a graduated cylinder with nitrogen gas at 80 psi to measure the percent retention of the stationary phase.

RESULTS AND DISCUSSION

The efficiency and sample peak resolving capacity of each column was evaluated by the use of a standardized pair of dipeptides and a pure sample of bovine insulin.

Twenty-five milligrams (25 mg) of each dipeptide (trp-leu and val-tyr) were dissolved in 1 ml of upper phase and 1 ml of lower phase from an equilibrated solvent system composed of n-butanol/glacial acetic acid/water at a volume ratio of 4:1:5, respectively. A total of 50 mg dissolved in 2 ml (2.5 g%) was introduced as the test sample solution for each column. The flow rate remained at 50 ml/hr at room temperature (24°C).

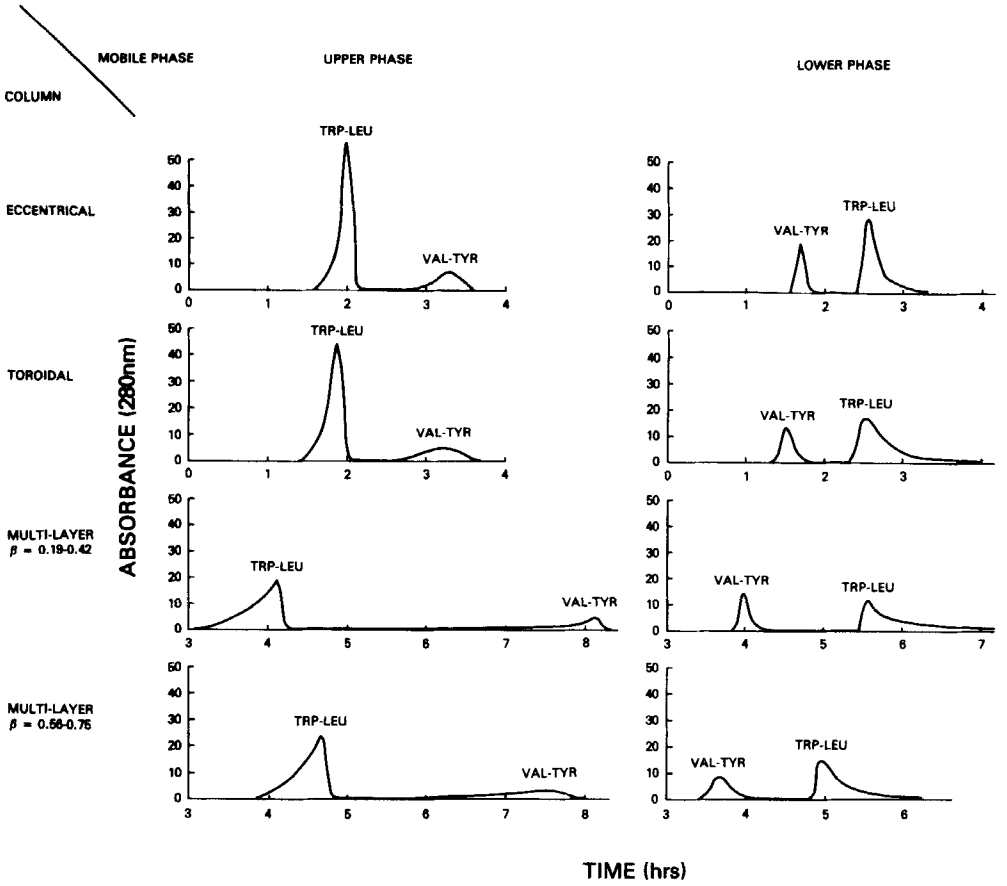

Fig. 4: Separations of val-tyr and trp-leu at an rpm setting of 600.

