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

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SUMMARY

Hydrodynamic distribution of two-phase solvent systems in a rotating helical column subjected to centrifugal fields produced by two different types of synchronous planetary motion has been studied by the use of the combined horizontal flow-through coil planet centrifuge. With continuous elution of the mobile phase, the simpler type of motion resulted in low retention of the stationary phase in the column whereas a more complex motion, which produces a quasi-radial centrifugal field varying in both intensity and direction, yielded high stationary phase retention for commonly used solvent systems having a wide range of hydrophobicity. These solvent systems display highly complex modes of hydrodynamic interaction in the coil according to their particular physical properties.

INTRODUCTION

Counter-current chromatography (CCC)[1-3] is characterized by the use of separation columns free of a solid supporting matrix. Retention of the stationary phase and partitioning of the solute within the column are influenced by subtle aspects of the hydrodynamic equilibrium between the two immiscible solvent phases. Consequently, studies on the hydrodynamic behavior of the solvent phases in the column is essential for further development and application of the CCC technology.

High-speed CCC, recently developed in our laboratory[4], has a unique capability to retain a large quantity of the stationary phase in the presence of a high mobile phase flow-rate, thus yielding efficient separations in short periods of time. Potential capabilities of the method have been demonstrated by separations of various biological samples with conventional two-phase solvent systems[5].

In order to extend the capability of high-speed CCC, a series of experimental

studies has been performed to examine the hydrodynamic distribution of the two-phase solvent system in the rotating coiled column. The present paper, which is Part I of the above experimental series, describes the hydrodynamic distribution of the two immiscible solvent phases in a helical column under the two different types of synchronous planetary motion provided by the combined horizontal flow-through coil planet centrifuge constructed in our laboratory. Using a set of volatile solvent systems covering a wide range of hydrophobicity, the retention of the stationary phase was determined for various revolutional speeds to obtain phase distribution diagrams which are useful for the understanding and practical application of high-speed CCC. For Parts II and III, see refs. 6 and 7, respectively.

APPARATUS

Principle

        The design principles of the combined horizontal flow-through coil planet centrifuge[8-10], which was used in the present studies, are illustrated in Fig. 1. Schemes I–IV indicate various orientations and motion of a cylindrical column holder connected to a bundle of flow tubes, having their terminus tightly supported at X on the central axis of the centrifuge. In Scheme I, the holder revolves around the central axis at angular velocity ω, while counter-rotating on its own axis at the same angular velocity. The synchronous counter-rotation of the holder continuously unwinds the twist of the tube bundle caused by its revolution, hence the tube bundle becomes free from twisting. A similar anti-twisting effect is produced by the remaining Schemes II, III, and IV. Because of the symmetrical relationship of the holders, Schemes I and
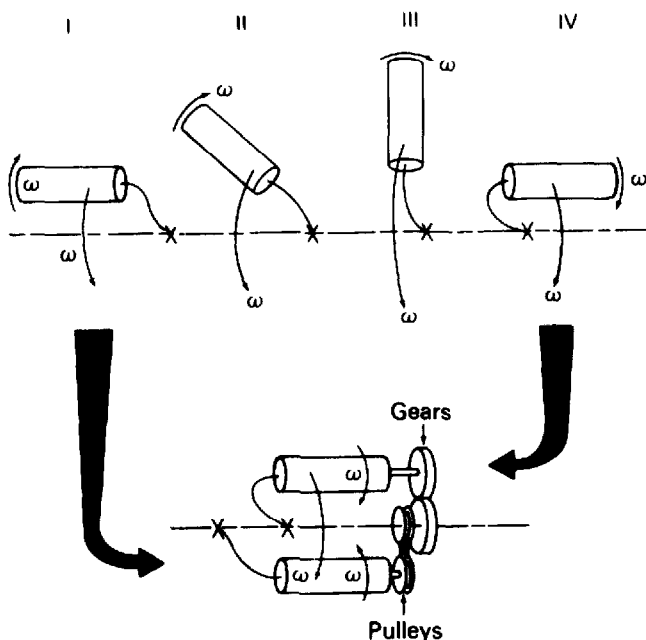

Fig. 1. Principle of the combined horizontal flow-through coil planet centrifuge.