Experimental variables included choice of mobile phase and revolutional speed (600 or 800 rpm). Figure 4 depicts chromatograms obtained from the separation of the dipeptides at an rpm setting of 600. The abscissa indicates time in hours while the ordinate indicates absorbance level monitored at 280 nm. The right hand column indicates a lower mobile phase while the

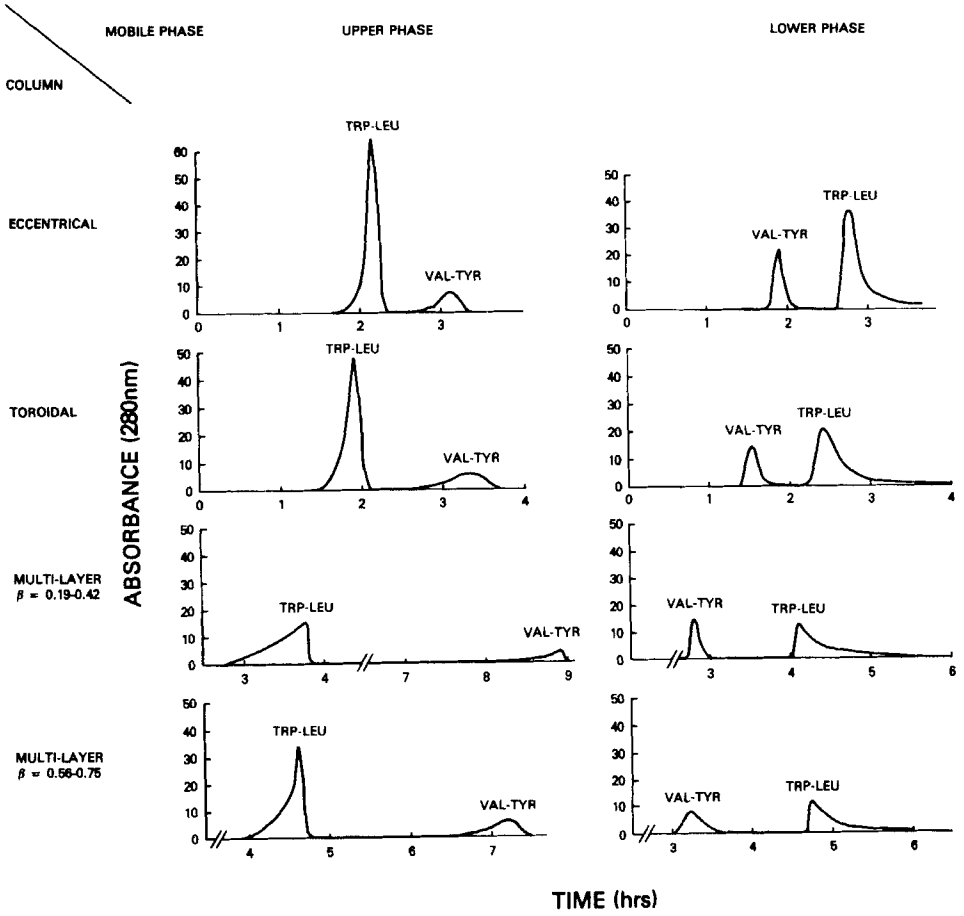

Fig. 5: Separation of val-typ and trp-leu at an rpm setting of 800.

left hand column indicates an upper mobile phase. The far left hand column indicates column orientation. Figure 5 represents chromatograms obtained under identical experimental conditions as seen in Figure 4 at an rpm value of 800.

From Table 1 it may be seen that overall, stationary phase retention percentages were not significantly altered by