IV can be paired in one apparatus as shown in the lower diagram, while still maintaining the anti-twisting feature. Scheme I synchronous planetary motion is produced by driving a timing pulley on the column holder with a toothed belt coupled to an identical stationary pulley on the central axis whereas the Scheme IV synchronous planetary motion is produced by coupling a planetary gear on the column holder with an identical stationary sun gear on the central axis. The term synchronous designates a fixed ratio between the angular velocity of rotation of the holder on its own axis and the velocity of revolution of the holder about the center axis. The term distinguishes the apparatus used here from a more complex non-chronous device described elsewhere[1].

Analysis of centrifugal force field

Simple mathematical analysis of the centrifugal force fields produced by these two synchronous planetary motions has been reported earlier[1,8,11]. The modes of the synchronous planetary motion (A) and the distribution of the centrifugal force vectors (B) momentarily acting at various locations on the holder are illustrated in Fig. 2. In order to define the location of the point, P, on the holder, a single parameter, $\beta = r/R$, is introduced where r is the rotational radius (distance from the axis of the holder to point P) and R, the revolutional radius (distance between the axis of the holder and the central axis of the centrifuge).

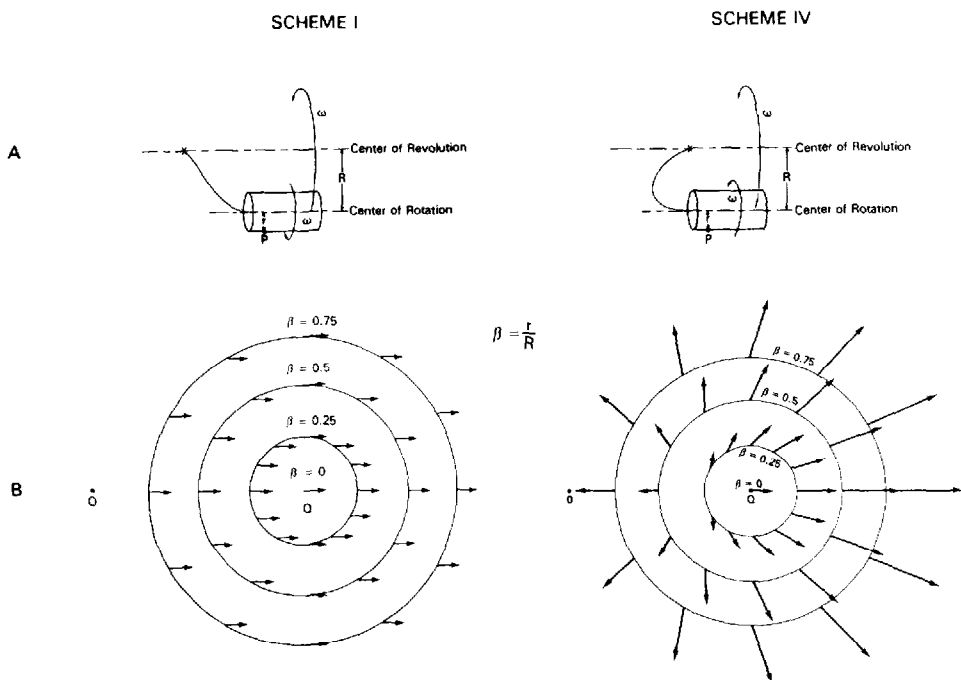In the Scheme I synchronous planetary action (pulley-driven holder), every

Fig. 2. Centrifugal force field produced by two typical synchronous planetary motions of Schemes I and IV. (A) Planetary motion of the holder. (B) Distribution of the centrifugal force vectors at a given moment. Centrifugal force field produced by each synchronous planetary motion was calculated by the aid of an x-y coordinate system where 0 indicates the center of revolution and Q the center of rotation.

location on the holder is subjected to an identical force field regardless of the β values. As the holder revolves around the central axis of the centrifuge, the field rotates at the same angular velocity and in the same direction. The rotation of the centrifugal force field at a high rate produces efficient mixing of the two solvent phases in a coiled column regardless of its location on the holder.

The Scheme IV synchronous planetary motion (gear-driven holder) produces a highly complex heterogeneous force-field which varies in both magnitude and direction according to the location of the point on the holder. However, when the β value exceeds 0.25, the centrifugal force vectors are always directed outwardly from the holder. High-speed CCC has been developed by utilizing this unique centrifugal force field with the column coiled coaxially around the holder. With this coil orientation, the two solvent phases are subjected to vigorous agitation in those segments of the coil in the vicinity of the central axis of the centrifuge and are separated into two layers in coil regions distant from the centrifuge axis. This results in a repetitive mixing and settling process occurring over 13 times per sec at a revolutional speed of 800 rpm[12], which is well suited for solute partitioning. The scheme provides high retention of the stationary phase against a high flow-rate of the mobile phase and produces highly efficient separations in short periods of time.

Design of the apparatus

Fig. 3 illustrates a cross-sectional view through the central axis of the combined horizontal flow-through coil planet centrifuge. The motor (Bodine Electric Co., Chicago, IL, U.S.A.) drives the rotary frame around the central stationary pipe (shaded) by a pair of toothed pulleys coupled with a toothed belt. To stabilize the centrifuge system, the other end of the rotary frame is coaxially connected to a short coupling pipe which is in turn supported by a ball bearing mounted in a stationary wall member of the centrifuge. The rotary frame holds column holders and countershafts on each side in symmetrical positions. On the lower side of the rotary frame (pulley-drive), a toothed pulley mounted on the central stationary pipe is coupled with a toothed belt to an identical pulley on the countershaft. This coupling produces synchronous counter-rotation of the countershaft with respect to the rotary frame. This motion is conveyed to the column holder by a belt coupling a pair of identical toothed pulleys one mounted on the countershaft and the other on the column holder shaft. Consequently, the column holder undergoes synchronous planetary motion identical to that of Scheme I illustrated in Fig. 1. On the upper side of the rotary frame (gear-drive), the stationary gear mounted around the central stationary pipe is coupled to an identical gear on the countershaft to produce synchronous rotation of the countershaft with respect to the rotating frame. This motion is similarly conveyed to the holder by a belt and a pair of identical timing pulleys. The gear-driven column holder, then, rotates with respect to the rotary frame once about its own axis per each revolution of the rotary frame around the central axis of the centrifuge as in Scheme IV illustrated in Fig. 1.

The column holder on each side is removable from the rotary frame and may be positioned at 15 or 20 cm from the axis of the centrifuge by selecting the respective bearing holes on the pair of bearing blocks. For each position of the holder the β value can be varied by changing the holder diameter up to a maximum β value of 0.5, limited by the presence of the countershaft on each side of the rotary frame.
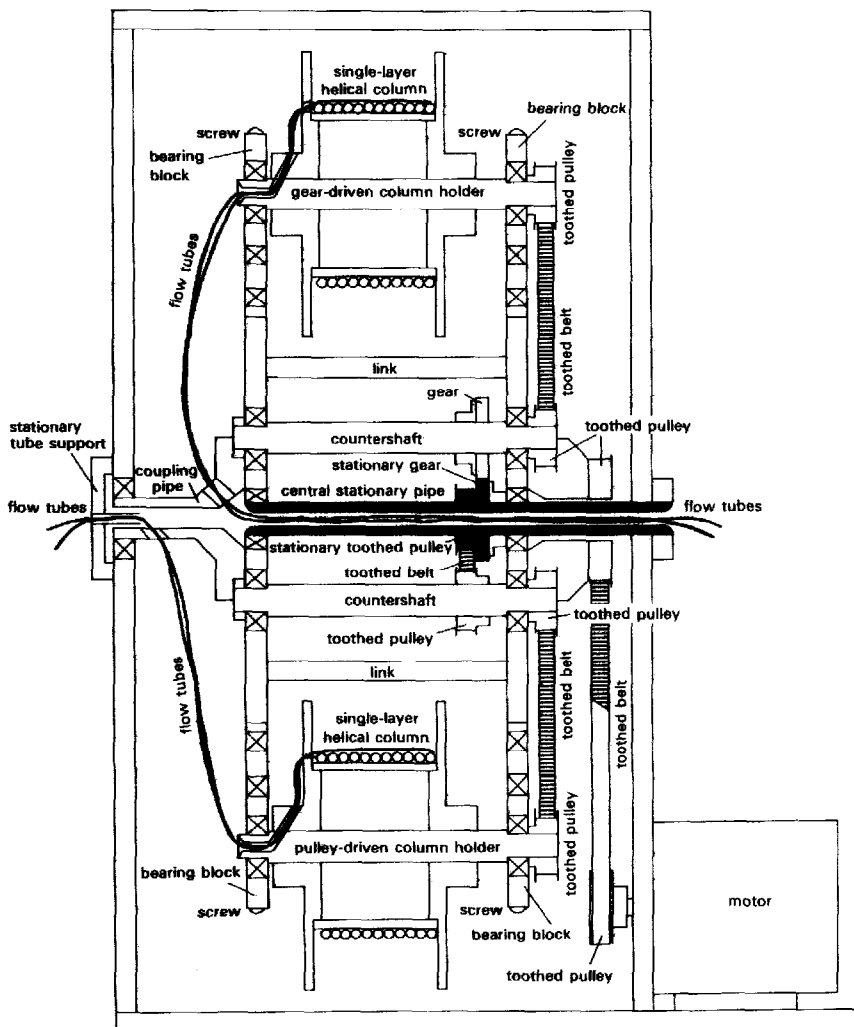
Fig. 3. Design of the combined horizontal flow-through coil planet centrifuge.