Table 1

Stationary Phase Retention Values

Coiled Column	Upper Phase Mobile 600 rpm	800 rpm	Lower Phase Mobile 600 rpm	800 rpm
		Peptides		
Eccentric	39%	29%	40%	30%
Toroidal	41	42	41	39
Multilayer				
β=.19-.42	53	64	25	23
β=.56-.75	38	36	14	14
		Insulin		
Eccentric	40	-	38	-
Toroidal	42	-	39	-
Multilayer				
β=.56-.75	33	-	48	-

increasing the rpm value from 600 to 800 with respect to the eccentric and toroidal columns. This might be expected since the range in retention values of 39-41% are already approaching the maximal retention values of 50%. Increasing the revolutional speed may in fact produce too much phase mixing and result in loss of stationary phase from the column via emulsification and carryover of the stationary phase with the eluting mobile phase. This is evident more in the eccentric configuration than the toroidal at the higher rpm setting (Table 1). However, the increased phase mixing associated with the eccentric configuration may account for the improved partition efficiency in terms of theoretical plates at both rpm settings when compared to the toroidal orientation (Figs. 4,5).

With respect to the multilayer column, stationary phase retention is more dependent upon the β value of the apparatus. From previous studies the phase distribution of the chloroform/acetic acid/water (2:2:1) system is such that at a small β value of 0.25, the upper phase distributes to the tail of the column while the lower phase distributes to the head. An intermediate β value of 0.25 produced a transitional hydrodynamic state such that distribution trends did not exist. However, at β values greater than 0.5, a reversal in the distribution pattern exists. The upper phase now distributes to the head while the lower phase distributes to the tail (5). Similar observations have been made with the n-butanol/acetic acid/water system (4:1.5), (5). At the lower β values the phase distribution is similar to that seen with the chloroform/acetic acid/water system. As the β values increase, the distribution patterns begin to change but a complete reversal of these trends were not observed at the β values tested. Table 1 indicates a decrease in the stationary phase retention values at the higher β value range of 0.56-0.75. This also corresponds to a decrease in the peak resolution of the dipeptides (Figs. 4,5) at this same β value. During the studies, both multilayer columns (β = 0.19-0.42 and 0.5-0.75) were tested under identical experimental conditions such that the elution mode with the upper phase mobile was from head to tail and with the lower phase mobile tail to head. According to these hydrodynamic observations, a possible way to increase stationary phase retention and peak resolution in the multilayer column would be to decrease the β value with the same

elution mode profile or to reverse the elution mode while increasing the β value until a favorable phase distribution reversal is obtained. The baseline separation is increased with the multilayer columns when compared to both toroidal and eccentric configurations but efficiency is decreased. This loss in efficiency may be related to the increased stationary phase retention. With increased retention the sample solution is subjected to the partitioning processes within the column for longer period of time. Subsequently, while peak resolution is enhanced efficiency drops due to band broadening of the sample peaks. Manipulation of flow rate and elution mode may help to resolve this loss of efficiency.

Figure 6 illustrates chromatograms obtained from the purification of bovine insulin at an rpm setting of 600. The abscissa and ordinate represent time and absorbance level respectively while the right and left hand columns indicate lower and upper mobile phases, respectively. All procedures were conducted at 50°C to reduce solvent viscosity and settling time (6) and DCA was added to the solvent system to adjust the partition coefficients. At a concentration of 0.5% DCA, the partition coefficient (UP/LP) equaled 0.57 while at a concentration of 2.0% DCA yielded a partition coefficient of 1.52. DCA concentrations of 0.5% and 2.0% were used with upper and lower mobile phases, respectively. One hundred milligrams (100 mg) of insulin were added to equal volumes (1.5 ml) of upper and lower phases for a final sample volume of 3 ml (~ 3g %). At the higher rpm setting of 800 a white precipitate would form with all