The helical column was prepared by winding a piece of PTFE tubing directly onto the holder making multiple helical turns of uniform diameter. The flow tubes from the pulley-driven column holder (bottom) are first passed through the center hole of the holder shaft and then led through a side hole of the short coupling pipe to reach the stationary tube support as illustrated. The flow tubes from the gear-driven column holder (top) are similarly passed through the center hole of the holder shaft and then led through another side hole of the short coupling pipe to enter the opening of the central stationary pipe. Each pair of flow tubes is lubricated with silicone grease and the portions rotating with the frame are protected by a flexible plastic sheath to prevent abrasion by direct contact with metal parts.

The revolutional speed of the apparatus is continuously adjustable up to 1000 rpm with a speed control unit (Bodine Electric, Chicago, IL, U.S.A.).

EXPERIMENTAL

Preparation of two-phase solvent systems

Glass-distilled organic solvents such as hexane, ethyl acetate, chloroform, *n*-butanol, *sec.*-butanol and methanol were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) and reagent-grade glacial acetic acid from Fisher Scientific (Fair Lawn, NJ, U.S.A.). In the present study nine different types of two-phase solvent systems were employed which include hexane–water, hexane–methanol, ethyl acetate–water, ethyl acetate–acetic acid–water (4:1:4), chloroform–water, chloroform–acetic acid–water (2:2:1), *n*-butanol–water, *n*-butanol–acetic acid–water (4:1:5) and *sec.*-butanol–water. Each solvent system was prepared by equilibrating the solvent mixture in a separatory funnel at room temperature and separated before use.

Measurement of phase retention

The present series of experiments was performed with helical columns, 5 m × 1.6 mm I.D., coiled around a 10-cm diameter holder mounted at 20 cm revolutional radii on either side of the rotary frame (β = 0.25). As described earlier[1], a rotating coil exerts an Archimedean screw force which sends the contents toward one end of the coil. This end is called the head and the other end, the tail (for detailed illustration see Fig. 1 in Part II, ref. 6). This head–tail relationship plays a key role in the hydrodynamic distribution of the two solvent phases in the coil. Runs were carried out in two different elution modes for each mobile phase, *i.e.*, the upper phase flowing from the head to tail and from the tail to head, and the lower phase from the head to tail and from the tail to head.

For each measurement, the column was first entirely filled with the stationary phase. Then the apparatus was run at a desired revolutional speed while the mobile phase was pumped through the column at a given flow-rate. The effluent from the outlet of the column was collected in a graduated cylinder to measure the volume of the stationary phase eluted from the column as well as the total volume of mobile phase eluted. The run was continued until an effluent volume of about one and a half times the total column capacity was collected. Then, the apparatus was stopped and the column was emptied by connecting the inlet to nitrogen gas line pressured at 80 p.s.i. The column was then flushed with several milliliters of a solvent such as methanol mutually miscible with both the organic and aqueous phases. Finally, the column was flushed with several milliliters of a fresh stationary phase to be used in the next experiment. During emptying and flushing the column with nitrogen gas, the apparatus was rotated at a moderate speed of 100 to 200 rpm in a direction making the column outlet the head to promote the drainage of the column contents.

Phase distribution diagrams

In each experiment the volume of the stationary phase retained was calculated from the volume of the stationary phase eluted from the column and expressed as a percentage of the total column capacity according to the expression $100(V_c + V_f - V_s)/V_c$, where V_c denotes the total column capacity; V_f, the volume of the flow tubes; and V_s, the volume of the stationary phase eluted from the column. The hydrodynamic distribution of the two solvent phases in the column was summarized by a

phase distribution diagram constructed by plotting the percentage retention of stationary phase as a function of revolutional speed for a particular mobile phase flow-rate. A group of retention curves produced by different elution modes but otherwise identical experimental conditions can be illustrated in a single phase distribution diagram.

RESULTS AND DISCUSSION

Fig. 4 summarizes the phase distribution diagrams for a helical column subjected to both Scheme I and Scheme IV synchronous planetary motions, using the combined horizontal flow-through coil planet centrifuge.

Nine different solvent systems were examined by using each phase as the mobile phase at a flow-rate of 120 ml/h except for the butanol phase systems in which, because of its high viscosity, the flow-rate was reduced at 60 ml/h. Other important experimental conditions such as the column I.D., R, r and β values, and the revolutional speeds, etc., are indicated in Fig. 4. Two retention curves are drawn in each diagram, the solid line for head to tail elution and the broken line for tail to head elution. These phase distribution diagrams are arranged, from left to right, in the order of hydrophobicity of the major organic solvent, i.e., hexane, ethyl acetate, chloroform and butanol. Within each organic solvent group, the phase composition
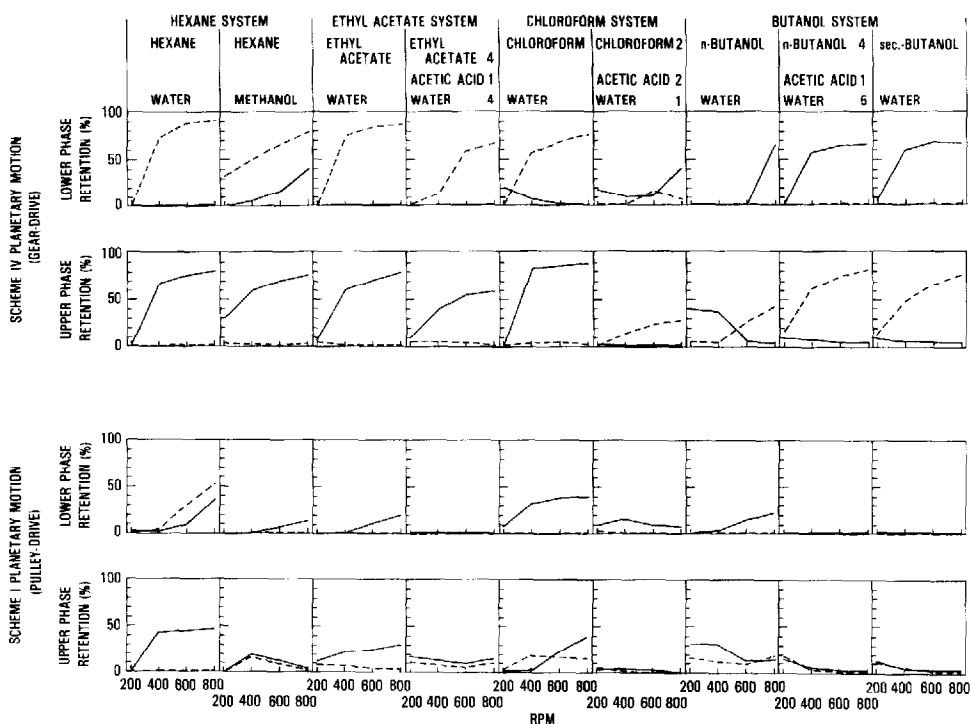

Fig. 4. Phase distribution diagrams in single-layer coil of various two-phase solvent systems obtained by the Schemes I and IV planetary motion (1.6 mm I.D., R = 20 cm, r = 5 cm, β = 0.25). (——) Head to tail elution, (---) tail to head elution.

is modified by addition of a mutually miscible solvent to moderate the interfacial tension of the solvent system.

The phase distribution diagram of the hexane–water system obtained by the Scheme IV planetary motion, upper left diagram in Fig. 4, will serve to illustrate the interpretation of these diagrams. In the upper diagram, plotting lower-phase retention on the ordinate implies that the upper phase is mobile. The retention of the lower phase sharply increases with revolutional speed, reaching over 90% at 800 rpm with the tail to head elution mode (broken line), while no retention occurs in the head to tail elution mode (solid line). This retention behavior can be described as unilateral, to indicate that the upper phase has a strong hydrodynamic tendency to move to the head and lower phase to the tail of the column. Thus with tail to head elution of upper phase, the lower phase is well retained and the flowing upper phase quickly moves through it. On the other hand, with head to tail elution of upper phase, the natural tendency of lower phase to move to the tail is enhanced and the lower phase is simply washed out of the column. In the diagram second from the top, where the lower phase is mobile, high stationary phase retention results from the head to tail elution mode (solid line) because the natural tendency of the upper phase to flow to the head is opposed by the mobile phase flow. Again poor retention of upper phase is obtained if lower phase is pumped from tail to head. These findings agree with the previous observation[13] that the hexane–water system, if confined in a similar column, establishes a unilateral hydrodynamic distribution where the two phases are completely separated in the direction of the column axis, the upper phase entirely occupying the head side and the lower phase, the tail side of the column. This unilateral hydrodynamic distribution of the two phases is ideal for performing CCC, because a high retention of the stationary phases is attainable with either phase used as the mobile phase by selecting the proper mode of elution.