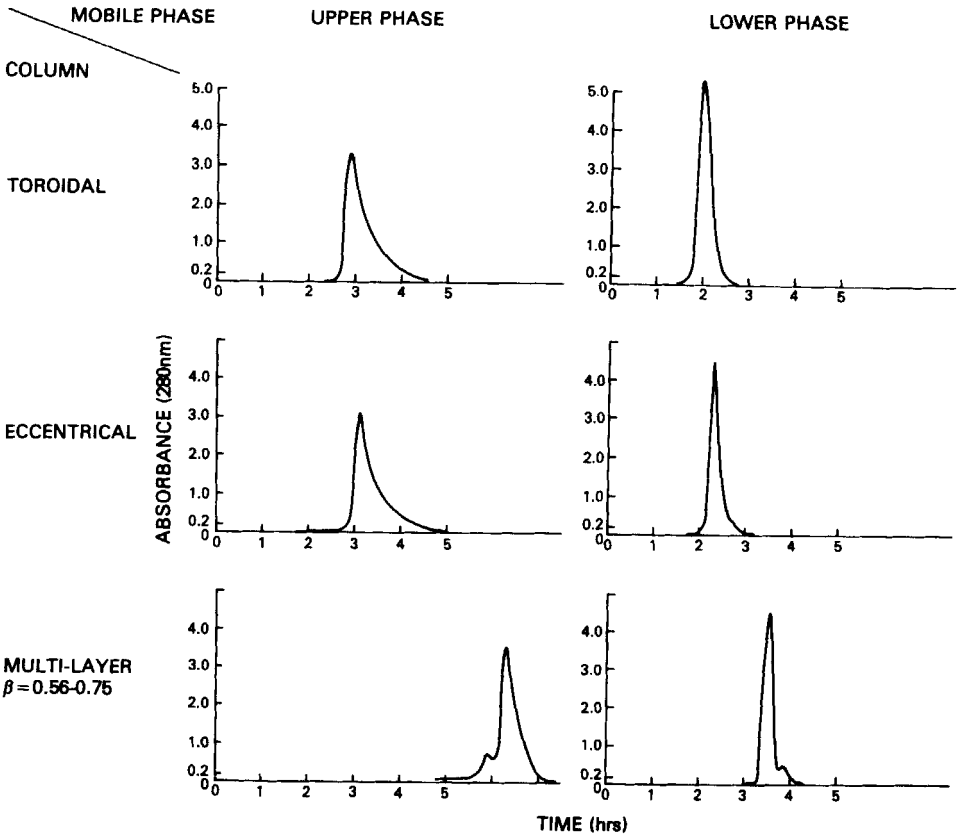

Fig. 6. Separation of bovine insulin at an rpm setting of 600 at 50°C.

columns tested. Possible denaturation of the protein due to excess mixing necessitated the use of only the lower rpm setting at 600. From table 1, stationary phase retention values for all column configurations were similar. Upon comparing the three column configurations in the separation of insulin it appears that the multilayer orientation was best suited for resolving the

impurities from the original sample. This may be secondary to the greater length and volume capacity of this column.

While both the eccentric and toroidal columns had similar volume capacities of 100 ml and tubing length of 4 meters, the multilayer column had approximately 3 times the volume capacity and length. By increasing the length and volume of the eccentric and toroidal columns, in addition to their increased phase mixing ability, these two configurations may produce results superior to that of the multilayer coil but the tradeoff would be the increased time expenditure.

REFERENCES

1. Ito, Y. and Bowman, R. L., Horizontal flow-through coil planet centrifuge without rotating seals, Anal. Biochem. 82, 63, 1977.

2. Ito, Y., and Bowman, R. L., Preparative countercurrent chromatography with horizontal flow-through coil planet centrifuge, J. Chromatogr., 147, 221, 1978.

3. Ito, Y., Toroidal coil planet centrifuge for countercurrent chromatography, J. Chromatogr., 192, 75, 1980.

4. Ito, Y., New continuous extraction method with a coil planet centrifuge, J. Chromatogr., 207, 161, 1981.

5. Ito, Y., High-speed countercurrent chromatography, CRC Critical Reviews in Analytical Chemistry, 17, Issue 1, 1986.

6. Ito, Y. and Conway, W. D., Experimental observations of the hydrodynamic behavior of solvent systems in high-speed countercurrent chromatography: Part III. Effects of physical properties of the solvent systems and operating temperature on the distribution of two-phase solvent systems, J. Chromatogr., 301, 405, 1984.