Two other solvent systems, ethyl acetate–water and chloroform–water, also exhibit the ideal retention curves similar to those of hexane–water, when subjected to Scheme IV planetary motion. These three solvent systems are characterized by high interfacial tension and a strongly hydrophobic organic phase.

Ideal retention curves, but with completely reversed hydrodynamic trends, are observed in the two butanol solvent systems n-butanol–acetic acid–water (4:1:5) and sec.-butanol–water. Both organic phases are very hydrophilic and possess high viscosity and low interfacial tension to the aqueous phase. As seen from the retention curves, these solvent systems display a reversed unilateral hydrodynamic motion in the column; the upper phase moves toward the tail and the lower phase, toward the head. If the two phases are confined in the column without introduction of external flow, they establish a unilateral hydrodynamic distribution or complete separation along the axis of the column with the upper phase on the tail side and the lower phase on the head side.

The remaining solvent systems in the Scheme IV group show phase distributions intermediate between those of the above two extreme groups. These intermediate solvent systems are usually prepared by adding methanol or acetic acid to a hydrophobic solvent system to moderate the interfacial tension and hydrophobicity of the organic phase. The relatively hydrophilic n-butanol–water also falls in this intermediate group. All solvent systems treated with Scheme IV planetary motion display a trend of the unilateral hydrodynamic distribution in which one phase moves

toward the head and the other phase, toward the tail of the column, though some are similar to hexane–water while others resemble the reversed hydrodynamic equilibrium of *sec.*-butanol–water.

Phase distribution diagrams obtained from the Scheme I planetary motion under the otherwise identical experimental conditions are illustrated in the lower half of Fig. 4. As these phase distribution diagrams clearly indicate, the Scheme I planetary motion fails to yield a satisfactory retention level of the stationary phase in all solvent systems. The fact that the solid curve is generally higher than the broken curve indicates that higher retention levels are attained by the head to tail elution mode regardless of which phase is used as the mobile phase. These findings strongly suggest that the two solvent phases establish a basic hydrodynamic equilibrium in the column in which each phase occupies nearly equal volume on the head side as previously demonstrated in the coiled column with much smaller helical diameters[14]. Under this basic hydrodynamic equilibrium, the head to tail elution mode always favors the retention of the stationary phase regardless of the choice of the mobile phase, and the maximum attainable retention is usually less than 50% of the total column capacity. The reversed mode of elution from the tail toward the head of the column in these circumstances is usually not feasible because of poor stationary phase retention and steady carry-over of the stationary phase resulting in loss of peak resolution.

Comparison of the phase distribution diagrams for the two different types of the synchronous planetary motion clearly reveals that the Scheme IV planetary motion produces much higher stationary phase retention for all solvent systems under otherwise identical experimental conditions. This surprising contrast between the retention capability of the two schemes must be attributed to the difference in the centrifugal force field produced by their planetary motions illustrated in Fig. 2B. As stated earlier, the Scheme I planetary motion produces a homogeneous centrifugal force field which synchronously rotates with the high revolutional rate of the column. The steadily-rotating force field would introduce constant and vigorous agitation of the two solvent phases in a coiled column especially with large internal diameters.

In the Scheme IV synchronous planetary motion, the centrifugal force vectors always directed outwardly from the holder provide a force component which steadily acts perpendicularly to the coiled tube. Under this circumstances the two solvent phases are separated to form two layers along the helical flow path with the heavier phase in the outer portion and the lighter phase in the inner portion except for one local mixing zone for each helical turn as observed under the stroboscopic illumination[12]. Thus, the Scheme IV planetary motion provides a suitable condition for the two solvent phases to flow smoothly against each other without risk of emulsification.

However, both the mathematical analysis of the centrifugal force field and the actual observation of the hydrodynamic interaction of the solvent phases in a running column fail to explain the mechanism of the unilateral hydrodynamic distribution of the two solvent phases in the coiled column in which one phase travels toward the head and the other phase toward the tail. It is most likely that the unilateral phase distribution is a manifestation of highly complex hydrodynamic interaction involving various physical factors which remain to be elucidated in future investigations.

ACKNOWLEDGEMENT

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